# ELECTRON AUTORADIOGRAPHIC STUDY OF INTRACELLULAR CONVERSION OF FATTY ACIDS INTO GLYCOGEN IN RATS WITH ALLOXAN DIABETES

N. P. Lebkova, O. E. Kolesova,
V. D. Gorbunova, Yu. I. Bobkov,
Yu. A. Petrovich, and M. F. Bondarenko

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The formation of fats from carbohydrates in higher animals and man is now accepted as an undisputed fact. Yet the opposite process as a rule is dismissed [8, 10], although conversion of fats into carbohydrates was noted many years ago. In 1927, Shaternikov et al. reported this conversion in a study of respiration of adipose tissue after removal of a considerable part of it [11]. In 1950, Lorber et al. labeled short-chain fatty acids (FA) with radioactive carbon and discovered the label in glycogen in intact rats [15]. In 1952, Abraham et al. used the same method to demonstrate incorpoation of $^{14}$C-labeled long-chain palmitic acid into glucose in the urine of dogs with alloxan diabetes [12]. However, transformation of long-chain FA with an even number of carbon atoms, the main component of intracellular neutral fat, into carbohydrates has met with objections from biochemists [6], whereas the conversion of glycerol, another component of neutral fat, into carbohydrates is accepted by all investigators [1]. However, glycerol is formed during catabolism of intracellular fat in very small quantities compared with FA [3], and the glycogen which arises from it cannot therefore play an essential role in the energy metabolism of the cell.

Conversion of intermediate breakdown products of FA along the path of glycogen synthesis is possible through the so-called glyoxylate cycle, which exists in oil-bearing plants and many lower animals [13]. It has hitherto been considered that this cycle cannot take place in the cells of higher animals and man, but relatively recently it has been found in some mammalian organs also [14].

Indirect evidence of the possibility of conversion of FA into glycogen is given by many ultrastructural data, which were subjected to detailed analysis by the writers previously [5]. Nevertheless, electron-autoradiographic data are essential for the direct proof of conversion of FA into glycogen, and no such data are to be found in the literature.

For the reasons given above, an electron-autographic study was undertaken of the intracellular distribution of hydrogen of FA in alloxan diabetes. There is much evidence that glycogen accumulates in the cells of various tissues of diabetic animals and man [5]. However, since this pathology is characterized by a reduction in the passage of glucose into the cells from the blood, intracellular glycogen formation may take place under these conditions mainly by glyconeogenesis. Under these circumstances the principal substrate for it must probably be FA, the passage of which into the cells is considerably intensified in diabetes [2]. Accordingly we counted on finding the labeled isotope of FA in the structure of glycogen.

## EXPERIMENTAL METHOD

Alloxan diabetes was induced by the method described previously [4] in male noninbred albino rats weighing 200-300 g. Between 2 weeks and 2 months after development of the disease 0.1 ml of [$^3$H]oleic (specific radioactivity 5 mCi/ml) or [$^3$H]arachidonic (1 mCi/ml)

Central Research Laboratory and Department of Pathophysiology, Central Postgraduate Medical Institute, Moscow. Department of Biochemistry, Moscow Medical Stomatologic Institute.
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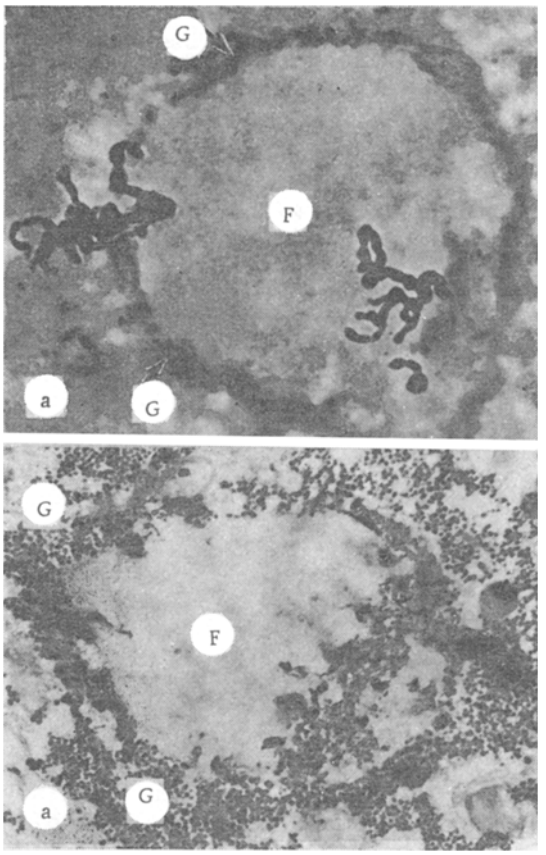


Fig. 1. Glycogen formation around fat in the process of resorption. a) Localization of radioactive label of oleic acid in glycogen surrounding fat in hepatocyte of rat 2 weeks induction of alloxan diabetes. 10,000 ×; b) accumulation of glycogen around and inside lipid inclusion, in process of resorption, in hepatocyte from a patient with chronic cholecystitis [5]. 15,000 ×. G) Glycogen, F) fat.

acid was injected into the caudel vein of the rats. The animals were decapitated under ether anesthesia 2 h after injection of arachidonic (two rats) and 24 h after injection of oleic (five rats) acid.

Myocardial tissue from the subendocardial zone of the left ventricle, liver tissue, and glycogen isolated from the liver by a biochemical method [7], were taken for electron-autoradiographic investigation. Specimens were fixed by Millonig's method in a 2.5% solution of glutaraldehyde and then postfixed in 2% $OsO_4$. The tissues were dehydrated with acetones of increasing concentration, and embedded in a mixture of equal volumes of Araldite and Epon 812. Ultrathin sections cut on the LKB 8802A Ultrotome were mounted on copper grids with formvar film, coated with type M photographic emulsion, and exposed for 1 month [9]. The sections were examined in the JEM-7A electron microscope.

The presence of autoradiographs in the glycogen fraction also was determined by light microscopy in semithin sections treated beforehand by the PAS method to identify glycogen. The number of tracks was counted in the zone of distribution of glycogen and also in the "background" zone, and the significance of differences was then calculated by Student's test.

## EXPERIMENTAL RESULTS

Analysis of the data showed that radioactive isotope, injected into the blood stream of the animals in the form of oleic or arachidonic acids, is incorporated into various structures of hepatocytes and cardiomyocytes. The label was found in mitochondria, where FA are oxidized, and in the nuclei and membranes of several hepatocytes and stellate endotheliocytes.

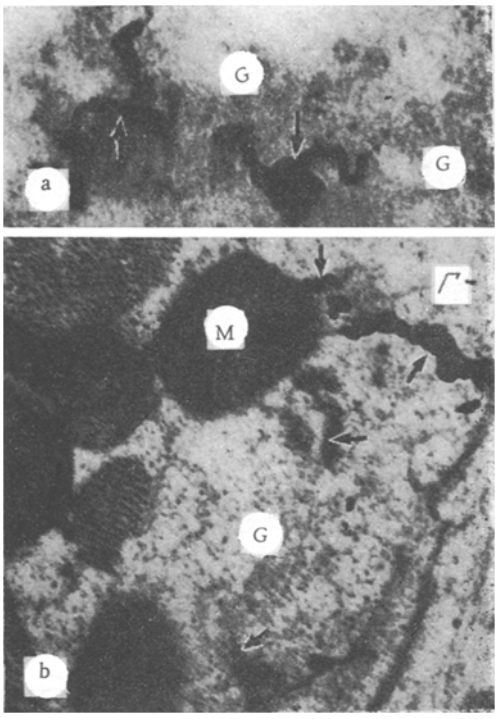


Fig. 2. Distribution of radioactive label of arachidonic acid at site of accumulation of cytoplasmic glycogen. a) Fragment of hepatocyte, diabetes, 2 months. 48,000 ×; b) subsarcolemmal zone of cardimyocyte, diabetes, 2 weeks. 15,000 ×. G) Glycogen, M) mitochondrion.

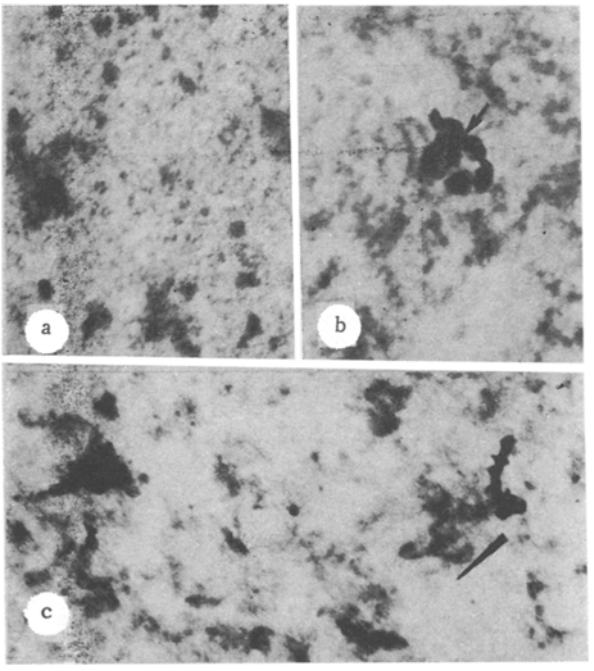


Fig. 3. Location of radioactive label of oleic acid in purified fraction of liver glycogen. a) Glycogen fraction before application of emulsion; b, c) glycogen fraction with autoradiographs after exposure of sections for 1 month (30,600 ×).

Autoradiographs also were found in lipid inclusions present mainly in hepatocytes, which is perfectly understandable because FA are the main component of intracellular triacylglycerides.

At the same time the hypothesis that FA participate in glycogen synthesis was confirmed. As Fig. 1a shows, the radioactive label was located on the boundary between fat and the glycogen surrounding it. The glycogen concentration around and inside the lipid drops undergoing resorption, incidentally, was demonstrated previously by the writers in hepatocytes and other cells in various pathological and experimental states, and also in normal tissues (Fig. 1b), where it was interpreted conjecturally as the ultrastructural manifestation of transformation of intracellular fat into glycogen. Complete agreement between structural data obtained in the present investigation and the data cited above (the formation of a glycogen layer around a lipid inclusion) and, more especially, the discovery of labeled isotope of FA in glycogen surrounding the fat, are evidence in support of the validity of this hypothesis. The location of the radioactive label in glycogen adjacent to fat, in our opinion, indicates the origin of the glycogen from the labeled FA, which probably first became incorporated into the triglyceride, and later, in the course of catabolism, was released again and then transformed into glycogen. An indication that lipid inclusions (Fig. 1a) were in a state of "resorption" was given by their weak osmiophilia. Since glyconeogenesis from glucose is unlikely in diabetes [5], when Fig. 1a is analyzed it is impossible to imagine any other source for the newly formed glycogen than the fat adjacent to it.

Incorporation of the radioactive FA label into glycogen molecules could also be deduced from the presence of autoradiographs in the region of concentrations of glycogen granules in areas of cytoplasm of hepatocytes and cardiomyocytes remote from fat (Fig. 2).

Radioactive isotopes of FA also were found in glycogen isolated from the liver (Fig. 3). The PAS reaction on semithin sections and electron microscopy demonstrated the high degree of purity of the glycogen. Morphometric analysis of semithin sections showed high statistical significance ($P < 0.01$) of differences between the number of tracks in the glycogen fraction ($5.3 \pm 0.8$ tracks per field of vision of the microscope) and in the pure emulsion without glycogen ($1.5 \pm 0.4$ tracks per field of vision).

The results of the electron-autoradiographic study described above are thus direct proof that glycogen in hepatocytes and cardiomyocytes of diabetic rats may be formed from FA. They confirm at the subcellular level the analogous data of Lorber et al., cited at the beginning of this paper, and obtained by biochemical and radiographic methods, and they provide supporting evidence for the writers' earlier hypothesis [5] that fat may be transformed into glycogen in cells of various mammalian and human tissues.

Acceptance of the possibility of intracellular conversion of fat into glycogen provides new approaches to the understanding of the pathogenetic mechanism of development of diabetes and of other diseases connected with disturbances of lipid and carbohydrate metabolism, and also to the search for ways of their prevention and treatment.

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