DEPENDENCE OF INTENSITY OF PROTEIN SYNTHESIS IN FIBROBLASTS ON WIDTH OF CHANNELS OF THE ROUGH ENDOPLASMIC RETICULUM

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The view has often been expressed in the literature that fibroblasts with dilated channels of the rough endoplasmic reticulum are cells intensively synthesizing protein [1, 2, 8-10, 12]. What are the facts on which this opinion is based? They are first, the well-known biochemical, autoradiographic, and electron-autoradiographic data showing that synthesis of a protein secretion takes place in the rough endoplasmic reticulum [11, 13-15]. Second, the discovery of fibroblasts with dilated channels (lacunae) of the rough endoplasmic reticulum filled with electron-dense contents during the electron-microscopic study of wounds, keloid scars, and other states connected with intensive collagen formation. It must be emphasized that a simple compiling of these facts does not prove that intensive production of protein takes place in the wide channels of the rough endoplasmic reticulum, and this interpretation of electron-microscopic observations began to appear in the literature most probably simply under the influence of the common-sense notion that the more capacious a working system, the greater it productive capacity. In electron-autoradiographic studies of wound healing, by the use of a double labeling method described previously [4] the present writers were able to measure the rate of protein synthesis in fibroblasts differing in the structure of their rough endoplasmic reticulum, and thus to put the problem under discussion to experimental proof.

EXPERIMENTAL METHOD

On the 3rd day after the formation of a wound on the leg of four mice, with a tissue defect measuring 3 × 3 × 3 mm, a mixture of precursors was injected subcutaneously, uridine-5-^{3}H in a dose of 4 μCi/g (specific activity 22.5 Ci/mmole) and proline-3,4,5-^{5}H in a dose of 20 μCi/g (specific activity 115 mCi/mmole). The wound tissue was fixed 1 h after injection of the precursor with 2.5% glutaraldehyde solution in phosphate buffer, pH 7.4. This was followed by washing with phosphate buffer for 24 h, postfixation with 1% osmium tetoxide solution, and embedding in Epon. Depending on the results of investigation of light-microscopic autoradiographs (semithin sections) cut from each block, a region containing granulation tissue cells was selected, and a pyramid was cut out in this area for cutting ultrathin sections. Electron-microscopic autoradiographs were then prepared by the method described previously [7]. All fibroblasts completely included in an electron-autoradiograph and with no important technical defects were photographed in the JEM-100B electron microscope. The number of grains of silver above the nucleus and cytoplasm of the cell was counted in the negatives and the area of the cross section of the nucleus and cytoplasm (the product of length and width in millimeters) was measured in conventional units. The density of labeling was determined by dividing the number of grains of silver above a particular part of the cell by its area of cross section. From 350 cells treated in this manner fibroblasts were chosen in which the channels of the rough endoplasmic reticulum could be confidently classed as narrow (group 1) or as wide (group 2). Altogether 27 fibroblasts of group 1 and 28 of group 2 were found.

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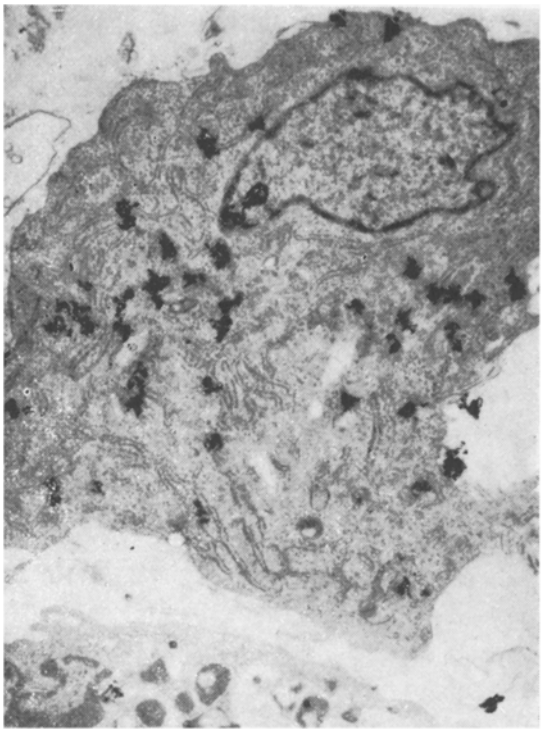

Fig. 1

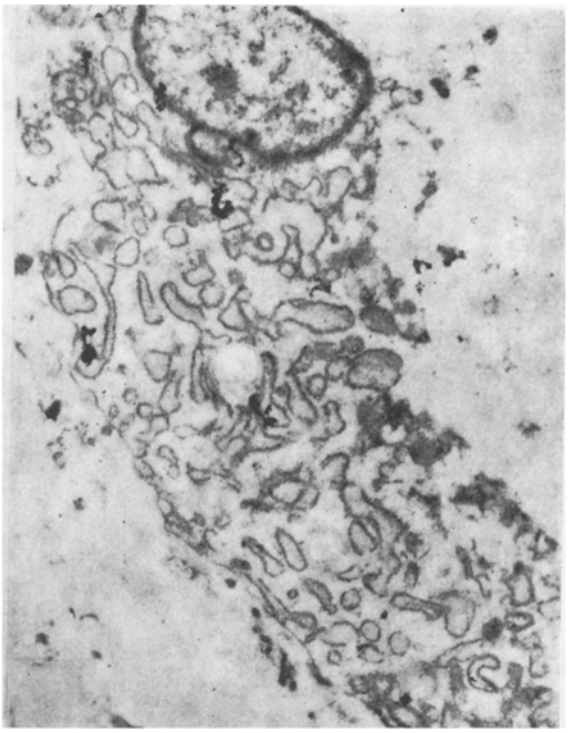

Fig. 2

Fig. 1. Fibroblast with narrow channels of granular endoplasmic reticulum. Considerable accumulation of grains of silver above cytoplasm indicates intensive protein synthesis. 18,000 ×,

Fig. 2. Fibroblast with wide channels of rough endoplasmic reticulum. Concentration of grain of silver much lower than in cells shown in Fig. 1. 20,000 ×.

EXPERIMENTAL RESULTS

The distribution of label in the fibroblasts was not strictly standard, and in each group cells with higher and lower densities of distribution of grains of silver above the cell as a whole and with different ratios between the density of label above the nucleus and cytoplasm were found. Nevertheless, even before quantitative analysis, simply from the appearance of the two distinct groups of fibroblasts, the view that cells with wide channels of the rough endoplasmic reticulum have high ability for protein synthesis could be disputed. Fibroblasts of group 1 in most cases were distinguished by a higher concentration of label in the cytoplasm (Figs. 1 and 2). Quantitative analysis confirmed these observations. The mean density of grains of silver above the cytoplasm of the group 1 fibroblasts was 0.012 ± 0.001, and above the cytoplasm of the group 2 fibroblasts it was 0.009 ± 0.001. The density of grains of silver above the cytoplasm reflects chiefly the rate of incorporation of proline [4]. Consequently, the figures given above point to a higher rate of protein synthesis in fibroblasts with narrow channels of their rough endoplasmic reticulum.

The use of the double labeling method in the experiment just described not only allowed the absolute rate of protein synthesis in the cytoplasm of the two groups of fibroblasts to be compared, but also allowed the ratio between the intensity of protein synthesis in each group and the intensity of another key intracellular process, namely RNA synthesis, to be determined. The possibility of determining the relative rate of protein synthesis first enables mistakes in the experimental results connected with irregularity of distribution of the precursors between the cells to be avoided and, second, enables a decision to be made on whether this was just a difference in the rate of protein synthesis in the groups compared or a difference in the level of metabolic processes in general.

When the experimental technique described above is used the concentration of grains of silver above the nucleus reflects chiefly RNA synthesis [4]. In group 1 this value was 0.007 ± 0.001, and in group 2 it was 0.010 ± 0.001. In the group of fibroblasts with wide tubules of

the rough endoplasmic reticulum the density of grains of silver above the cytoplasma was lower, whereas above the nucleus it was higher, than in the first group. Consequently, these fibroblasts differed from cells combined into group 1, not by a decrease in the intensity of their metabolic processes in general, but by a decrease only in the rate of protein synthesis, i.e., in one of the specific functions of the rough endoplasmic reticulum. The reason for the disperity between the levels of RNA synthesis and protein synthesis in fibroblasts was examined in a previous communication [3].

The significance of the difference between the two groups of fibroblasts was determined from the relative rate of protein synthesis because this reflects more accurately the true nature of the problem under discussion — the intensity of function of the rough endoplasmic reticulum. The value of the relative rate of synthesis was obtained by dividing the density of grains of silver above the cytoplasm by the density of grains of silver above the nucleus. In group 1 its value was 1.83 ± 0.23, and in group 2 it was 0.98 ± 0.11. Comparison by Wilcoxon's criterion showed that the difference between these values is statistically significant ($P < 0.05$).

It must be emphasized that this difference found between fibroblasts with narrow and wide channels of their rough endoplasmic reticulum in the rate of protein synthesis does not by any means imply the existence of a similar difference in protein content. Most probably the electron-dense contents of the wide channels of the rough endoplasmic reticulum consist of previously synthesized protein, and that such cells may actually have a higher protein content than fibroblasts with narrow tubules of their rough endoplasmic reticulum.

The possibility cannot be ruled out that the work of a "unicellular gland," such as the fibroblast, like that of typical gland cells such as pancreatic cells [8], takes place in phases: 1) synthesis, 2) accumulation of secretion, 3) discharge of the secretion. In this case cells of group 2 are at the stage of development of fibroblasts at which, against the background of a relative fall in the rates of synthesis and secretion of protein, marked accumulation of protein takes place in the dilated channels of the rough endoplasmic reticulum. The wave-like changes discovered in the rate of protein synthesis in fibroblasts [5] suggest that other phases of secretion dependent on synthesis also are characterized by periodic changes in intensity and do not proceed at a constant level.

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