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RHYTHM OF DNA SYNTHESIS IN HUMAN ADIPOCYTES

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Physiologists have long been of the opinion that during functional loading of average intensity only a proportion of the structural units of a given type in a tissue or organ participates in the corresponding activity. For a long time this view rested on an essentially indirect piece of evidence: the ability of organs and tissues to change their level of functional activity considerably in organisms in different states. More recently direct evidence of differences in the structural and functional state of homonymous cells has appeared. This evidence consists chiefly of histochemical and immunohistochemical data on enzyme activity and the content of glycogen and certain other substances [1, 3, 6], and also the very clearly demonstrable difference in the phases of liberation of secretion in glandular cells [2]. Pathologists are well aware of the different degrees of severity of lesions in different parts or individual cells of the same tissue in many diseases. This fact is nowadays also explained by the unequal functional state of the cells. Since cell activity is controlled by the genetic apparatus, differences in this activity in neighboring cells ought to be linked with corresponding differences in regulating influences. No direct proof in support of this hypothesis has so far been published. This situation is most probably due to the fact that qualitative changes in RNA (base composition, size of molecules) are by their nature unsuitable for analysis by morphological methods; biochemistry, however, cannot detect differences in single cells. In some tissues, however, states arise in which structural and functional differences between homonymous cells are so considerable that they give rise to quantitative changes in the level of RNA synthesis that are detectable by electron-microscopic autoradiography. The results of such observations on fibroblasts and mast cells have been published by Sarkisov et al. [5]. Data on adipocytes are reported in the present communication.

EXPERIMENTAL METHOD

The unchanged fatty areolar tissue of two men aged 55 and 59 years undergoing operations for lung cancer and desmoid was studied. Pieces of adipose tissue measuring 1 × 1 × 0.5 mm were incubated for 90 min at 37°C in medium No. 199 with ^{3}H-uridine in a dose of 100 μCi/ml (specific activity 26 Ci/mmole). After incubation the pieces were washed with cold medium No. 199 and phosphate buffer, pH 7.4, to remove unincorporated ^{3}H-uridine. After fixation with 2.5% glutaraldehyde solution and 1% osmium tetroxide solution the material was embedded in Epon. Semithin sections were coated with M emulsion and exposed for 3 days. Regions for ultrathin section cutting were chosen on the basis of the results of analysis of autoradiographs on semithin sections. Electron-microscopic autoradiographs were prepared by the method described previously [4, 5].

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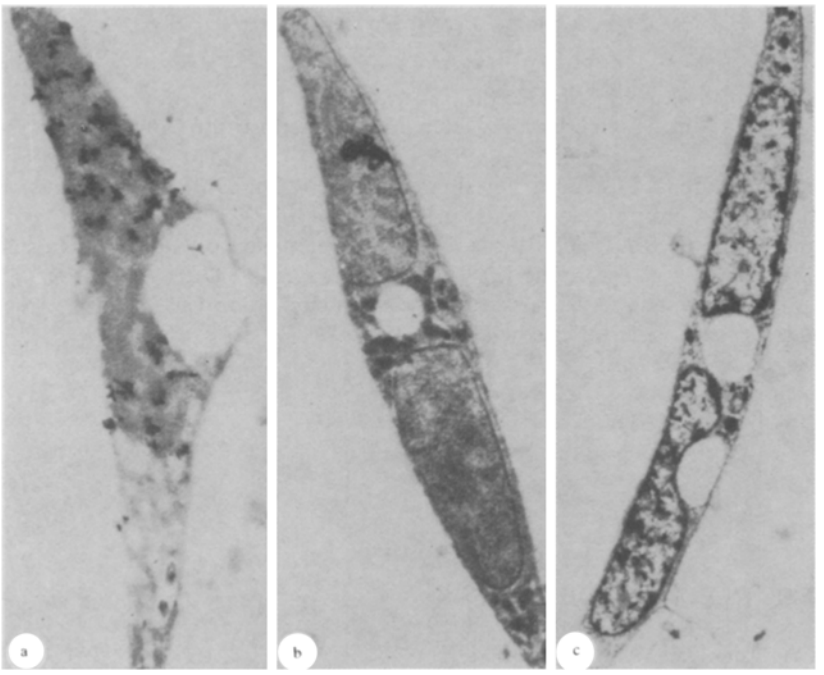

Fig. 1. Different variants of ^{3}H-uridine incorporation into mature adipocytes. a) Concentration of many grains of silver above nucleus with homogeneous chromatin structure, 10,000 X; b) grains of silver scattered singly above nucleus of adipocytes with dispersed distribution of chromatin, 15,000 X; c) unlabeled nucleus with condensation of chromatin near nuclear membrane, 12,000 X.

EXPERIMENTAL RESULTS

Examination of autoradiographs prepared on semithin sections showed that among the adipocytes there were some with an intensively labeled nucleus and others completely unlabeled. However, it must be remembered that in observations under the light microscope it is impossible to be absolutely sure that the nucleus being examined is in fact the nucleus of an adipocyte. Sometimes long nuclei of stromal cells are oriented parallel to the cell surface of the adipocyte and lie at a very short distance from this surface, which can be distinguished only in the electron microscope, so that observed labeling differences may relate to cells of different types (to adipocytes and stromal cells). However, electron-autoradiographic analysis confirmed the results of light-microscopic autoradiography and showed that the adipose tissue contained intensively labeled (Fig. 1a), weakly labeled (Fig. 1b), and completely unlabeled (Fig. 1c) adipocytes. The labeling variants described above applied to mature fat cells with no appreciable morphological differences between them other than the structure of their chromatin. Condensation of chromatin near the nuclear membrane was more marked in the unlabeled cells. What may be the cause of this difference in ^{3}H-uridine incorporation by adipocytes? The first factor to be taken into account is the possibility of a technical error, due to the fact that it was not isolated adipocytes that were incubated but fragments of adipose tissue. Under these conditions access of the uridine to adipocytes in the depth of the fragment may be more difficult than to adipocytes on the surface. Consequently, it can be tentatively suggested that the difference discovered in labeling density did not reflect differences in the velocity of RNA synthesis, but merely the fact that uridine penetrated into some cells in a higher concentration than into others. Here it must be pointed out that adipose tissue proved to be a very convenient object with which to judge the distance from the surface of the fragments at which a particular cell lay. Adipocytes on the surface of the fragment were exposed longer to the action of osmium and, as a result of this intensive treatment, fat bound with osmium did not dissolve when the fragment was subsequently taken through alcohols and propylene oxide. Osmium did not act for such a long time on adipocytes located in the center of the fragment, and it perhaps acted also in a lower concentration, and for that reason the fat in these cells dissolved during treatment with alcohol. The presence or absence of fat could be readily distinguished in light (Fig. 2) and electron microscopes. Considering this fact, it was to be expected that if the density of labeling was determined by the degree of penetration of ^{3}H-uridine, there ought to be more grains of silver above peripheral, fat-containing adipocytes, and fewer above the central, fat-free adipocytes. These expectations were not confirmed. Labeled and unlabeled adipocytes could be seen both in the center and at the extreme periphery of the incubated fragment. Absence of label in peripheral, fat-filled adipocytes is evidence against the hypothesis that differences in labeling were due to the degree of uridine penetration. Osmium is known to coagulate protein and to penetrate slowly into tissue, whereas uridine, on the other hand, penetrates readily through the intact cell membrane. That is why if a cell proved to be accessible for osmium solution, it must have been even more accessible for uridine, and, consequently, absence of label in it could not be attributed to an insufficiency of uridine.

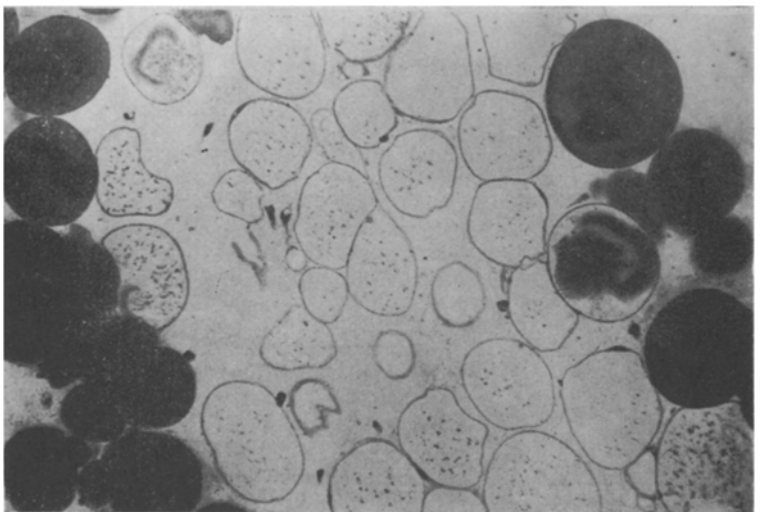

Fig. 2. Semithin section of incubated fragment of adipose tissue. Fat preserved as dark drops, filling the whole cell, in adipocytes at periphery of fragment fixed with osmium. Fat spaces in central adipocytes almost empty, and contain only single, very tiny droplets. Toluidine blue and azure, 320 X.

The cause of the sharp differences in content of label between the adipocytes is thus the different rate of RNA synthesis in these cells. Is it possible that this great difference in the rate of RNA synthesis is a constant property of individual adipocytes? Functional activity of the genome, as expressed in RNA synthesis, is a fundamental process which determines all the structural and functional features of the cell. It is well known that cells synthesizing RNA intensively usually have dispersed chromatin and an extensive cytoplasm with numerous ribosomes, polysomes, and elements of the rough endoplasmic reticulum. That is why a lasting change in the level of RNA synthesis is accompanied by such a radical reorganization of the intracellular architecture so that related cells acquire sharp morphological differences. One such example is the lymphocyte and plasma cell. In this connection the morphological similarity between labeled and unlabeled adipocytes is evidence that the difference between them as regards the level of their RNA synthesis is not a constant, but only a temporary state of these cells, with a period so short that there is no time for visible changes in structure of the cytoplasm to take place during its course. Fluctuations in the level of RNA synthesis in neighboring cells are asynchronous, and for that reason at the moment of fixation of the specimen a maximal level in one adipocyte may coincide with a minimal level in another, and this is reflected in differences in the concentration of grains of silver above their nuclei. The minimal level in the present experimental material was expressed as complete absence of label. In other words, the rate of RNA synthesis in a certain segment of the cycle was most probably zero, or at least below the threshold of sensitivity of autoradiography. The rhythm of genome function revealed in the adipocytes evidently serves as a trigger mechanism which determines the rhythm of all other cell functions under genome control.

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