and the formation of numerous discrete thin nerve bundles, whose fibers began to be myelinated at the 25th-30th days after transplantation. Isolation of the graft from the CNS was evidently the reason for the uncontrolled growth of the newly formed myelinated fibers in all directions and throughout long periods of observation (until nine months). The results indicate high functional activity of transplanted differentiated neurons and the potential powers of regeneration of differentiated nerve cells of adult animals. This long-term transplantation of sensory neurons can serve as a model for the study of the morphological and functional potential of differentiated neurons and for the creation of new nerve centers in the body.

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DIFFERENTIATION OF A TRANSFORMED CULTURE OF L-CELLS

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L-cells arising from subcutaneous connective tissue and adipose tissue of C3H mice were cultured in nutrient medium with the concentration of bovine serum increased to 60%. Differentiation of some of the cells into fat cells took place, with the formation of structures similar to normal adipose tissue. The occurrence of differentiation was judged from the formation of characteristic signet-ring cells, whose cytoplasm was filled with neutral fat, giving a positive reaction for lipids when stained with a mixture of Sudan III and Sudan IV. It is concluded that the long existence of the cells in vitro and the intracellular changes accompanying the transformation process, including those of mutation character, do not render the cells incapable of differentiation. KEY WORDS: L-cells; differentiation; adipose tissue; signet-ring cells; neutral lipids.

The concept of reversibility of malignant properties, affording new prospects for influencing the tumor process [3, 7, 9], is based on the epigenetic theory of malignant transformation of cells [5]. In some cases, however, malignant transformation is accompanied by changes of mutation character. For example, in normal cells in culture, kept in vitro for a long period of time, and during malignant transformation metabolic [6], antigenic [14], and genome [8] changes arise. In particular, for cultures of L-cells, which are extensively used in scientific research, considerable changes characteristically take place in the karotype, which distinguish these cells from their normal precursors [13]. Accordingly the problem of preservation of their ability to differentiate arises.

To study this problem experiments were carried out and their results are described below.

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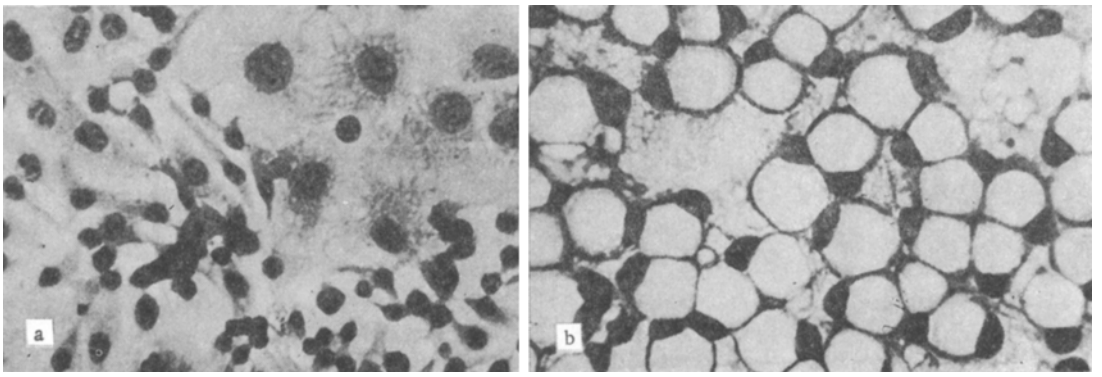

Fig. 1. Culture of L-cells: a) control; b) culture grown in medium with 60% serum: signet-ring cells. Stained with hematoxylin-eosin, 160×.

EXPERIMENTAL METHOD

L-cells were grown on coverslips in flasks under penicillin at 37°C. The number of cells seeded was 2000-300 per flask. Control cultures were grown in medium consisting of medium 199 (45%), lactalbumin hydrolysate (45%), and bovine serum (10%). The nutrient medium used for the experimental cultures consisted of medium 199 (20%), lactalbumin hydrolysate (20%), and bovine serum (60%). The high concentration of serum was used to induce differentiation [10]. Into each flask 4 ml of nutrient medium was poured. One batch of cultures was fixed in 96% ethanol, the other in the vapor of 10% neutral formalin on the 10th and 25th days of explanation. The preparations were stained with hematoxylin and eosin. Histochemical tests also were carried out for polysaccharides by the PAS reaction and for lipids with a mixture of Sudan III and Sudan IV, and also with Sudan Black B, by methods recommended in [1].

EXPERIMENTAL RESULTS

By the 7th-10th day of explanation cell colonies consisting of round, stellate, polygonal, or fusiform cells of different sizes were formed in both the control and the experimental cultures. The cell nuclei of the control cultures were round or oval in shape, varied in size, and central in position. The chromatin consisted of fine or coarse granules. The 2 to 10 nucleoli were polymorphic and clearly outlined. Individual colonies consisted of cells containing one or more round vacuoles in their cytoplasm, the contents of which gave a positive reaction for neutral lipids with Sudan III and IV. In individual cells vacuoles occupied the greater part of the cytoplasm and compressed the nucleus. Under these circumstances the chromatin became more compact.

In the experimental cultures vacuolation of the cells was more marked and was observed in more cells. The vacuoles varied in size and were round in shape. They gave a positive reaction for neutral lipids. The nuclei of these cells were round or oval, with coarsely granular chromatin, and located in the central part of the cells. In some cells the nuclei were compressed by the lipid inclusions. The nucleoli varied in size and shape and were clearly outlined.

The PAS reaction was positive in both the control and the experimental cultures. However, it was more strongly positive in the experimental series. For instance, whereas in the control cultures polysaccharides were concentrated as small but numerous granules mainly around the nuclei, in the experimental cultures large concentrations of these substances were discovered throughout the cytoplasm. In control experiments with salivary amylase a weak reaction was obtained, evidence of the presence of glycogen and of other PAS-positive compounds in the cells.

By the 20th-25th days of growth individual colonies in the control cultures began to merge with each other to cover whole areas of the slide. In some places stratified growth was observed. During this period large and small, round, polygonal, and fusiform cells were found in the explants. The small round cells had a narrow rim of cytoplasm. The nuclei were round and hyperchromic. The 3 to 5 nucleoli were clearly outlined. In cells located on the surface of the monolayer the nucleoli were indistinctly outlined. The large polygonal and round cells had round nuclei located in the center of the cell, coarsely granular chromatin, and 5 to 10 clearly outlined nucleoli. The fusiform cells had oval nuclei. Their chromatin was finely granular or dust-like. In the large cells the nucleoli were clearly outlined, but in the small cells indistinctly. They varied from 3 to 6 in number. Nucleoli in all cells were polymorphic (Fig. 1a). In most cells neutral lipids were detected as small

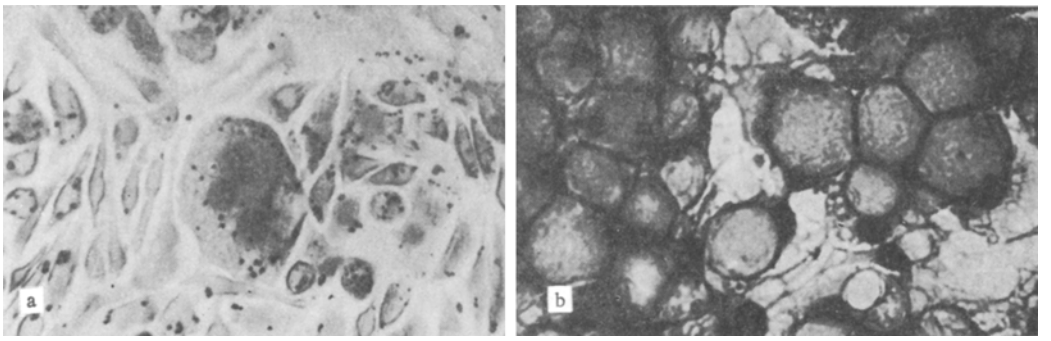

Fig. 2. Demonstration of lipids in L cells: a) control culture: small drops of neutral fat in cytoplasm of cells; b) culture grown in medium with 60% serum: unilocular type of cell differentiation. Here and in Fig. 3, stained with Sudan III mixed with Sudan IV; 160×.

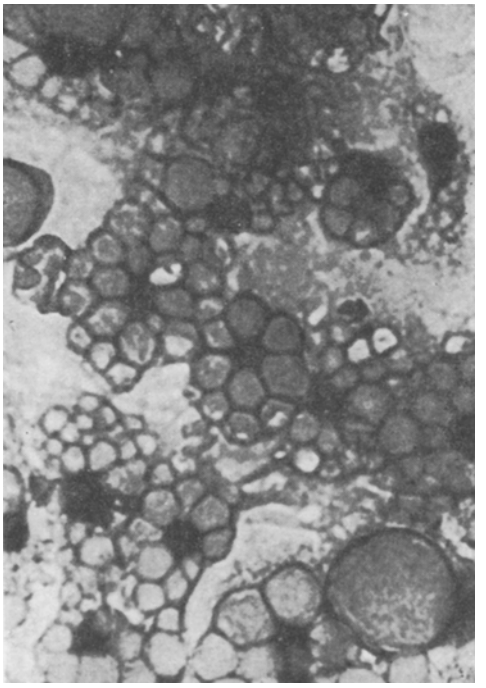

Fig. 3. Multilocular type of differentiation of L cells.

droplets, 10 to 15 in number, situated in different parts of the cytoplasm (Fig. 2a). Larger lipid drops were found in a few cells.

In the experimental cultures by this time continuous fields of large, spherical cells, resembling signet-rings in appearance, were formed. The cells were arranged mainly in a single layer. The nuclei of these cells were displaced to the periphery of the cytoplasm and appeared flattened or sickle-shaped. Their chromatin was no longer granular in character but more compact, and no nucleoli could be detected (Fig. 1b). The contents of the cytoplasm stained bright orange with a mixture of Sudan III and IV, evidence of the accumulation of neutral lipids in them. The large fat drop in each cell was surrounded by a rim of cytoplasm, forming a membrane around it (Fig. 2b). In different parts of the culture giant multinuclear (from 2 to 5 nuclei) cells filled with fat could be seen. Their nuclei were located at the periphery of the cytoplasm and were flattened or sickle-shaped in appearance, hyperchromic, and dense. No nucleoli could be detected. The cytoplasm of the multinuclear cells was spherical in shape and was completely filled with neutral fat.

Besides unilocular fat cells, in the experimental cultures a multilocular type of differentiation of the cells also was observed. The cytoplasm of these cells was vacuolar in appearance and azure in color. The nu-

clei, located in the center of the cell or a little eccentrically, became pycnotic and it was impossible to distinguish any structures in them. The shape of the nuclei also changed. They were flattened or became star-shaped as a result of the formation of indentations from adjacent fat drops (Fig. 3).

Phospholipids, detectable with the aid of Sudan Black B, were revealed as material stained grayish-blue, mainly in the membranes of the fat cells. In control cultures phospholipids were found as small droplets in the cytoplasm of round cells, and also in the cell membranes.

The reaction for glycogen was positive in both the experimental and the control series. In the control cultures it differed from the earlier periods of culture by the weaker reaction in all the cells. In mature fat cells of the experimental cultures glycogen could be demonstrated only in the membranes. The contents of the cytoplasm gave a negative reaction.

Among the collections of fat cells single fibroblast-like cells or thin fibers of different lengths could be seen. In the intervals between the collections of fat cells there were long or polygonal cells with undifferentiated nuclei, among which pictures of mitotic division could be seen. In some cultures these areas were occupied by fibroblast-like cells intervening between the collections of fat cells and separating them. On the whole the picture was similar with that observed in normal subcutaneous adipose tissue [2].

The results of these experiments suggest that as a result of the increase in the serum concentration in the nutrient medium used to culture the L cells, differentiation of part of the cell population into fat cells takes place, with the formation of structures similar to those of normal subcutaneous adipose tissue. This suggestion was confirmed by the presence of characteristic signet-ring cells, pursuing the unilocular type of differentiation, and of azure vacuolated cells, pursuing a multiocular type of differentiation, and the contents of whose cytoplasm gave a positive reaction for neutral lipids. That differentiation had taken place was also shown by the fact that the nuclei assumed the characteristic shape of fat cells, the nuclear chromatin was condensed, and the nucleoli were indistinctly outlined. In addition, the actual origin of the cells of this strain, which was derived from cells of the subcutaneous connective and adipose tissue of C3H mice [11, 12], may also serve as an argument in support of the view that differentiation took place. It also evidently explains the presence of cells differentiating into fat cells in this particular culture. It must be added that lipid formation could be the result of the accumulation of glycogen in the cells of the experimental cultures in the early periods of their growth. A conversion of this type takes place during lipid synthesis by normal fat cells in the healthy organism.

As already stated, besides fat cells, the test culture also contained fibroblast-like cells, whose differentiation can be induced by reverse transcriptase [4]. Accordingly a culture of L-cells can be regarded as a population consisting of cells whose differentiation can take place in different directions.

The results of these experiments thus indicate that, despite their existence in vitro for 34 years and the intracellular, including genome, changes which have taken place during this period, L-cells have remained capable of differentiation in response to a change in the conditions of their existence.

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