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EXPERIMENTAL MODEL OF THE INITIAL STAGES OF ALIMENTARY OBESITY IN RATS

A. L. Pozdnyakov, A. V. Pogozheva, Z. M. Gadzhieva, and V. A. Tutul'yan

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Interest in the problem of alimentary obesity has risen sharply in recent years on account of the wide distribution of this disease among the population of economically developed countries [1, 2]. The mechanisms of development of obesity and, in particular, the role of such lipolytic enzymes as the phospholipases, have not been adequately studied.

The aim of this investigation was to create an experimental model of the initial stage of alimentary obesity and, at the same time, to undertake a parallel study of the morphological and biochemical characteristics of different types of cells.

EXPERIMENTAL METHOD

Male Wistar rats weighing initially 60-70 g were used. The group of control animals received a diet containing by calorific value 20% protein, 50% carbohydrates, and 30% fat (lard and sunflower oil 1:1), together with a vitamin and salt mixture. In the diet of the experimental rats the fat content was increased to 50% (butter) and 1% cholesterol was added. The animals were allowed water and food ad lib.

After 7 months the rats were decapitated and the liver, kidneys, spleen, heart, aorta, and epididymal adipose tissue were removed for subsequent morphological and biochemical investigations. The material was fixed in 10% formalin solution and Carnoy's fluid and embedded in paraffin wax and gelatin. Sections were stained with hematoxylin–eosin, with picrofuchsin by Van Gieson's method, for lipids with Sudan III + IV according to Goldman, and with Oil red, for RNA by Brachet's method, for neutral mucopolysaccharides after Shabadash, for acid mucopolysaccharides with alcian blue after Steedman and with toluidine blue at pH 4.0 and 7.0 after preliminary treatment of some of the sections with methyl alcohol for 2 h, for calcium salts by silver nitrate after Cossa, for amyloid with Congo red, for elastic fibers with fuchselin after Weigert, and for argyrophilic fibers by Snesarev's silver impregnation method.

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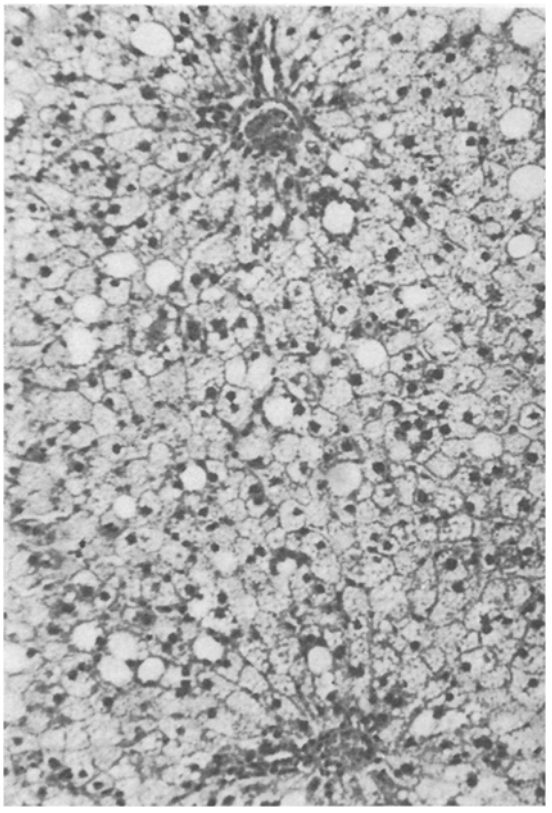

Fig. 1. Liver of rat kept for 7 months on diet containing 50% butter (by calorific value). Marked fatty infiltration of liver cells. Hematoxylin–eosin, 160 ×.

Homogenates of liver, kidneys, spleen, and epididymal adipose tissue were prepared by the standard method using 0.25 M sucrose solution (pH 7.4), containing 0.01 M EDTA as the suspending medium [4]. Activity of lysosomal phospholipases A_1 (EC 3.1.1.32) and A_2 (EC 3.1.1.4) was determined in the homogenates by a radioisotope method [7] using 1-acyl-2[1-^{3}H]-arachidonoyl-3-sn-glycerophosphorylchlorine and 1-acyl-2[1-^{14}C]-oleoyl-3-sn-glycerophosphoryl-choline, synthesized by the writers by the method of Robertson and Lands [6], and substrates. Protein was determined by the method of Lowry et al. [3]. Enzyme activity was expressed in nanomoles substrate hydrolyzed per minute per milligram protein. The level of fat deposition in the rats was determined by a radioisotope method [5], based on dilution of ^3H_2O, injected beforehand (2 h before sacrifice) intraperitoneally in a concentration of 1.0 μCi/ml, in the body weight of the animals.

EXPERIMENTAL RESULTS

Rats kept on a high-fat diet developed a syndrome of overweight (their weight exceeded that of the control rats by 11.5%); the level of fat deposition was 57.9% higher than in the control. Massive depositions of fat in the subcutaneous areolar tissue, omentum, mesentery, and retroperitoneal areolar tissues of the pelvis were found in animals of the experimental group. During histological investigation of the epididymal adipose tissue attention was drawn to an increase in number of the larger adipocytes: Their size ranged to 121 μ compared with 109 μ in the control (along the long axis). The general structure of the adipocytes was unchanged and the fat cells had the appearance of round or slightly oval polygons, filled with lipids consisting of single drops with centers of crystallization, evidently of a cholesterol nature.

The liver of the experimental animals was yellowish brown in color, flabby in consistency, and on section the lobular pattern was indistinct. Histologically the changes in the liver bore the character of fatty infiltration of diffuse type. Lipid inclusions in the form of large- and medium-sized drops distended the cytoplasm of the hepatocytes, increasing their size and displacing the nucleus toward the periphery. Liver cell nuclei were characterized

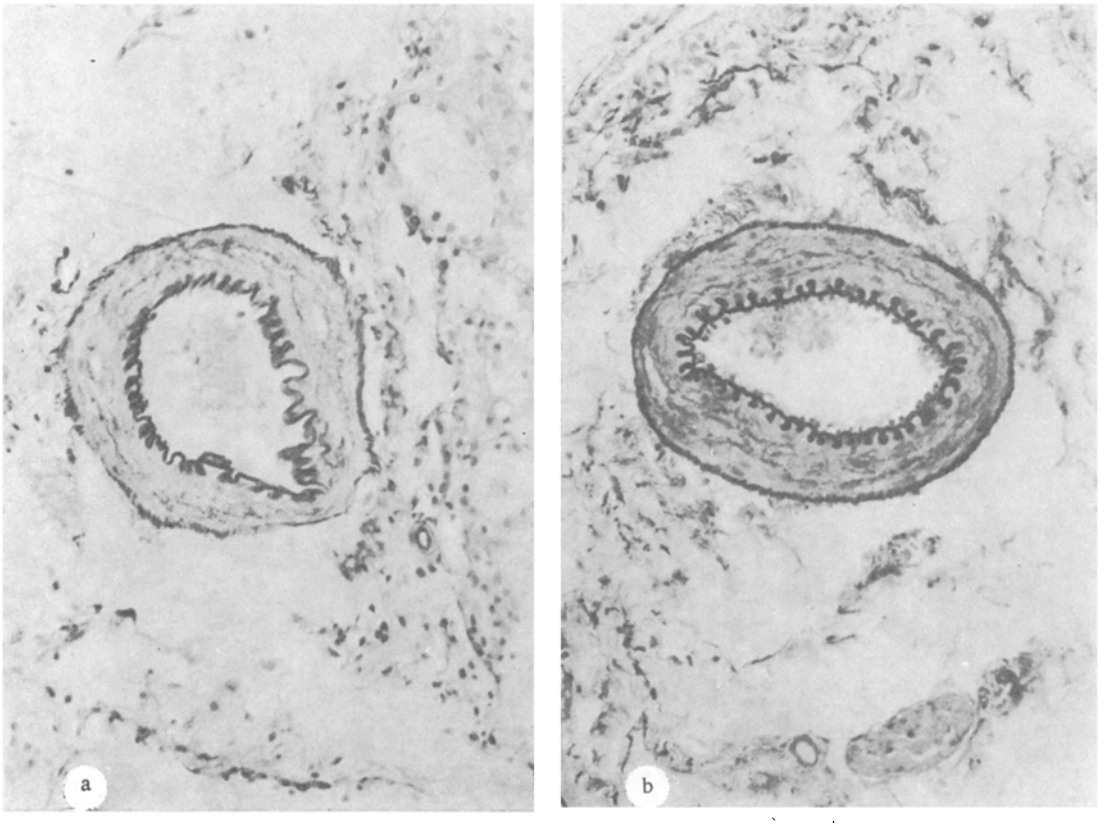

Fig. 2. Structure of intrarenal artery: a) loss of elasticity of inner elastic membrane of intrarenal artery of rat kept for 7 months on diet containing 50% butter (by calorific value); b) unchanged inner elastic membrane of intrarenal artery in kidney of control animal. Weigert's fuchselin stain. 200 ×.

by polymorphism, deformation, the presence of enlarged nucleoli and, in some cases, of vacuoles. In some places, mainly at the periphery of the lobules, hepatocytes saturated with fat were fragmented and joined together to form small fat cysts. In some cases the cysts contained cholesterol crystals, whereas triglycerides were found in the Kupffer cells (Fig. 1). The content of glycogen and RNA in the hepatocytes was sharply reduced.

In the remaining viscera studied (heart, kidneys, and spleen) structural changes were observed chiefly in the inner elastic membrane in the walls of the intramural arteries and took the form of disappearance of its characteristic tortuosity (Fig. 2), with evidence in some cases of multiplication, and with the accumulation of neutral and acid mucopolysaccharides at sites of destruction of the elastic fibers. At the same time vacuolation of the smooth-muscle cells of the media was observed. Similar changes also were found in the aorta.

A parallel study of lysosomal hydrolases in these organs and tissues revealed an increase in phospholipase A activity in the rats with an overweight syndrome (Fig. 3). An increase in phospholipase A_1 activity was observed in all tissues except the spleen, where the level of activity of this enzyme fell to 67% of the control. The maximal increase in phospholipase A_1 activity was recorded in the liver. Phospholipase A_2 activation in rats with an overweight syndrome was detected in all tissues with a maximum in the spleen (179% of the control). Characteristically phospholipase A_1 was distinguished by greater lability than phospholipase A_2 in the liver and epididymal adipose tissue of the experimental rats, whereas the opposite relationship between the levels of activity of these enzymes was observed in the kidneys and spleen. It is worth noting that in joint investigations conducted with L. I. Avren'eva and A. V. Vasil'ev on the same experimental model no change was found in the activity of other lysosomal hydrolases involved in the degradation of proteins (cathepsin D) and polysaccharides (aryl sulfatases A and B, β-glucuronidase).

The results of these morphological and radioisotope studies thus confirm that rats kept on a diet with a high fat content provide an experimental model of the overweight syndrome.

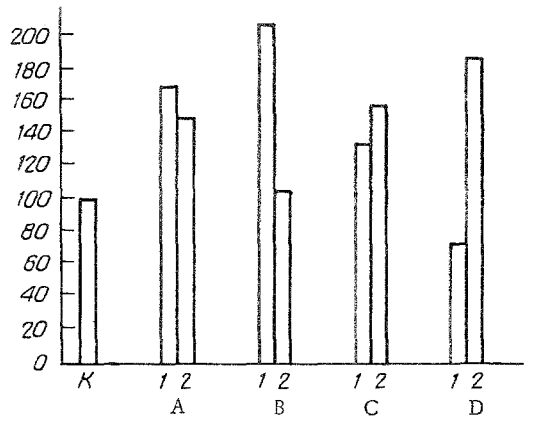

Fig. 3. Phospholipase A activity in adipose tissue, liver, kidneys, and spleen of rats with overweight syndrome. Ordinate: phospholipase activity (% of control). Abscissa: 1) phospholipase A_1 activity, 2) phospholipase A_2 activity. K) Control, A) adipose tissue, B) liver, C) kidneys, D) spleen.

The discovery of the most marked changes in both morphological and biochemical parameters in the hepatocytes and adipocytes means that, with certain reservations, these can be classed as target cells that are most sensitive to an imbalance in the fat component of the diet. Increased phospholipase A activity for all the types of tissues studied is evidently an adaptive reaction of the body, aimed at intensifying degradation processes of lipids, especially phospholipids, when the fat intake with the diet is excessive. This increase in phospholipase activity in the liver and adipose tissue probably also takes place as a result of intensification of intralysosomal degradation of the membranous structures of the cells as a result of their fatty degeneration.

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