COMPARATIVE MORPHOMETRIC STUDY OF THE ULTRASTRUCTURE OF FIBROBLASTS AND CELLS OF EXPERIMENTAL RAT FIBROSARCOMAS

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Details are given of a morphometric and electron-microscopic analysis of the mitochondrial, ergastoplasmic, and polysomal systems of the cells of experimental rat fibrosarcomas and normal rat fibroblasts at various levels of differentiation. Normal fibroblasts at different levels of differentiation are shown to differ in the ratio between extracellular and intracellular protein synthesis. No statistical differences were found in the level of organization of the mitochondrial system or of total protein synthesis. The ultrastructural organization of fibrosarcoma cells is indistinguishable in all morphometric parameters from that of embryonic cells; i.e., in the structure of the apparatus for protein synthesis fibrosarcoma cells are undifferentiated.

KEY WORDS: fibrosarcoma; morphometry of cells; ultrastructure of cells; fibroblasts.

The problems relating to the similarity and difference between the ultrastructure of neoplastic and normal cells still remain far from completely solved. A widespread shortcoming during the study of these problems is that the cells compared are not histogenetically equivalent [5]. Moreover, normal cells in different states of function and at different levels of differentiation may differ sharply from each other in their ultrastructure [4, 7, 8], and this is often not taken into account. Finally, although many workers have described quantitative changes in the ultrastructure of malignant cells by comparison with normal [9, 12], they usually have not subjected these changes to quantitative analysis and they have assessed the results purely on the basis of visual tests.

It was accordingly decided to make a comparative morphometric study of the ultrastructure [1] of the cells of experimental rat fibrosarcomas and of rat fibroblasts at various levels of differentiation, as histogenetically equivalent to normal cells and their neoplastic analogs [2].

EXPERIMENTAL METHOD

Three groups of cells (30 cells from 3 animals in each group) were chosen as normal fibroblasts: 1) fibroblasts of the skin of rat embryos at the end of pregnancy as cells already fully established on the road of fibroblastic differentiation, but whose collagen-forming function was still in an undeveloped state; 2) fibroblasts from the intestine of an adult rat as cells most of which are in an active state (analogous skin cells are 70-80% inactive and degenerated) [4, 13]; 3) fibroblasts of a 7-day aseptic granuloma obtained by implanting a sterile glass disc beneath the skin of an adult animal. The ultrastructure of 30 cells from three experimental fibrosarcomas induced in rats by subcutaneous injection of DMBA (1,2-dimethyl-9,10-aminoazobenzene) in a dose of 1 mg/100 g body weight, also was investigated.

The methods used to take the material, for fixation, dehydration, and embedding in epoxy resins, and for staining the sections for electron microscopy were those in general use. Ultrathin sections were cut with the LKB-111 ultratome and photographed on the JEM-7A electron microscope with a standard magni-

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TABLE 1. Morphometric Parameters and Coefficients of the Level of Morphological Organization of Organoids of Normal and Tumor Cells

Parameters and coefficients	Fibroblasts of adult rats	Fibroblasts of rat granuloma	Fibroblasts of rat embryo	Cell of rat fibrosarcoma
V_V^M	0,101±0,027	0,057±0,016	0,063±0,020	0,063±0,020
S_V^M	0,86±0,12	0,65±0,19	0,58±,017	0,66±0,17
S_V^K	1,80±0,26	1,30±0,56	1,24±0,45	1,35±0,32
L_A^K	1,41±0,20	1,02±0,44	0,97±0,36	1,05±0,25
N_A^K	3,00±,062	1,79±0,61	1,97±0,52	2,55±0,73
AM	0,51	0,61	0,41	0,49
V_V^E	0 28±0,06	0,16±0,03	0,13±,003	0,11±0,03
S_V^E	6,10±1,10	3,87±0,92	3,15±0,79	2,80±0,65
L_A^E	4,79±0,93	3,04±0,72	2,47±0,62	2,20±0,51
N_A^E	2,53±0,85	1,25±0,31	1,23±0,36	1,24±0,28
N_L^{RE}	27,4±2,1	29,0±1,9	30,1±2,1	24,0±1,5
K_M	3,8±1,0	4,3±1,5	2,5±1,0	3,0±0,9
K_E	110±39	68±50	34,5±24,8	15,2±7,9
K_P	27,0±7,3	45,6±11,4	100±17	86±12
K_E/K_P	5,3±3,2	1,68±1,12	0,42±0,35	0,24±0,14
K_E+K_P	138±40	114±50	135±29	103±11

Legend. Confidents limits given for P = 0.05.

fication of 9000×. The morphometric parameters of the organoids were recorded [3, 11, 14] on 8 × 24 cm photographic prints with a final magnification of 27,000×. Since it is difficult to judge differences in the organoids as a whole in normal and tumor cells on the basis of single parameters, coefficients reflecting the level of morphological organization of each organoid were calculated from its morphometric parameters [10]. Since these coefficients were derived from data in the literature for morphofunctional correlations between the corresponding organoids, each coefficient reflects to a certain degree the level of the functional state of the particular organoid. The principal coefficients obtained were the following:

the coefficient of the level of morphological organization of the mitochondria:

$$K_M = S_V^K \cdot AM \cdot (S_V^M : V_V^M) \cdot (L_A^K : N_A^K),$$

where S_V^K is the surface area of the cristae of the mitochondria in 1 μ^3 of cytoplasm, AM the percentage of conventionally active mitochondria of average size, S_V^M the surface area of the mitochondria in 1 μ^3 of cytoplasm, V_V^M the fraction by volume of the mitochondria in 1 μ^3 of cytoplasm, L_A^K the track length of the cristae per 1 μ^2 cross section of cytoplasm, and N_A^K the number of mitochondrial cristae cut across in 1 μ^2 cross section of cytoplasm;

the coefficient of the level of morphological organization of the granular ergastoplasm:

$$K_E = S_V^E \cdot V_V^E \cdot N_L^{RE} (L_A^E : N_A^E),$$

where S_V^E is the surface area of the ergastoplasmic membranes in 1 μ^3 of cytoplasm, V_V^E the fraction by volume of the granular ergastoplasm in 1 μ^3 of cytoplasm, N_L^{RE} the number of ribosomes in 1 μ length of track of the ergastoplasmic membranes, L_A^E the track length of the ergastoplasmic membranes per 1 μ^2 cross section of cytoplasm, and N_A^E the number of closed contours of cisterns of the ergastoplasm per 1 μ^2 cross section of cytoplasm;

the coefficient of the level of morphological organization of free polysomes:

$$K_P = N_A^{RP},$$

i.e., the number of ribosomes combined into polysomes per 1 μ^2 cross section of cytoplasm; and

the derived coefficients:

$$K_E/K_P \text{ and } K_E + K_P,$$

where the former reflects the ratio between the external and internal synthesis of the cell, and the latter the combined synthetic activity of the cell. The values of the morphometric parameters and their coefficients are given in Table 1.

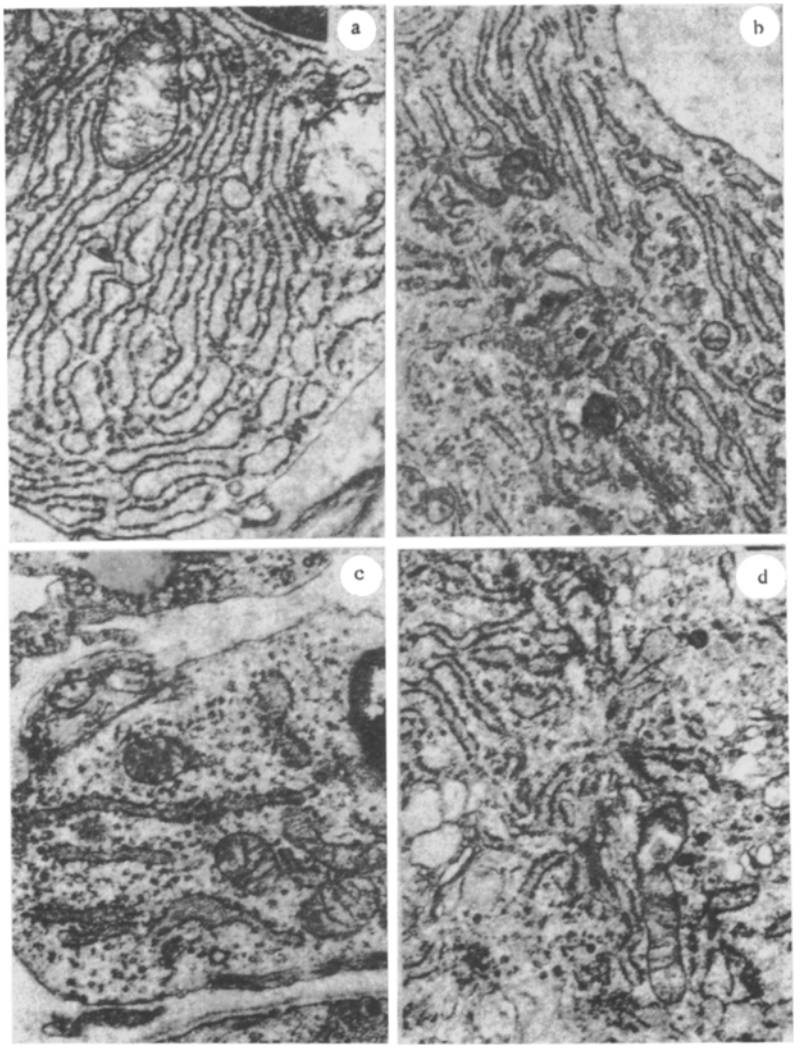

Fig. 1. Electron-microscopic picture of cells most typical of the groups studied (30,000×): a) ultrastructure of adult rat fibroblast; b) ultrastructure of fibroblast of experimental aseptic granuloma of rat; c) ultrastructure of fibroblast of rat embryo; d) ultrastructure of cell of experimental rat fibrosarcoma.

EXPERIMENTAL RESULTS AND DISCUSSION

The electron-microscopic picture of the most typical cells of the groups studied is illustrated in Fig. 1. The detailed statistical analysis of the morphometric data yielded the data given in Table 1. The first point to be noted is that differences between the fibroblasts of the adult rats, of the aseptic granuloma, and of the embryos were discovered in the biosynthetic apparatus: specifically in the ratio between the intracellular and extracellular synthesis, reflected in the coefficient K_E/K_P. The intensive intracellular synthesis in the embryonic cells was accounted for by the high level of development of the polysomal system, the "factory" of protein used chiefly for the needs of the cell itself [6, 9]; the collagen-forming function of these cells, however, is low compared with that of adult animals [4]. The coefficients reflecting the biosynthetic activity of fibroblasts of the experimental granuloma occupied an intermediate position between the corresponding coefficients for the fibroblasts of the embryos and the adult animals, also in agreement with data in the literature on the functional activity of cells of this type. Meanwhile the differences in K_E of the cells of these groups were the result of differences in the surface area of the membranes of the ergastoplasm and in the volume of its cisterns, but not in the density of granulation of the membranes [4]. It is an interesting fact that the level of organization of the mitochondrial apparatus in all the groups of cells was the same whether based on K_M or on its component parameters, in contradiction with the results described by other workers who observed analogous groups of cells visually and found differences in the

ultramorphology of the mitochondria. The total synthetic activity of the cells investigated also was identical, as reflected in the virtual equality of the coefficients $K_E + K_P$. Analysis of the data obtained by treatment of the fibrosarcomas showed that in terms of all parameters, both basic and derived, the tumor cell was indistinguishable from the embryonic; i.e., the synthetic apparatus is structurally undifferentiated.

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