HISTOCHEMICAL FEATURES OF LIPID METABOLISM AND LIPOLYTIC ACTIVITY IN OLD ANIMALS

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Many of the biochemical studies of lipid metabolism in animals have been made in connection with aging [2, 4, 6, 7, 10-14], but there are no references to histochemical work on this problem.

We have studied the histochemical features of lipid metabolism in 30 male white rats aged about two years, weighing 275 ± 15.2 g. As controls we studied the histochemical indices of lipid metabolism in 20 animals from the same group aged one year (mean weight 195 ± 13.2 g).

EXPERIMENTAL METHOD

The animals of both groups were kept under the normal conditions and on the normal food (food pellets, milk, and meat), and were killed by decapitation after starvation for 18 h. At post-mortem examination and after the usual histological procedures we used the following histochemical methods: staining with Sudan IV and with Sudan black B for total lipids; staining with Nile Blue sulphate for unsaturated triglycerides; Schultz and Vindaus for total and for free cholesterol (with Feigin's method for differentiation of cholesterol and its esters); staining with Suday black in paraffin sections after fixation in Beker, and the PAS reaction for the determination of unsaturated lipids (chiefly phosphatides and cerebrosides). We also determined the strength of the protein-lipid bonds in the tissue lipo-proteins using Weil's method and polarization microscopy. To compare the activities of the lipolytic enzymes in old and young animals, we carried out the Nakhlas-Zeligman reaction as modified by Gomori for nonspecific esterase, and the Gomori-Mark reaction for lipase.

EXPERIMENTAL RESULTS

We observed considerable differences of tissue lipid metabolism between old animals and those one year old. Even in the stain for total lipids, in the old animals the amount was increased in most of the organs investigated. In the liver there was a diffuse simple accumulation of fat in the different liver cells in the peripheral and intermediate portions of the lobe, while in most of the animals there was also an accumulation of anisotropic lipids (cholesterol and its esters) in the Kupfer cells and in the macrophage elements of Glisson's capsule. In the same cells there were also PAS-positive inclusions which stained with Sudan black in paraffin sections, and which were apparently phosphatides. In the control group there were practically none of these changes. In one field of view a section of the parenchyma of the liver 10 μ thick contained 27.2 ± 2.2 lipid-containing cells (the result of an examination of 50 fields of view in each of 30 animals at a magnification of 10 x 20 with statistical treatment of the results); in the control group the total number was only 3.22 ± 0.28. This difference is statistically significant.

In old animals, the increase of lipid-containing cells was found in the spleen also; here there was an accumulation of neutral fat, cholesterol esters, and a small amount of unsaturated lipids (apparently phosphatides) in the cells of the red pulp and in the reticular cells of the embryonic centers of the follicles. A count of the number of lipid-containing cells in the spleen (made by the method described) showed that in one field of view of the section, in the old animals, they numbered 58.1 ± 5.3 as compared with 15.8 ± 3.48 in the control group.

Whereas in the renal cortex of the old animals there was practically no fatty dystrophy of the epithelium (only occasionally was there a simple deposition of fat in the separate groups of primary convoluted tubules), in the medulla there was a considerable accumulation of lipids. In the epithelium of the straight tubules, and to a great extent in the

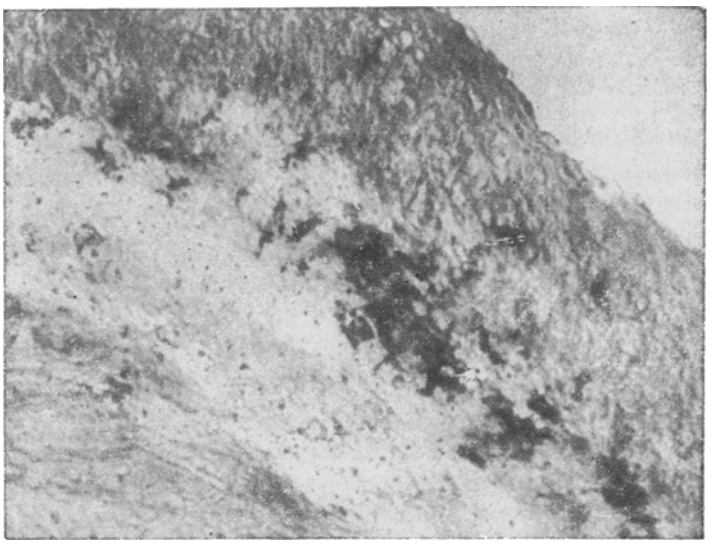

Fig. 1. A large number of cells containing nonspecific esterase (black clumps and spots) in the adventitia of the aorta in an animal one year old. Gomori's modification of the Nakhlas-Zeligman reaction. Ocular 10x, objective 40x.

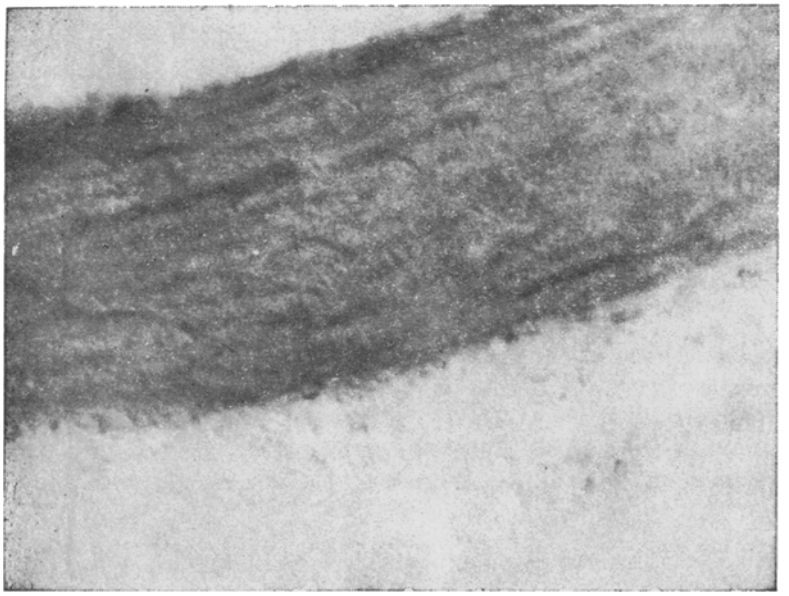

Fig. 2. Almost complete absence of cells containing nonspecific esterase in the wall of the aorta of an old animal. Magnification and histochemical treatment as in Fig. 1.

surrounding connective tissue, there was a fatty infiltration consisting of zones of small droplets some of which had merged, giving rise sometimes in the peritubular zones to an appearance of "fatty lakes". A histochemical analysis of the lipids contained in these zones showed that most of them consisted of neutral fats and, to a very small extent, of free or esterified cholesterol. In the gaps between the tubules of the medullary layer and particularly in the collecting tubules, in most of the animals cylinders were found which stained with Sudan, and a detailed histochemical study showed that they consisted of cholesterol esters. Apparently the liberation of cholesterol esters by the kidney is related to the relatively high content of total cholesterol in the blood of old animals, as has frequently been shown. According to our results the mean amount of cholesterol in the blood of the older animals was 104 ± 16.3 mg %; in the one-year-old group it was 68 ± 12.2 mg %. The cholesterol esters (free form) were also found in the brush border of the straight tubules of the intermediate zone of the kidneys, possibly because of reversed absorption.

Characteristic features of lipid metabolism were shown in the lungs of the older animals. According to many authors [3, 5] this organ plays an important part in lipid metabolism, acting as a depot and a site for the possible breakdown and synthesis of neutral fats and of cholesterol. With the appropriate dyes, in the old animals a considerable number of phagocytic lipid-containing cells were found in the alveoli (particularly in the subpleural regions of both lungs), and also in the interstices of the lungs and in the submucosal membrane of the bronchi. The macrophages contained both neutral fats and unsaturated lipids which stained in paraffin sections with Sudan and gave a PAS reaction. In these cells an even greater amount of cholesterol esters and free cholesterol were found. There were far more of these cells in the old animals than in the young. In the control group, in one field of view there were 9.5 ± 0.15 lipid-containing cells, whereas in the old animals the number was 23.5 ± 5.80. A well-marked simple fattening in the old rats was observed also in the cytoplasm of the cartilagenous cells (chondroblasts and chondrocytes of the hyaline cartilage of the tracheae and bronchi), whereas in the control group the number was insignificant.

No accumulation of lipids was observed in the myocardium of the old animals, but their amount was greatly increased in the fibro-elastic tissue of the heart and vessels, which has been shown [1] to be a site for the selective deposition of cholesterol and its esters in atherosclerosis. In rats it appears that atherosclerosis does not develop spontaneously, and they are relatively resistant to the experimental induction of this condition [9]. However, in old rats the condition here differs from that found in atherosclerosis: the lipid deposits at the base of the valves of the heart and in the subendothelial layer of the aorta take the form of scarcely noticeable zones staining slightly with Sudan, and consist only of neutral fat. A very small zone of simple fattening is sometimes found in old animals in the muscular tunic of the large arteries – the common carotids, the innominate, pulmonary, etc. There are some reports [7, 9] that a lipidosis of the tunics of the arteries is characteristic of this species and can be observed even at an early age, though we did not find it.

In the adrenals of the older animals there was a greater amount of lipids consisting chiefly of free and bound cholesterol than in the young. We also observed an accumulation of lipids, particularly of neutral fat, as well as unsaturated lipids (apparently phosphatides) in the macrophages of the submucosal membranes of the gastrointestinal tract, trachea, large bronchi, ureters, and urinary bladder. Many similar macrophage cells were present in the lymphatic nodes, and (as has already been pointed out) in the red pulp of the spleen. The lipid-containing macrophages in the connective tissue of the viscera in animals of the control group was considerably smaller.

It is interesting to make a comparative study of the stability of the protein-lipid bonds in the lipoproteins of the myocardium (Weil's method). It was found that to break these bonds in old animals a much shorter time of incubation of the tissue in 15% ammonium chloride was required than in the control group. In the control animals the time was 31 ± 2.1 hours, and in the older group only 16.7 ± 1.7 hours; the difference was statistically significant.

To decide on the possible reasons for the far greater amount of lipids in the older group it is important to know the age changes of activity of the lipolytic enzymes. It is for this reason that we studied the activity of the enzymes of nonspecific esterase and lipase in the viscera. The enzymes show a resemblance to each other, and had previously been identified. However, in many reports [16, 17] it has been shown that nonspecific esterase has a broader spectrum of action than lipase: it breaks down (and synthesizes) esters not only of true fatty acids but also of short-chain carbonic acids.

In studying the activity of the lipolytic enzymes in various organs it was found that in the old animals the number of cells containing active enzymes was considerably smaller than in the younger group (Figs. 1 and 2). We have found only one reference [18] to such biochemical findings, and it applied only to the wall of the aorta.

Our mean results and their deviations (see table) show that the differences in lipolytic activity between the old animals and the control group are statistically significant for all organs investigated. We obtained a similar result in an examination of preparations of other organs, without making a count of the enzyme-containing cells; it could be seen that there was a great reduction with age of the lipolytic activity. Thus, in the liver which shows the highest lipolytic activity, where almost every parenchymatous cell contains these enzymes (in both the young and old animals) we were also able to demonstrate an age difference in the activity of the lipolytic enzymes; the cells of the older group were less intensely stained in each reaction.

Apparently the reduction of activity of the lipolytic enzymes with age plays an essential part in the physiological lipidosis of the viscera of the older animals.

Number of Cells in One Field of View of Sections of Different Organs Containing Active Nonspecific Esterase

Group of animal	Spleen	Lung	Myocardium	Heart valves	Wall of aorta
Experimental.....	22,2±3,15	34,6±2,61	12,8±1,39	7,5±0,33	1,17±0,04
Control	36,1±5,52	48,2±5,4	19,5±1,08	11,2±0,61	2,23±0,15

SUMMARY

Lipid metabolism and lipolytic enzyme activity were studied histochemically in old albino rats. The old animals differed from those under one year of age in showing a marked rise in the lipid content of the organs and tissues and a peculiar distribution and qualitative composition of the deposited lipids. The considerable fall with age of lipolytic activity which we observed in all tissues investigated is the probable cause of the physiological lipidosis of the viscera of old animals.

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