ELECTRON-AUTORADIOGRAPHIC STUDY OF NUCLEIC ACID SYNTHESIS IN NORMAL ADIPOSE TISSUE AND LIPOMAS

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In the normal and even the obese adult the number of adipocytes is considered to remain constant [7, 10, 15]. Adipocytes which have completed differentiation do not divide and do not synthesize DNA [2, 8, 11, 12, 14]. These observations, however, do not rule out the possibility of renewal of adipocytes. If their life span is shorter than the individual person's life span, constancy of their total number may be ensured by equilibrium between the rate of death of adipocytes and the rate of formation of new adipocytes from precursor cells. It is not yet known exactly to what concrete type these precursor cells belong. It has been suggested that they are fibroblasts [2, 16], primitive mesenchymal cells [9], or histiocyte-like cells [13].

To determine the type of the precursor cells and the level of their metabolic activity, information on DNA and RNA synthesis in the cells composing adipose tissue is essential. To obtain it, in the investigation described below the method of electron-microscopic autoradiography with the DNA precursor [^{3}H]thymidine and the RNA precursor [^{3}H]uridine was used. Besides normal adipose tissue, tissue from lipomas at different stages of maturity was also studied.

EXPERIMENTAL METHOD

As normal adipose tissue, unchanged subcutaneous cellular tissue from the region of a surgical incision was studied from four men aged 31-58 years undergoing surgery for gastric ulcer (two cases), lung cancer, and a desmoid. Lipomas which had not increased in size for several years were removed from two women aged 35 and 54 years respectively. A slowly growing lipoma ("Madelung's lipoma") was removed from the neck region in three men aged 40-54 years. Material for histological investigation was fixed in 10% formalin. Paraffin sections were stained with the ordinary histological stains, and frozen sections with Scharlach red. Specimens for electron-autoradiographic investigation were incubated for 1.5 h at 37°C in medium 199 with [^{3}H]thymidine (specific activity 21.6 Ci/mmole) in a dose of 10 μCi/ml or with [^{3}H]uridine (specific activity 26.0 Ci/mmole) in a dose of 100 μCi/ml. After incubation the pieces were washed with cold medium 199 and phosphate buffer, pH 7.4. The material was fixed with 2.3% glutaraldehyde solution and 1% OsO_4 solution, then embedded in Epon. Semithin sections for analysis were prepared by the method of light-microscopic autoradiography. Depending on the results of analysis of the autoradiographs, areas for cutting ultrathin sections were chosen on the semithin sections. Electron-microscopic autoradiographs were prepared by the technique described previously [4, 5]. Since a semithin section of an incubated fragment of adipose tissue contains few interstitial cells and adipocyte nuclei, to determine the number of labeled elements among them, 10-20 staggered sections were studied from each fragment. The number of labeled cells in the vessel wall was counted in each separate tissue fragment. The values obtained were divided into three groups: 1) normal fatty cellular tissue; 2) nongrowing lipoma; 3) Madelung's lipoma. The significance of differences between these groups was determined by Wilcoxon's test.

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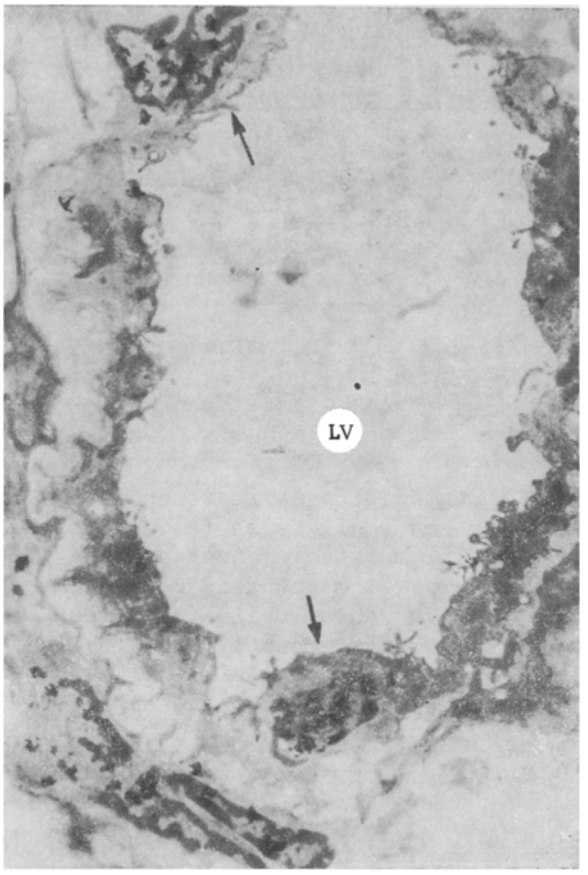

Fig. 1. Tissue of Madelung's lipoma incubated with [^{3}H]thymidine (12,000 ×). Grains of silver above nuclei of endothelial cells (arrows) indicate DNA synthesis in them. Here and in Figs. 2 and 3: LV) lumen of vessel.

EXPERIMENTAL RESULTS

The histological structure of the three objects compared (normal fatty cellular tissue, nongrowing lipomas, and Madelung's lipomas) was similar in type, in agreement with data in the literature [1]. Only in some areas of Madelung's lipomas was moderate proliferation of the cells observed. The only morphological difference between the cells in the objects compared was the presence of a few young adipocytes in the samples of Madelung's lipoma (Table 1). In the early stage of development these cells were characterized by the presence of several cavities containing fat (multilocular cells). A gradual increase in size of these cavities leads to thinning of the walls between then and fusion of the cavities into one large fat vacuole, the size of which at first is not much different from that of the nucleus (unilocular) cell). Later the fat vacuole becomes many times larger than the nucleus and displaces it, together with a narrow rim of cytoplasm, to the periphery of the cell. Dying and dead cells were found among the mass of adipocytes. The main distinguishing feature of the latter was an increase in contrast of the cell pattern: All the intracellular structures became compact and condensed in appearance and the spaces between them appeared as an electron-transparent space devoid of material. This picture was evidently due to denaturation of proteins. In the nuclei of these cells, unlike normal cells, the heterochromatin was not concentrated chiefly near the nuclear membrane, but was distributed in the form of floccules throughout the volume of the nucleus. The spaces between the floccules of condensed chromatin were electron-transparent and did not contain dispersed chromatin, as in the normal cell. Condensed residues of organelles in the cytoplasm were separated by wide "empty" zones. Tears appeared in the plasma membrane. Fragmented adipocytes in normal fatty cellular tissue and the nongrowing lipoma were found only as single cells. They were much more frequently seen in Madelung's lipoma.

In all types of adipose tissue studied the cells labeled with [^{3}H]thymidine were almost exclusively endotheliocytes and pericytes (Figs. 1 and 2). Only in a few cases was the label

TABLE 1. Comparison of Incorporation of [^{3}H]Thymidine and [^{3}H]Uridine into Types of Adipose Tissue Investigated

Tissue studied	Content of young adipocytes	Content of [^{3}H]-thymidine-labeled cells in vessel walls	Content of [^{3}H]-thymidine-labeled adipocytes	Content of [^{3}H]-uridine-labeled adipocytes
	%			
Normal fatty cellular tissue	0	0,5	0	11,4
Nongrowing lipoma	0	0,45	0	9,0
Madelung's lipoma	0,2	1,6*	0,3	10,2

Legend. Young adipocytes represented by multilocular and small cells (size of fat vacuole similar to size of nucleus). Asterisk indicates that content of [^{3}H]thymidine-labeled vascular cells in Madelung's lipoma differed significantly from their number in normal fatty cellular tissue and nongrowing lipoma (in both cases $P < 0.01$).

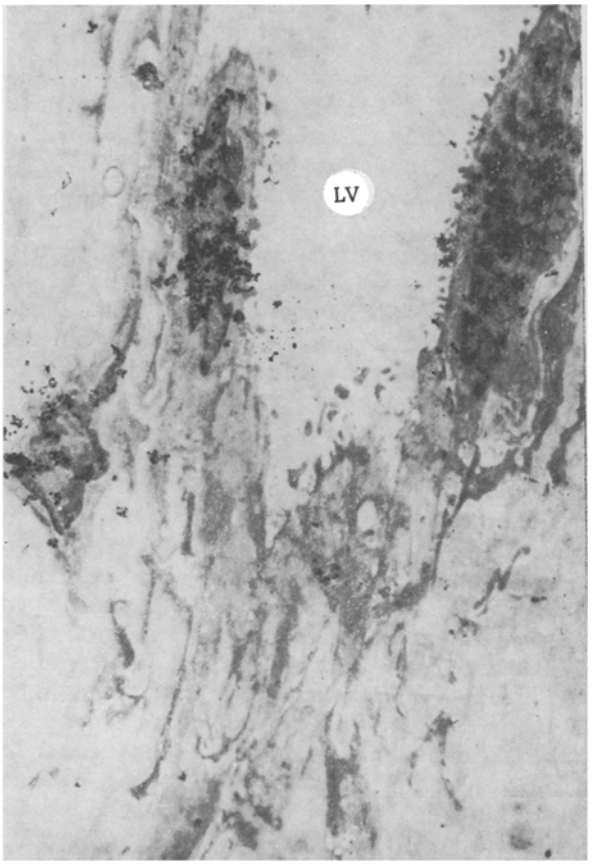

Fig. 2. Incorporation of [^{3}H]thymidine by vessel wall cells in Madelung's lipoma (12,000 ×).

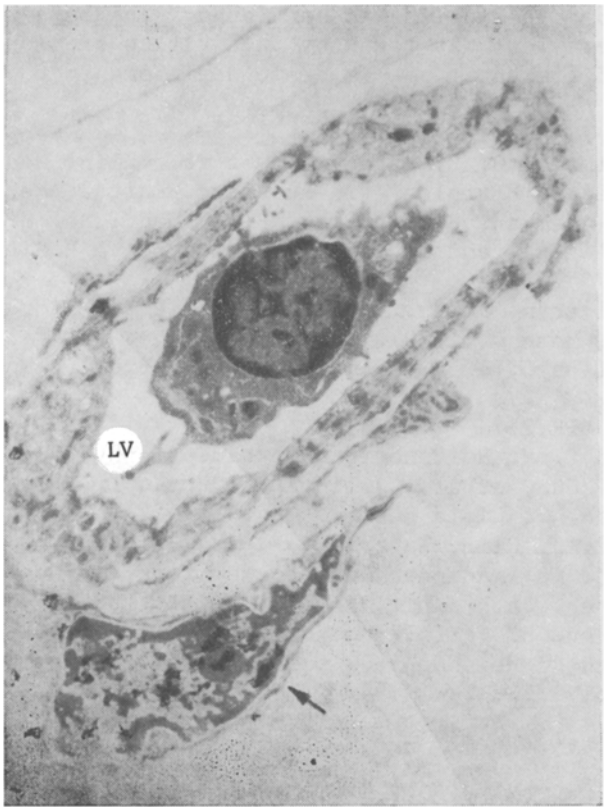

Fig. 3. RNA synthesis (incorporation of [^{3}H]uridine) in pericyte (arrow) of Madelung's lipoma (18,000 ×).

found in undifferentiated stromal cells, the position of which relative to the vessel wall was unclear. Adipocytes labeled with [^{3}H]thymidine were found just as infrequently, and only in Madelung's lipoma. The number of [^{3}H]thymidine-labeled cells in the vessel walls in Madelung's lipoma was significantly higher than in normal fatty cellular tissue and in the nongrowing lipoma (Table 1).

Incorporation of [^{3}H]uridine in all specimens studied was uniform. All endotheliocytes and pericytes were intensively labeled (Fig. 3). Most adipocytes did not take up [^{3}H]uridine, but in each object studied about 10% of the adipocytes were labeled. The amount of label was small in adipocytes containing a thick layer of heterochromatin near the nuclear membrane and, conversely, cells with a more dispersed distribution of chromatin were very intensively labeled. The presence of a few adipocytes labeled with [^{3}H]uridine among the unlabeled cells is evidence that RNA synthesis in these cells takes place only at certain periods of their life cycle. Further investigations are needed to identify these periods: a phase of development of the cell which it passes through only once in its life, or a frequently and regularly repeated change in functional activity connected with the rhythm of many physiological processes [3].

The results have yielded additional material for the study of histogenesis of tumors of adipose tissue. Constancy of the number of adipocytes in the adult human and our discovery of dying adipocytes can be explained only by compensation of their death by new formation. The appearance of new adipocytes is indicated also by growth, albeit slow, of Madelung's lipoma. Since adipocytes themselves do not divide, their new formation must be the result of proliferation of certain precursor cells. The observations made in this study indicate that the only type of cells which can perform the function of this precursor are cells of the vessel wall. This follows from the fact that virtually no other proliferating cells were found in specimens of adipose tissue studied. Endotheliocytes and pericytes also were labeled most intensively and constantly with [^{3}H]uridine, and this may reflect their more rapid differentiation. Further confirmation of the role of vascular cells as the source of formation of new adipocytes and growth of fatty tumors is given by the fact that we found a significant increase in the number of [^{3}H]thymidine-labeled vessel wall cells in Madelung's lipoma, characterized by an increase in mass of adipose tissue and by the largest number of dying adipo-

cytes, but proliferation of any other cells was not found. The same role, i.e., tumor growth on account of pericyte proliferation, was found by the writers previously when studying a connective-tissue tumor of a different type — a desmoid fibroma [6].

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DEPENDENCE OF MONONUCLEAR INFILTRATION OF THE LIVER ON HEPATOCYTE PROLIFERATION

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After injection of microbial polysaccharides into mice, multiple foci of mononuclear infiltration are formed in the liver. Their origin is linked with primary stimulation of Kupffer cells (KC) and secondary "recruiting" of mononuclear cells — monocytes and macrophages, lymphocytes and their derivatives from the bloodstream and lymph [6]. Foci of mononuclear accumulation are evidently formed, on the one hand, under the influence of chemoattractants, secreted initially by stimulated hepatic mononuclear phagocytes (MP), and on the other hand, by induction of medullary monocytopoiesis [3]. It has been shown that restoration of the structure of the liver after partial resection depends on the initial functional state of the KC [4]. The proliferating parenchyma of the liver is also known to inhibit the fibroplastic process (FP) in the same organ [5]. However, the mechanisms of this effect have not been studied. Realization of FP largely depends on reactivity of resident marcophages in the stroma [2].

It was accordingly decided to study how reactivity of MP in the liver stroma and the MP system as a whole change when hepatocyte proliferation is disinhibited. The investigation described below was devoted to these problems.

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