A MORPHOLOGICAL AND HISTOCHEMICAL STUDY OF HUMAN DIPLOID CELLS

S. Ya. Zalkind, N. V. Borisoglebskaya, and R. I. Rapoport

UCD 611-018.1+612.014.1

Strains of diploid cells obtained from human embryonic tissues are now extensively used. They can be cultivated for long periods (up to one year), they are capable of rapid growth, they preserve the diploid set of chromosomes typical of the original tissue, they possess a broad spectrum of sensitivity to the action of viruses, and they have no oncogenic properties [6-9]. The life cycle of such a strain may be divided into three phases: development, florescence and decline.

In the present investigation a dynamic study was made of the morphology and metabolism (by means of of histochemical reactions) of one strain of diploid cells taken from the first passages, from the moment of obtaining them until their natural death.

EXPERIMENTAL METHOD

Strain L-42, obtained at the Institute of Virus Preparations from human embryonic lung tissue, was investigated. The cells were studied in the 1st, 5th, 14th, 16th, 20th, 26th, and 40th passages on the second or third days of cultivation. The cells were grown in tubes on mica disks by the usual method [1].

The morphological investigations were carried out on preparations stained with hematoxylin-eosin and by Brachet's reaction, and morphological observations were made with the luminescence microscope after staining with acridine orange to reveal RNA, by Shabadash's method for glycogen, and with bromphenol blue for protein. A reaction for total lipids (staining with Sudan black B) was carried out selectively.

EXPERIMENTAL RESULTS

Morphological investigations. Strain L-42 consists mainly of fibroblastlike cells. The fusiform shape of the cells, their marked polarity, and their attachement in "streams" create the characteristic picture of the cell layer (see Figure, a). In the conditions of cultivation used, the cells as a rule grew in one layer, although in some parts of the preparation a double layer could be seen. The double-layer appearance was particularly conspicuous in the later periods of cultivation (see Figure, b).

The nuclei of the cells were round or oval in shape; their cytoplasm contained tiny granules or fibrils, lying in the direction of the long axis of the cell (see Figure, c).

The cells beginning to divide became rounded and were joined to the layer by long, thin processes. Exceptionally, division of elongated cells was observed. Irregular mitoses were rarely seen.

Irregular (lobular) forms of some late telophases were observed.

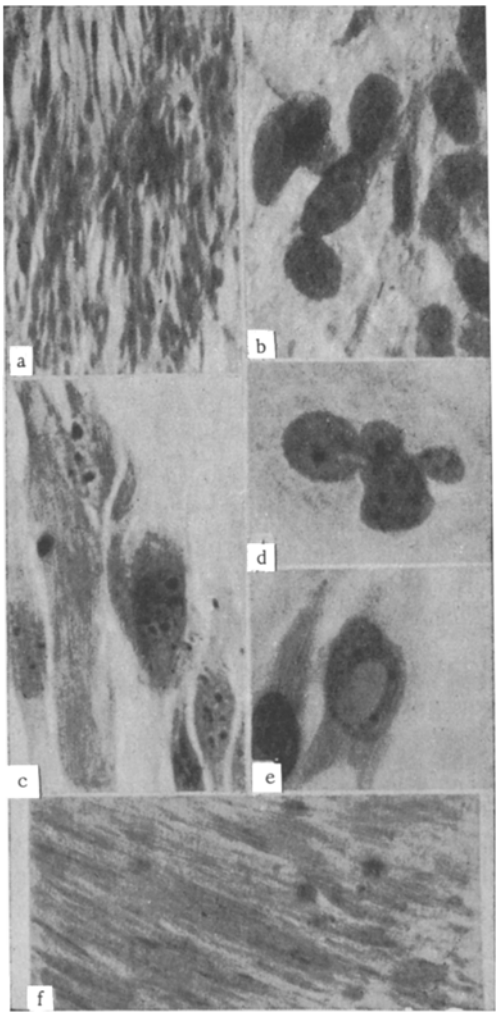
Mitoses were present in all passages, including the 40th. The mitotic activity reached a maximum in some passages on the second, and in others on the third day.

The values of the mitotic activity of L-42 cells of different passages, expressed in promille, are given below:

No. of passage	1	5	14	16	26	40
Mitotic activity	40	62	38	29	19	30

The mitotic activity of the diploid cells as a whole was lower than that of transplantable lines (according to the results obtained, 60-90%), but slightly higher than the mitotic activity of primary cultures (25-35%). A tendency for the mitotic acitvity to diminish in the later passages was observed.

Moscow Research Institute of Virus Preparations (Presented by Active Member of the Academy of Medical Sciences of the USSR A. P. Avtsyn). Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 63, No. 3, pp. 100-103, March, 1967. Original article submitted August 2, 1965.



Human diploid cells in cluture (strain L-42). a) 1st passage, second day of culitvation. The cells are arranged in regular streams; b) 1st passage, *day of cultivation. Formation of a double layer; c) 16th passage, second day of cultivation, the structure of the cells can be seen; d) 40th passage, second day of cultivation, fragmentation of nucleus; e) 26th passage, third day of cultivation, vacuole in nucleus; f) 5th passage, second day of cultivation. a-e) hematoxylin-eosine, f) bromphenol blue; a, f) objective 8x, ocular 5x; b-e) objective 40x, ocular 5x.

Number of Atypical Cells during Cultivation of Strain L-42 (in %)

No. of passages	Cells				Total
	With vacuoles in nucleus	With fragmented nuclei	Degenerative, with pycnotic nuclei	Giant and binuclear	
1	0,2	0,1	1,1	0,4	1,8
5	0,2	—	0,7	—	0,9
14	0,1	0,1	0,5	0,1	0,8
16	1,5	0,1	0,1	0,4	2,1
20	1,0	—	2,1	0,4	3,5
26	0,2	0,1	2,2	0,7	3,2
40	0,6	0,1	0,5	1,0	2,2

Despite the homogeneity of the diploid cells, some deviations from the typical morphology were observed. A few tiny, round or oval, degenerating cells with dark, pycnotic nuclei and a small quantity of cytoplasm were found. These could be ingested by healthy cells. In the later periods of cultivation, the number of degenerative cells increased. A few cells were found with irregular fragmented, or vacuolated nuclei (see Figure, d and e), and also giant and binuclear cells. The number of these atypical cells increased after the 16th passage, but they remained few throughout the period of observation (see Table).

Starting with the 20th passage, the cell processes became appreciably thinner.

At the 40th passage, besides an increase in the number of atypical structures, a disturbance of the regular arrangement of the cells in the layer was observed, and wide cells appeared with their nuclei situated eccentrically and lying transversely. The cytoplasm lost its fine structure, and a dense and intensively stained material could be seen near the nuclei. A study of the cultural properties of the strain up to the 26th passage showed considerable activity of multiplication, with an index of proliferation of S. From the 27th passage, the intensity of growth declined, and because of this the interval between passages was increased to 6 days (3-4 days for the earlier passages). The signs of aging gradually became more marked, and at the 46th passage the strain ceased to exist.

Histochemical investigations. The morphological homogeneity of the diploid cells described above suggests that their metabolism was similar.

Glycogen Throughout the period of observation, as in the case of other fibroblast-like cells [2, 5], an extremely low glycogen content was found, uniformly distributed as tiny granules throughout the cytoplasm. No lioglycogen was found. A marked increase in the intensity of the reaction was observed in the degenerating cells, which began to stain diffusely.

* The Russian orginal omitted the day — Publisher's note.

RNA. Brachet's reaction and luminescence microscopy revealed tiny delicate granules, often arranged longitudinally, with more intensive staining at the poles of the nucleus and less intensive in the distal parts of the cell processes, with a distinct reaction in the nucleoli.

In the first passage, some tightly packed groups of cells stained relaively intensively. From the 5th passage and later, an increase in the uniformity of the reaction and, at the same time, a decrease in its intensity, were observed. At the 40th passage, a weak reaction was found in the great majority of cells.

Protein. A finely granular structure was found in the nucleus, occasionally accompanied by larger granules; the most intensive reaction was given by the nucleoli. The cytoplasm reacted to show tiny granules and thin fibrils. The reaction in the first passage was relatively weak, and slightly stronger in the 5th, especially in the groups of tightly packed cells (see Figure, f). In the 14th-26th passages the reaction in most of the cells became weaker. It increased again in intensity in the 40th passage, expecially in the degenerating cells.

The results of the selective study of the lipids showed that diploid cells have a low content of lipids, visible as tiny droplets scattered throughout the cytoplasm.

The histochemical findings show that the intensity of the fundamental metabolic processes in the diploid cells is relatively low and uniform. However, in the more tightly packed "streams" of cells the reaction for all the substances investigated is somewhat higher. The intensification of some reactions is associated with the adaptation, florescence, and aging of the strain. Changes in the histochemical reactions for glycogen [3] and protein [4] have been described previously in aging primary and transplanted cultures.

In their metabolism and intensity of propagation the diploid cells occupy an intermediate position between primary trypsinized cultures and transplantable lines. The moderate intensity of metabolism corresponds to the low, but very contstant, level of vital activity characteristic of diploid cells.

The atypical nuclei (fragmentation, lobulation, vacuolation) observed in early passages evidently reflect some form of cell patholgy connected with cultivation in vitro. It is interesting that these disturbances of the structure of the nucleus were found in a culture of diploid cells where the conditions of existence were most favorable, the genomes possess high homogeneity and stability, and where there is good reason to suppose that latent virus infection was excluded.

LITERATURE CITED

1. S. Ya. Zalkind and L. G. Stepanova, Byull. éksp. Biol., No. 6, 110 (1959).
2. S. Ya. Zalkind, R. I. Rapoport, and V. M. Dorofeev, Tsitologiya, No. 1, 81 (1964).
3. L. P. Izakova, Tsitologiya, No. 4, 427 (1962).
4. L. P. Izakova, Tsitologiya, No. 3, 337 (1964).
5. V. Ya. Karmysheva, A. N. Mustafina, et al. In the book: Morphology of the Cytopathogenic Action of Viruses [in Russian], p. 69, Moscow (1963).
6. R. I. Rapoport, K. I. Kokovikhina, N. B. Varshaver, et al. Vopr. Virusol., No. 2, 187 (1965).
7. V. I. Khozinskii, V. B. Seibil', L. B. Tsypkin, et al. Acta virol., 8, 454 (1964).
8. L. Hayflick and P. S. Moorhead, Exp. Cell. Res., 25, 585 (1961).
9. T. Puck, S. Cieciura, and A. Robinson, J. Exp. Med., 108, 945 (1958).