MICROSPECTROPHOTOMETRIC MEASUREMENT OF DNA ON ISOLATED EPIDERMAL CELLS, COMBINED WITH ³H-TdR AUTORADIOGRAPHY

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- (1) Feulgen cytophotometry was performed selectively on sufficient number of single whole nuclei of epidermal cells which were isolated from a sheet of stripped epidermis.
- (2) *The two-wavelength-scanning-method* using 550nm and 450nm made it possible to detect and eliminate non-specific light loss.
- (3) *The two-wavelength-scanning-method* using the Olympus-MMSP-TU-S spectrophotometer enables us to identify the DNA synthetic cells with their intermediate values of absorbance.
- (4) In normal epidermis of a rabbit's ear, 90.2% of all basal cells exist in G₁-phase (including G₀-phase), and 2.9% of them are in G₂-phase. The remaining 6.9% are DNA synthetic cells.
- (5) The absence of "G₂-population of Gelfant" was confirmed in normal epidermis of a rabbit's ear.
- (6) The rate of DNA synthesis is once decreased in the middle of S-phase and it is accelerated again toward the end. This phenomenon may be related to the late-replicating DNA.
- (7) Autoradiographic study with ³H-TdR on a sheet of stripped epidermis enabled us to obtain a bird's eye -view of distribution of proliferating basal cells.

In the Feulgen cytophotometry, even with visible light (550 nm)¹⁾, a part of light traversing medium, cytoplasmic and nuclear substance, is lost out of its lense system by scattering due to reflection and refraction.^{2,3,4,5)} The light energy which is lost by scattering is referred to as "non-specific" light loss in contrast with the specific absorption by Feulgen dye. Non-specific light loss is usually reduced to certain extent by making a smear directly from raw tissue material.^{2,3,5,6)} Optical method to detect non-specific light loss is to carry out measurement with a beam of light at a wavelength that is not absorbed practically by Feulgen dye.^{4,5)} For this purpose the *two-wavelength-scanning-method* using Olympus-MMSP-TU-S spectrophotometer was developed by Fujita and Sawamura.⁸⁾ This method enabled an automatic elimination of non-specific light loss from the so called "total absorption" at 550 nm.^{9,10)}

In order to improve accuracy of measurements, it is necessary to take considerable care for refinements of material preparation besides the development of suitable photometric apparatus. Material to be measured by this method must contain sufficient number of single whole nuclei without contamination of other tissue cells.

This report deals with the improved method of isolating the epidermal cells and the Feulgen cytophotometric DNA determination by *two-wavelength-scanning-method*. The combination of ^3H -TdR autoradiography and cytophotometric DNA measurement of basal cells on a same slide assured that the DNA synthetic cells (cells in the S-phase) can be identified by the values of Feulgen absorbance.^{10,11)}

MATERIALS AND METHODS

Skin of inner side of ears of adult male rabbit (2 kg) was used.

(1) *Stripping the sheets of epidermis from the underlying dermis*

After removing hairs and horny substance by using depilatory cream, boiled water (90–100°C) was poured directly on the epidermal surface. On pouring boiled water, the whole ear begins to roll inward due to the marked contraction of dermal tissue. The difference of contraction between epidermis and dermal tissue produces immediate and complete dissociation of epidermis from the underlying dermis. A sheet of epidermis ($2 \times 2 \text{ cm}^2$) was then removed from the dermis with a sharp pointed pincette after the fringe was cut by a razor blade. Heating at 90–100°C for 5–10 seconds was sufficient to separate the epidermis in a good condition.

(2) *Feulgen stain*

Feulgen stain was carried out according to a slightly modified method of Leuchtenberger.¹²⁾ Stripped sheets of epidermis were hydrolyzed in 1N HCl for 5 minutes at $60 \pm 1^\circ\text{C}$, after fixing in absolute methanol for 30 minutes. They were immediately dipped in Schiff's solution and stained for 1 hour, with successive rinses in 0.5% NaHSO_3 solution for 6 minutes.

(3) *Isolation of each cell from a stripped sheet of epidermis*

Hair follicles were trimmed away from a sheet of epidermis which was stained reddish purple by Feulgen technique, under a stereomicroscope. Epidermal cells were isolated from the stained epidermal sheet by the following method.

Mechanical liberation after maceration with acetic acid

A stained epidermal sheet was stretched on a quartz slide glass with the basal layer upward. A sheet of epidermal cell layers was swept off mechanically by a razor blade after maceration by adding a few drops of 50% acetic acid. Epidermal cells were further isolated by gentle tapping between two quartz slide glasses. Although possibility was suspected that the cells in the upper layers might be hydrolyzed more slowly in comparison with those in the basal layer due to probable hindrance of infiltration of hydrochloric acid by the horny substance, no significant difference was found in the total absorbances of both types of cells, without apparent proportionality error¹³⁾ in the cells of each layer.

(4) *Microspectrophotometric measurement of nuclear DNA on isolated epidermal cells*

Feulgen cytophotometry was carried out on isolated epidermal cells with Olympus-MMSP-TU-S spectrophotometer by *two-wavelength-scanning-method*.^{7,8)}

(5) *^3H -TdR autoradiography on the stripped sheets of epidermis and isolated epidermal cells*

^3H -TdR was locally injected intra-dermally (50 μCi of ^3H -TdR in 5% glucose solution) one hour before stripping the epidermal sheet (flash labeling method).¹⁴⁾ After Feulgen staining, epidermal cells were isolated from the half of a stripped sheet and cytophotometric measurements were carried out on them and the other

half was pressed to extend between two slide glasses adding a few drops of 50% acetic acid. The two kinds of materials were dipped in Sakura-NR-M₂ emulsion and developed in FD-111 after exposure of 4 weeks.

(6) *An autoradiographic study of reactive proliferation of epidermal cells after cut wounding*¹⁵⁾

A linear cut wound of 1 cm in length running parallel to the long axis of the ear was made by a razor blade in the inner surface of a rabbit's ear as deep as to reach the perichondrium. ³H-TdR was injected subepidermally to the wounded area 3 times at intervals of 5 hours, starting at 19.6 hours after wounding (cumulative labeling method).¹⁴⁾ Total duration of the labeling was 11 hours. Paraffin sections were cut serially at a thickness of 3 μ and autoradiographs were made.

RESULTS AND OBSERVATIONS

(1) *Microscopic examinations of stripped sheets of epidermis*

When stripped sheets of epidermis were examined in cross sections (3–5 μ) perpendicular to the surfaces, thin membranous substance which is slightly stained by PAS is found attaching to the bottom of the basal layer, but the dermal connective tissue is almost completely removed (photo. 1). Two to three layers of epidermal cells are identified in ordinary sections of normal epidermis of rabbit's ear. Hair follicles are lost just below the basal cell layer. Microscopic observation of a Feulgen-stained epidermal sheet from the basal side gave a bird's eye-view of a sheet of basal cells, which are arranged like pavement stones. Broken hair follicles are seen like craters on the moon. From the ridge of a crater, sometimes radial lines of elongated nuclei of basal cells are trailing to the foot. Going down the slope of a crater, the nuclei stand up gradually and they are almost perpendicular against the basement membrane toward the end of the skirt (photo. 2.a.). In some parts of basal cell layer, many small lacunar formations are noted like sprinkled stars in the dark sky. The inner rim of a lacuna is surrounded by some long curved nuclei of basal cells, and the larger pale nuclei of spinous cell layer are seen through it (photo. 2.b.). Gradually changing focus, the cells of each layer can be identified separately (photo. 3.a.b.). The cell nuclei in the upper layer were definitely

PHOTO 1. A section cut perpendicular to the surface of a sheet of stripped epidermis. Two to three layers of epidermal cells are identified as observed in ordinary sections of normal epidermis of a rabbit's ear. A membranous thin substance which is slightly stained by PAS staining is attached to the bottom of the basal layer, but the dermal connective tissue is almost completely removed. $\times 300$. PAS stain.

FIGURE 2. (a) A bird's eye-view of a sheet of stripped epidermis from the basal side. $\times 100$. Feulgen stain. Numerous small dark nuclei of basal cells are densely arranged like pavement stones. A broken hair follicle is seen in the left side (HF). (b) Small lacunar formations which are found in some parts of basal cell layer (indicated by arrows). The inner rim of a lacuna is lined by some long curved nuclei of basal cells. Some of the larger pale nuclei of upper layers (arrows) are seen through them. Autoradiograph after the flash labeling with ^3H -TdR. $\times 400$.

larger and paler than those in the lower layer. The nuclear area of the cells in the top-layer was 4 to 5 times as large as that of the basal cells, and the density of nuclear Feulgen dye is diluted inversely proportional to the nuclear area. The ratio of the number of cells in the basal and in the other layers is about 10 : 9.

By autoradiographic examinations with ^3H -TdR, heavily labeled basal cells were observed distributing randomly in normal epidermal sheets. The labeling indices in the fields except hair follicles are 1 to 9%, the mean being in 7%. Toward the foot of a hair follicle, labeled cells become more frequent and the mean labeling index on the slope is approximately 10% (photo. 4).

(2) *Observations on smears of isolated epidermal cells*

PHOTO 3. Higher magnification of photo 2. a., $\times 400$. (a) Basal cell layer. (b) By changing the focus in photo 3. a., the nuclei of 2nd layer are disclosed. They are stained paler by Feulgen staining and are about 2 times larger in size than the basal cell nuclei.

Numerous epidermal cell nuclei which are stained reddish purple with Feulgen dye are contained in a smear and the majority of them were single and intact without overlapping. Morphologically 3 types of nuclei can be distinguished. 1) Dark small nuclei are found most frequently and 2) paler nuclei which are 1.5 to 2 times larger and 3) the most pale nuclei which are approximately 4 to 5 times as large as the smallest ones. The ratio of the number of them is about 10:6:3 (photo. 5.). Hematoxylin and eosin stain was carried out on a smear to visualize the cytoplasmic detail of isolated cells. Almost all the nuclei are completely denuded but some of them are attached by remaining cytoplasm (photo.

PHOTO 4. An autoradiograph made on a sheet of stripped epidermis of a rabbit's ear, after the flash labeling with ^3H -TdR. H. E. stain, $\times 200$. DNA synthetic cells in the basal layer are heavily labeled. HF: hair follicle.

PHOTO 5. Isolated epidermal cells from a sheet of stripped epidermis of a rabbit's ear, after maceration with 50% acetic acid. Dark small nuclei are those of basal cells (B) and the others are the nuclei of the cells in the upper layers. Feulgen stain. $\times 300$ (upper), $\times 800$ (lower).

6.a.). In the cytoplasm of large pale nuclei, keratohyalin granules of various sizes which are stained blue with hematoxylin are sometimes noted (photo. 6.b.). It is evident that in this smear dark small nuclei are those of basal cells and the other types of larger nuclei belong to the cells in the upper layers. Especially the largest pale nuclei are thought to have been isolated from the cells which are observed as the flattened nuclei just below the horny layer in ordinary paraffin sections.

(3) *Feulgen cytophotometry on isolated epidermal cells*

a) Epidermal cells were isolated from a stripped sheet after the flash labeling with ^3H -TdR and Feulgen cytophotometry was carried out on 204 basal cell nuclei and 30 larger nuclei of upper layers. Three mitotic nuclei were also examined in the same slide (two in prophase and one in metaphase). As shown in fig. 1., and table 1, 90.2% of basal cells and the cells of upper layers had the DNA values of the same class and 2.9% of basal cells and mitotic cells were also revealed to have the same range of DNA values. Remaining 6.9% of basal cells had the intermediate DNA values and they were confirmed to be the cells in S-phase (DNA synthetic cells) by subsequent ^3H -TdR autoradiography which was made on the same slide after the cytophotometric measurements (fig. 1.). Autoradiographic studies on section materials of normal epidermis after the flash labeling with ^3H -TdR revealed

PHOTO 6. Isolated epidermal cells, H. E. stain, (a) Almost all the nuclei are completely denuded but some of them are attached by remaining cytoplasm. $\times 400$. (b) Keratohyalin granules of various sizes stained blue with hematoxylin are sometimes found in the cytoplasm of large cells. $\times 1,000$.

FIG. 1. Histograms of DNA values of epidermal cells: 204 basal cell nuclei, 30 nuclei of upper layers and 3 mitotic nuclei. 90% of all basal cells (blank area) were in G₁ and the cells of upper layers (obliquely lined area) had the DNA values of the same class of diploid. 2.9% of basal cells (dark area) and mitotic cells (indicated by arrows) had the double diploid values of DNA. The remaining 7% of basal cells showed intermediate DNA values and they were revealed to be in S-phase by subsequent examination of ³H-TdR autoradiography.

that the labeled cells distributed only in the basal layer (photo. 7.a.). The fact indicates that the cells in the upper layers are already differentiated and the DNA values of them are exactly diploid class. On the other hand, basal cells near wound were all heavily labeled within 11 hours by the cumulative injection of

PHOTO 7. (a) Autoradiograph of normal epidermis of a rabbit's ear after the flash labeling with ^3H -TdR. Labeled cells are rather scanty. H.E. stain, $\times 400$. (b) All the basal cells near a wound are heavily labeled within 11 hours by the cumulative injection of ^3H -TdR, having started at 19.6 hours after wounding. H. E. stain, $\times 400$.

FIG. 2. The relative number of DNA synthetic cells with various values of DNA content. Redrawn from the dotted area in fig. 1.. The curve is a probability function of a cell to have a certain value of DNA, A .

^3H -TdR having started 19.6 hours after wounding, thereby indicating that all the basal cells have the potency to proliferate, although many of them exist in some resting phase of the mitotic cycle in normal state¹⁴ (photo. 7.b.). Therefore, it can be concluded that 90.2% of basal cells exist in G_1 -phase (including G_0 -phase) and 2.9% of them are in G_2 -phase.

b) In the autoradiographs made on the same slide on which Feulgen sytophotometry was carried out, the densities of silver grains of DNA synthetic cells were counted and compared with the values of absorbance. The DNA synthetic cells which have the DNA values of nearly diploid and double diploid were heavily labeled but the number of labeled grains of those which have the intermediate DNA values around $1.5 \times 2n$ were evidently much less (table 1.a.). The relative number of cells at different stages of DNA synthesis were plotted against the values of DNA content in fig. 2.

DISCUSSIONS

(1) Various methods for separating the epidermis from the underlying dermis have been described. As classified by Alavaikko (1971)¹⁴, these methods have included the utilization of a) physical, b) chemical and c) enzymatic procedures. The purely mechanical methods (a) include cutting with a knife or a keratome.^{17,18,19} These manipulations, however, can not be completely free from the rudimentary hair follicles and dermal connective tissues remaining in the stripped epidermal sheets. The suction blister method for separating human epidermis was developed by Kiistala and Mustakallio.²⁴ The method enabled the complete separation of intact epidermis from the dermis without epidermal denaturation, but it takes one or two hours before a blister is formed by suction. Another purely mechanical method is the adhesive tape method described first by Wolf, and the keratinous layer of human skin was removed by successive strippings with adhesive tape by Pinkus (1951).²¹ But it is difficult to separate each epidermal cell layer selectively by this method. Harris²² in 1927 applied heat to separate the epidermis and Baumberger et al.²³ noted in 1942 that heating at 50°C for two minutes was sufficient to separate human epidermis in a good condition. (b) Even as early as 1852, separation of the epidermis from the dermis was achieved by maceration of the skin with diluted acetic acid (Kölliker).²⁴ That method has been successfully applied to mouse skin (Liang, 1948,²⁴ Goerttler and Friedmann, 1970),²⁴ yielding view of the three-dimensional complexity of the hair follicles and inter-follicular epidermis. Using hairless mice, Karatschai (1971)²⁴ succeeded in making a beautiful autoradiograph with ^3H -TdR on a stripped sheet of epidermis after maceration with diluted acetic acid. He noted that only using a hairless mouse relief autoradiography of the basal cells was possible. We reported in 1971 that the epidermis of hair-bearing skin of rabbit's ears were separated from the dermis satisfactorily and autoradiographs were made on their basal cells after flash labeling with ^3H -TdR, both in normal and regenerative conditions.¹⁵ Numerous chemical agents have been used for the same purposes. The enzymatic method (c) was first introduced in 1941 by Medavor,²⁸ who separated human epidermis by trypsin digestion. Since that time, several other enzymes have been employed. Giovanella and Heiderberger²⁹ managed to dissociate the epidermis of hair-bearing mice by cautious mechanical dispersion after successive incubation in hyaluronidase

and elastase. The most elegant enzymatic method at present available for separating the epidermis of hair-bearing animals is the chymotrypsin digestion method introduced by Scott (1958) and developed by Alavaikko.¹⁶⁾ Alavaikko succeeded in separating the epidermis of hair-bearing mice due to the chymotrypsin digestion method for the selective fluorocytophotometric DNA determination of the basal cells.

(2) Generally horny substance was attached to a side of a stripped sheet of epidermis, and it was thought to hinder the penetration of hydrochloric acid with the result of different speeds of hydrolysis in each cell layer. In our experiment, Feulgen stains were carried out both before and after isolating epidermal cells from the epidermal sheets, and Feulgen cytophotometry on both materials revealed no significant difference in the values of absorbance, without apparent

TABLE 1. Excerpt of cytophotometric data of 5 basal cells (one in G₁-, three in S-, and one in G₂-phase), one large cell and three mitotic cells (two in prophase and one in metaphase).

(a) Note the DNA value of a large matured cell is exactly in the diploid class, after eliminating the non-specific light loss. Compare the densities of labeled silver grains on the DNA synthetic cells against the values of absorbance, *A*.

(b) The DNA values of three mitotic cells were in double diploid class without significant deviations.

TABLE 1. (a)

Matured Cell		DNA SYNTHETIC CELLS (S)									
		G ₁		BEGINING		MIDDLE		END		G ₂	
450nm	550nm	450nm	550nm	450nm	550nm	450nm	550nm	450nm	550nm	450nm	550nm
0.1	0.1	0.1	0.1	0.0	0.0	0.1	0.1	0.0	0.0	0.0	0.0
1.1	2.5	0.3	0.9	0.5	0.8	0.2	0.8	0.9	3.6	0.0	0.5
2.1	2.8	0.5	3.3	0.5	1.0	0.7	5.0	1.1	6.2	0.3	3.6
5.1	6.5	0.7	3.4	0.5	1.7	1.1	11.1	1.3	7.5	0.4	3.6
6.1	8.1	0.9	4.2	0.5	2.8	0.9	10.1	1.4	8.6	0.5	3.9
6.2	8.5	0.9	4.9	0.6	3.9	0.5	5.8	1.3	8.2	0.5	4.3
6.2	8.5	0.9	4.6	0.7	4.7	0.1	2.7	1.0	6.3	0.7	4.4
6.2	8.9	0.7	4.5	0.9	5.0	0.1	0.1	0.8	4.7	0.6	4.9
5.2	7.9	0.7	3.0	0.9	4.8			0.5	1.9	0.6	4.9
5.1	7.7	0.4	1.3	0.7	4.1			0.2	0.2	0.4	5.1
3.0	5.1	0.0	0.0	0.5	3.1					0.7	6.3
2.1	4.4			0.5	2.2					0.7	5.6
1.2	2.5			0.3	0.6					0.5	5.4
0.1	0.1					0.3	0.3				
										0.1	0.2
										0.1	0.1
49.6	73.4	6.0	30.1	7.1	34.7	3.5	35.5	8.3	47.0	6.5	55.1
A: 23.8		A: 24.1		A: 27.6		A: 32.0		A: 38.7		A: 48.6

TABLE 1. (b)

PROPHASE				METAPHASE	
450nm	550nm	450nm	550nm	450nm	550nm
0.0	0.0	0.2	0.4	0.2	0.1
0.0	0.1	0.3	1.0	0.1	0.4
0.2	1.4	0.5	3.4	0.0	1.9
0.3	3.2	0.7	5.8	0.2	3.2
0.4	4.3	0.7	6.4	0.3	4.7
0.5	5.9	0.8	7.3	0.3	4.9
0.6	6.5	0.8	7.2	0.3	4.4
0.8	7.8	0.8	7.2	0.3	4.8
0.9	8.6	0.8	6.8	0.4	5.6
1.0	8.1	0.7	6.0	0.4	5.9
0.7	5.8	0.6	4.8	0.4	4.9
0.2	1.2	0.5	4.1	0.3	4.4
0.1	0.1	0.3	1.2	0.1	3.4
		0.2	0.4	0.0	1.8
				0.2	0.4
				0.2	0.1
5.6	52.9	6.6	54.5	3.3	50.7
A: 47.3		A: 48.0		A: 47.4	

proportionality^{4,6,13)} error in each cell layer. To isolate each epidermal cell after staining has the advantage that the hair follicles are clearly identified even to the naked eyes as darkely stained projections and easily removed selectively from the sheet under a stereomicroscope, with a sharp pointed pincette. Eliminating hair follicles before making a smear of isolated epidermal cells made it possible to avoid intermingling of heterogeneous cell populations. After thorough detachment of epidermal cells, a semitransparent sheet of horny substance remained and it was helpfull to assure again the complete removal of all the epidermal cells.

(3) As shown in table 1., even the most pale nuclei which are surrounded by much amount of cytoplasm were revealed to contain exactly diploid amount of DNA, after the elimination of non-specific light loss. Heterogeneous substances such as keratohyalin granules and mass of tonofilaments are richly distributed in the cytoplasm of these large cells of upper layers. In fact, the non-specific light loss which was quantitatively detected by the *two-wavelength-scanning-method* in such a cell was prominent and the overall absorbances measured at 550 nm vary considerably in indivisual cell. On the other hand, however, the cytoplasm of a basal cell is scanty and the non-specific light loss is, therefore, generally not so large (table 1). Including various amounts of large non-specific light loss, the nuclei of upper layers would be falsely considered to have larger amounts of DNA with the wide range of distribution if no correction were made for the non-specific light loss. As discussed above, the proliferating cells are restricted in the basal layer in rabbit's epidermis. And the contents of nuclear DNA of upper layers are constantly kept in diploid class. It is evident that the differentiation of an epidermal cell is accomplished in G₁-phase of mitotic cycle.¹⁴⁾ It also indicates that lysis of nuclear DNA in keratinizing cells should occur promptly, immediately before it changing to horny substance. In contrary to our finding Radley³⁴⁾ in 1965 measured the absorption of nuclear Feulgen dye of epidermal cells isolated from the ear of hairless mouse after maceration with diluted acetic acid and reported

that hypodiploid nuclei were evident indicating the reduction of DNA content of epidermal cells undergoing keratinization. It may be a characteristic feature for hairless mice. As for mitotic nuclei³¹⁾ in our experiment, two cells in prophase and one cell in metaphase, the DNA values of them distributed virtually in double diploid class without significant deviations.

(4) Using dorsal epidermis of mice, Gelfant³²⁾ has insisted the existence of so called "G₂-population" with very long duration of G₂ phase, being longer than 2 days in normal state. But in our experiment only 2.9% of basal cells in normal epidermis of rabbit's ear were found to be cells in G₂-phase which have the double diploid values of DNA. This finding assures that the duration of G₂ phase is short and confirms the absence of the "G₂-population of Gelfant".

(5) As shown in fig. 2., the relative numbers of DNA synthetic cells, $P_s(A)$, distribute showing a peculiar undulating curve. It may be regarded as a probability function of a cell to have a certain value of DNA, A . Therefore, $1/P_s(A)$ is the relative velocity of DNA synthesis at the time when the DNA content of a cell is A (fig. 3.). As shown in fig. 3., the velocity of DNA synthesis is minimum when about one third of scheduled DNA synthesis is accomplished. Then it is gradually accelerated again, reaching a maximum point when about two third of DNA amount is replicated. Even by the same duration of exposure to ³H-TdR, a cell which is rapidly synthesizing DNA is thought to incorporate larger amount

FIG. 3. The values of $1/P(A)$ were calculated and plotted from $P_s(A)$ in fig. 2.. It may be regarded as the rate of DNA synthesis (V_d) of a cell when the DNA content is A .

of ^3H -TdR. The grain count should be proportional to the velocity of DNA synthesis. In fact, the cells which are at the beginning and end stages of DNA synthesis were heavily labeled and the numbers of silver grains of those which have the middle DNA values were obviously small (table 1, a.). By the cytophotometric measurements of nuclear DNA of additional 177 basal cells, the number of cells in the middle of S-phase was significantly smaller and the tendency mentioned above (fig. 2.) was confirmed. Assuming steady state,^{33,34,35)} the undulating curve of $P_s(A)$ can be regarded to have a meaning as the inverted curve of DNA synthetic rate. And the maximum DNA synthetic rate toward the end might be related to late-replicating DNA.

(6) With the probability of $q=0.5$,^{33,34,35)} the daughter cells which are produced by cell divisions are destined to mature, migrate upward from the basal layer of generative cells. But some length of time seems necessary for a differentiated cell to be distinguished morphologically from an immature basal cell. Therefore, it is likely that already differentiated cells are included to some extent among the cells that are identified as basal cells in our cytophotometric measurements.

The nuclei in S- and G_2 -phase are larger in size and more densely stained by Feulgen technique, compared with those in G_1 -phase. Especially, the distribution of nuclear chromatin of a G_2 -cell is generally coarse and irregular.

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