CYTOCHEMICAL INVESTIGATIONS OF FATS AND LIPIDS IN HEPATIC CELLS IN TOXIC AND ALIMENTARY FATTY DEGENERATION OF THE LIVER IN RABBITS

E. V. Chernysheva, N. I. Chumakova, and F. L. Salimon

From the Hospital of Therapeutic Treatment (Dir., Active Member AMN SSSR A. L. Myasnikov), I. M. Sechenov I Moscow Order of Lenin Medical Institute
Presented by Active Member AMN SSSR A. L. Myasnikov
Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 54, No. 9, pp. 114-117, September, 1962
Original article submitted June 6, 1961

It is known that liver lipids are built up not only from neutral fat and cholesterol but also largely from phospholipids, which are substances resembling fat and formed from various fats or their derivatives, compounds containing phosphoric acid and nitrogen bases. The factors responsible for bringing about fat formation in the liver may enable various lipid components to accumulate and they may be formed in several ways.

A study of the living liver by cytochemical tests of samples taken by aspiration puncture has yielded information on the condition of lipid metabolism in certain cases of fatty degeneration of this organ. The experimental work was made on rabbits.

EXPERIMENTAL METHOD

In one group of rabbits, fatty degeneration was induced by one or two subcutaneous injections of 0.4 ml per kg of chloroform. Next, some of the rabbits so treated were cured by treatment with choline. All the rabbits were kept on the same diet, and tests were made when the stomach was empty.

The liver sample obtained by puncture was studied histologically and cytochemically before, 3-4 days after, and 1 and 2 months after the start of the curative treatment. Determinations were made of the amount of neutral fat (smears were stained with Sudan III), cholesterol was determined by Schultz's reaction, and lipids, mostly phospholipids, were revealed by the method of MacManus and by the stain with Sudan black. At the same time the serum was tested for phospholipids (by the method of Blyur, and by a modified Éltman method), while cholesterol was determined by the method of Grigo

Cytological liver studies were made on 16 rabbits which showed no liver abnormality before receiving the chloroform.

Thirteen of the rabbits received the poison. In ten, fatty degeneration of the third degree occurred, in two it was of the second degree, and in one of the first degree.

EXPERIMENTAL RESULTS

As a rule, there were no fatty inclusions in the cells of the reticuloendothelial system. In the hepatic cells stained with Sudan III there were drops of neutral fat. We found no cholesterol.

In the cytoplasm of the hepatic cells we found phospholipids. They were dark in color, irregular in shape, indefinite in outline and lay chiefly around the nuclei, and sometimes they filled almost the whole of the cytoplasm. We judged the amount of phospholipids from the intensity and extent of the stain.

There were no phospholipids in the hepatic or the reticular cells.

At the time when the droplets of neutral fat appeared, the amount of phospholipids present in the liver fell below normal.

The amount of phospholipids fell as the fatty droplets accumulated in the hepatic cells.

More phospholipids were found when neutral fat was absent, or when only a small amount was present. Phospholipids were absent when there was a very large accumulation of the fat. The phospholipid content of the blood in four of the ten animals fell during the period of accumulation of the fatty droplets.

TABLE 1. Phospholipids in the Liver

Amount of phospholipids	Before poisoning	After poisoning
	Number of rabbits	
Large	1	0
Moderate	5	1
Small	6	4
None	1	6

TABLE 2. Relationship between the Amounts of Fat and Phospholipids Accumulating in the Hepatic Cells

Amount of Phospholipids	Degree of fatty degeneration			
	III	II	I	0
Large			..	..
Moderate		...	.	....
Small	.....	...	..	....
None	....	..		..
				.

A 2-month treatment with 0.4 g per kg choline, (daily, by month) was given to five animals with marked fatty degeneration of the liver. One month after the start of the treatment, the cytological and cytochemical picture of the liver had changed (Table 3). In all the rabbits the amount of phospholipids had increased greatly, the number of droplets of neutral fat had decreased.

TABLE 3. Amount of Phospholipids in the Liver of Rabbits Before, During, and After Treatment with Choline

Number of rabbits	Phospholipids			
	Before poisoning	After poisoning	During treatment	After treatment
1	XX	XX	XX	XXX
2	XX	X	XXX	XX
3	X	–	X	XX
4	XX	X	XXX	Died
5	XX	X	XX	XX

After two months of treatment, the cytochemical picture had changed further. The hepatic cells showed no fatty droplets. The amount of phospholipids remained high, and at the same time, in all the treated rabbits there was a marked protein dystrophy.

In four out of the five rabbits, one month after the start of the treatment the amount of phospholipids had risen above normal. A further blood test two months after the treatment, during the period when neutral fat was disappearing from the liver and a protein dystrophy was developing, showed that the blood phospholipid content remained high; however, it had not risen further though during the treatment the blood cholesterol fell.

Thus during the toxic action on the liver there was a marked fall in the amount of phospholipid in the epithelial hepatic cells, and many droplets of neutral fat appeared. Under the influence of choline, the fatty degeneration of the liver cleared up. The amount of phospholipids in the liver and blood increased while the neutral fat in the liver and the cholesterol in the blood fell. Prolonged treatment with choline led to a marked protein dystrophy of the hepatic cells.

The undoubted reduction in the amount of phospholipids occurring together with an increased in the amount of neutral fat in the hepatic cells, and the fact that in some of the rabbits the amount of phospholipids in the blood also fell, compels us to inquire whether in fatty degeneration there may not be some fatty decomposition, in which at first the liproprotein bonds break and liberate lipids (without any increase in the absolute amount of fat); this stage would constitute the so-called lipid decompensation (stage I in the formation of fat). According to [3, 4], next the lipids split off, and from them fat and fatty acids are liberated, and stage II of the fat formation, fatty decomposition, takes place.

Many investigators hold that a reduction of liver glycogen does not prevent fat formation in the liver. Naturally a doubt arises as to whether the formation of fat in the liver occurring in response to toxin is an infiltrative process, i.e., whether it is the result of a transfer of neutral fat from the fat depots to the site of the disappearing glycogen.

It must be supposed that initially the toxin brings about a rupture of the lipoprotein bonds, with the result that the number of phospholipids in the liver, and possibly also in the blood, is reduced; the liver fat then becomes visible when stained, being liberated in the form of neutral droplets.

In eight rabbits with alimentary formation of fat in the liver, a cytological and cytochemical study was made on one occasion after the rabbits had been fed for two months with cholesterol.

Even a cytological investigation revealed a characteristic appearance of the protoplasm, which consisted of a mottled mass containing incomparably more small droplets than there were droplets of neutral fat in toxic fatty degeneration. In some cells, the very small merged droplets filled the whole of the cytoplasm, and the cells could be perceived only from the nucleus and the cell outline. With alimentary fat formation, unlike fat formation induced toxically, very small droplets were present in the cytoplasm of the reticular cells.

In all the rabbits, the stain for neutral fat gave a negative result. However, in five rabbits, staining for cholesterol revealed small dots of a pink-lilac shade lying in the cytoplasm of the cells. Unfortunately, we failed to observe the changeover of the initial color to green, as was described by Schultz. Nevertheless, in our view, this fine granularity was caused by cholesterol, or by cholesterol esterases deposited in the liver.

In five rabbits of this group there were no phospholipids in the hepatic cells, and in three there were only traces. Apparently, as S. M. Leites supposes, this result is associated with the fact that cholesterol suppresses phospholipid formation, and that the reduction in the amount of phospholipids, or, more accurately, the molecules of fatty acids incorporated in the phospholipids, in turn slows down the oxidation and liberation of the fat from the liver. In this group of rabbits, we could easily follow the transfer of the cholesterol by the reticular endothelial cells, i.e., fatty infiltration of the liver was occurring.

SUMMARY

Phospholipids were detected in the epithelial hepatic cells cytochemically by the MacManus reaction. Cytological and cytochemical alimentary cholesterol infiltration of the liver and toxic fatty degeneration of the liver was also detected in the rabbits. Fine vacuoles and a positive Schultz reaction are found in the former, and large coarse vacuoles and a positive Sudan III reaction were found in the latter. In both toxic fatty degeneration of the liver and alimentary cholesterol infiltration, the phospholipids of the epithelial hepatic cells decrease. The reduction of phospholipids in toxic fatty degeneration is evidently the primary cause, leading first to lipid and then to fatty decomposition, whereas in alimentary cholesterol infiltration, the reduction of phospholipids is secondary, and is due to the depressive effect of cholesterol. In toxic hepatic fatty degeneration, choline causes the disappearance of neutral fat from the liver, and a rise in the phospholipid content in both the liver and blood, but at the same time there is a marked protein dystrophy; this effect is likely to limit its application in medical practice. Simultaneous administration of substances for the prevention of protein dystrophy is not recommended.

LITERATURE CITED

1. A. I. Abrikosov and A. I. Strukov, Pathological Anatomy, Part 1 [in Russian], Moscow, 1953, p. 126.
2. S. M. Leites, Uspekhi sovr. biol., 1946, vol. XXII, No. 2.
3. S. M. Leites, Arkh. pat., 1947, No. 3, p. 80.
4. N. B. Medvedeva. Guide to Pathological Physiology [in Russian], Moscow, 1941, p. 183.
5. S. M. Ryss and V. G. Smogin, Ter. arkh., 1958, No. 2, p. 37.
6. A. V. Palladin, Foundations of Pathological Anatomy, Chapter XII [in Russian], p. 435.
7. E. V. Chernysheva and A. V. Burgman, Arkh. pat., 1958, No. 8, p. 38.
8. E. V. Chernysheva and N. I. Chumakova, Ter. arkh., 1959, No. 9, p. 68.
9. S. S. Khalatov, Cholesterol Disease [in Russian], 1941.
