702374

Reprinted from Experimental Cell Research, Vol. II, No. 1, 1951.

THE CHEMICAL NATURE OF THE SO-CALLED KERATOHYALINE GRANULES OF THE STRATUM GRANULOSUM OF THE SKIN

CECILIE LEUCHTENBERGER and HERBERT Z. LUND

Institute of Pathology, Western Reserve University, Cleveland, Ohio

Received August 2, 1950

THE chemical composition of the granules occurring in the cytoplasm of the cells of the stratum granulosum of the epidermis is unknown. It has been generally assumed that these granules are a result of the degeneration of the cells, the stratum granulosum being an "intermediate zone between life and death" (5). Since the granules stain in a similar fashion as does the chromatin; namely, with hematoxylin and other basic dyes, they were generally believed to be chromatin material derived from the breakdown of the nucleus.

In the following study evidence is presented which contradicts the concept that the keratohyaline granules are chromatin and which suggests an anabolic activity of the cells of the stratum granulosum.

Experimental Results and Observations: In order to test whether the granules contain chromatin material, they were examined for desoxyribosenucleic acid by means of the Feulgen reaction. The specificity of the Feulgen reaction for desoxyribosenucleic acid, if carried out under controlled conditions, has been established by numerous investigators (12, 3, 8, 10). In the epidermis of human and dog skin (fixed in Carnoy's acetic alcohol and 10 per cent formalin) the granules of the cells of the stratum granulosum were completely Feulgen-negative, while they stained strongly with hematoxylin. This finding agrees with observations made by T. Cornbleet (personal communication).

From these observations it can be concluded that the granules of the stratum granulosum do not contain desoxyribosenucleic acid. Since it seemed possible that in spite of the Feulgen-negative behavior of the granules there is a loss of desoxyribosenucleic acid from the nucleus of these cells similar to the loss of desoxyribosenucleic acid in pyknotic nuclei (6, 7), the amount of desoxyribosenucleic acid in nuclei of the stratum granulosum cells was compared with the amount of desoxyribosenucleic acid in nuclei of the malpighian cells. The desoxyribosenucleic acid was estimated by photometric measurements with an apparatus described by Pollister and Moses (9) and by the method of Schrader and Leuchtenberger (11). For the measurements of the desoxyribosenucleic acid in the nuclei, cells were selected in the stratum granulosum which were directly adjacent to the malpighian

REPOSITORY Argonne-CHR
COLLECTION A. Brues
BOX No. 12
FOLDER #20, Leuchten Berger Reprints

0018246

PARTIAL DOCUMENT

layer and which showed in their cytoplasm, on hematoxylin stain, distinct granules. Cells lying directly beneath the keratin could not be measured since, due to their extreme spindle form, a computation of the volume was not possible.

In the following table the results are presented.

TABLE I

Amounts of Desoxyribosenucleic Acid (Feulgen) in Arbitrary Units in Nuclei of Epidermal Cells of the Dog

Type of Cells	Number Measured	Mean Nuclear Volume in Cubic Microns	Mean Amount of Desoxyribosenucleic Acid per Nucleus
Malpighian Layer	20	230 ± 26	2.3 ± 0.19
Stratum Granulosum	20	196 ± 21	2.0 ± 0.20

It is apparent that the desoxyribosenucleic acid content of the nuclei of the stratum granulosum is approximately the same as that of the malpighian layer and therefore no loss of desoxyribosenucleic acid seems to have occurred during the process of formation of the granules in the cytoplasm of the cells of the stratum granulosum.

For a further characterization, the granules were tested for ribosenucleic acid. The methods employed were the one of Brachet (1), using a combination of toluidine blue and ribonuclease, and the one of Flax and Pollister (4) using azure A. In both sets of experiments the stainability of the granules with toluidine blue and with azure A was completely abolished if the sections were treated with ribonuclease prior to the staining. It can therefore be concluded that the granules contain ribosenucleic acid. It should be pointed out, however, that the intensity of the stain in the granules with azure A and with toluidine blue is considerably less than with hematoxylin. Whether this observation is an indication that the ribosenucleic acid constitutes only a small part of the granular material and the stronger staining with hematoxylin is due to its unspecific staining of other acidic components of the granules, or whether the weaker staining with azure A and toluidine blue is due to the fact that these dyes stain only a part of the ribosenucleic acid (4, 11) cannot be decided at the present time.

Discussion of the Result. The Feulgen-negative behavior of the granules in the cytoplasm of the cells of the stratum granulosum is against the view that the granules are chromatin and therefore a breakdown product of the nuclear material. The unchanged amount of the desoxyribosenucleic acid in the nuclei of these cells and the presence of ribosenucleic acid in the cytoplasmic granules are both inconsistent with the general concept that these cells are degenerating cells.

Accepting the above evidence, it may still be maintained that we deal here with a very early phase of degeneration. However, under pathologic conditions where all stages of degeneration have been observed in the epidermis and other tissues, there does not seem to be an equivalent picture. If chromatin is lost from the nucleus and appears in the cytoplasm, it is Feulgen-positive, and moreover the desoxyribose-

0018247

nucleic acid in such nuclei is considerably less than in nuclei of nondegenerating cells (6, 7). The stratum granulosum cells, furthermore, lack transitional stages so commonly observed in degeneration and necrosis. Pyknosis, which occurs occasionally in the uppermost layer of the stratum granulosum, is an abrupt change and appears to be unrelated to the presence or absence of granules.

The presence of ribosenucleic acid in the granules of the cells of the stratum granulosum rather suggests that such cells might be engaged in the synthesis of substances related to the process of keratinization for it has been shown in a wide variety of cells that such a chemical condition in the cytoplasm characterizes cells where protein synthesis is taking place (2). This concept seems to be supported by the fact that Caspersson *et al* (2) by the method of ultraviolet absorption found an accumulation of ribosenucleotides during the process of keratinization (2).

Cytochemical studies of intracellular nucleoproteins during disturbances of the normal process of keratinization (parakeratosis and hyperkeratosis) are now in progress.

It is a pleasure to acknowledge the technical help of Miss Ethel Lieb.

REFERENCES

1. Brachet, J., Embryologie Chimique, Masson et Cie, Paris, 1944.
2. Caspersson, T. O., Cell growth and cell function, W. Norton Co., New York, 1950.
3. DiStefano, H. S., *Chromosoma*, **4**, 282 (1948).
4. Flax, M. H., and Pollister, A. W., *Anat. Rec.*, **105**, 56 (1949).
5. Hueck, W., Morphologische Pathologie, Georg Thieme, Leipzig, 1937.
6. Leuchtenberger, C., *Cancer Research*, **9**, 600 (1949).
7. —— *Chromosoma*, **3**, 449 (1950).
8. Pollister, A. W., and Leuchtenberger, C., *Proc. Natl. Acad. Sci.*, **35**, 111 (1949).
9. Pollister, A. W., and Moses, M., *J. Gen. Physiol.*, **32**, 567 (1949).
10. Ris, H., and Mirsky, A. E., *J. Gen. Physiol.*, **33**, 125 (1949).
11. Schrader, F., and Leuchtenberger, C., *Exp. Cell Res.*, **1**, 421 (1950).
12. Stowell, R. E., *Stain Technol.*, **20**, 45 (1945).

0018248