TABLE 2. Results of Transplantation of Ovaries from Sterile hr^{rhY}/hr^{rhY} Females into Fertile Recipients

Expt. No.	Line and genotype of recipient	Number of recipients	Genotype of mating partner	Number of offspring		Average size of litter on weaning
				total	homozygotes	
1	B10 - hr^{rhY} +/+ or +/hr^{rhY}	4	+/hr^{rhY}	52	25 (48.8%)	4.7±0,5
2	F1(B10×129) +/+	4	+/hr^{rhY}	66	35 (53.0%)	5.1±0,8

To test the hypothesis that the cause of sterility of the hairless females lies outside gonads, experiments were carried out with transplantation of their ovaries into isogenic B10 females. The recipients were mated with males heterozygous for the hr^{rhY} gene. Half of the progeny was homozygous for the hr^{rhY} gene, i.e., hairless, whereas the other half had a normal hair cover and was heterozygous (+/hr^{rhY}). The same results were obtained on transplantation of the ovaries from homozygous mutants into (B10/Sn × 129/J)F_1 hybrid recipients with a similar subsequent mating (Table 2). The fertility of the females with transplanted ovaries was normal, and characteristic in the first experiment on the B10 line.

Ovaries transplanted from mutant females against the hormonal background of nonmutant recipients were thus shown to possess reproductive function and, consequently, the cause of sterility of hairless B10-hr^{rhY} females was proved to lie outside the gonads, possibly connected with steroid-dependent neuroendocrine disturbances [2]. Moreover, the positive results of experiments involving transplantation of ovaries into sterile mutants are of considerable practical importance, for they suggest the optimal method of maintaining a valuable mutant line of mice which are incapable of natural reproduction.

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SYNTHESIS AND CONTENT OF DNA IN EPIDERMAL CELL NUCLEI OF MOUSE SKIN DURING DIFFERENTIATION AND SPECIALIZATION

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KEY WORDS: DNA; autoradiography; cytophotometry; epidermis.

The keratinocytes of mammals have distinct morphological criteria of the successive stages of their multiplication, differentiation, and specialization and they provide a convenient model with which to study the mechanisms regulating proliferation and differentiation. It was shown previously on other model systems that during development of renewing cell populations the cells the mitotic cycle for differentiation not only in the G_1, but also in the G_2 phase and they form fractions of specialized cells with a double DNA content and with enhanced functional activity [4, 5]. This phenomenon, which is one form of hyperreplication of DNA [3], was particularly well marked in cases when extremal conditions of development of populations have necessitated a more rapid rate of cell differentiation and specialization [2]. It therefore seemed interesting to investigate a cell population in which, even under ordinary conditions,

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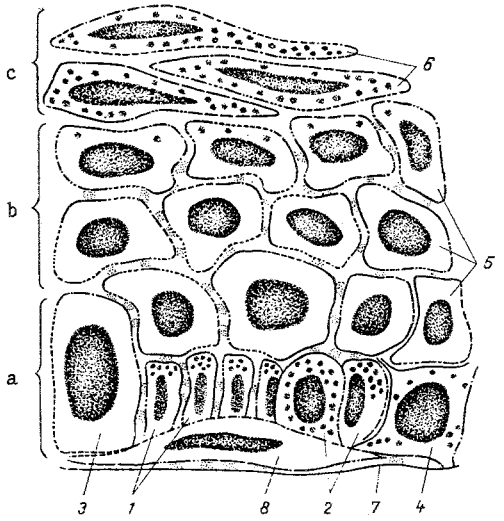

Fig. 1. Structure of cellular part of epidermis of mouse skin from interscapular region of spine. a) Stratum basale, b) str. spinosum, c) str. granulare. 1) Basal keratinocytes, 2) melanocytes, 3) Merkel's cells, 4) Langerhans' cells, 5) spinous keratinocytes, 6) granular keratinocytes, 7) basal membrane, 8) precursor cells of keratinoid series.

processes of proliferation, differentiation, and specialization take place at high intensity. The choice of the epidermis as a model was determined by the fact that previously polyploid cells were found in epithelial tissues of closely similar origin [8], and a G_2-cell population has been discovered in the epidermis of the mouse skin [6].

The aim of the present investigation was to study synthesis and the content of DNA during differentiation of epidermal cells.

EXPERIMENTAL METHOD

Experiments were carried out on 48 sexually mature male (C57BL/6 × CBA)F_1 mice. Each animal received an intraperitoneal injection of ^{3}H-thymidine in a dose of 0.08 μCi in 0.5 ml of 0.85% NaCl solution 6, 12, and 18 h before being killed by decapitation. Pieces of skin from the interscapular region of the spine were fixed in a formalin–ethanol–acetic acid mixture (9:3:1) for 2 h, and paraffin sections 5 μ thick were cut. Standard preparations were stained with azure and eosin. Sections for autoradiography were coated with type M emulsion (Research Institute of Photographic Chemistry Project), exposed for 20 days, developed, and stained with Mayer's hematoxylin; the labeling index was determined (in percent) by counting cells above whose nuclei there were at least 5 grains of reduced silver. Preparations for cytophotometry were stained by Feulgen's method (hydrolysis in 5 N HCl at 20°C for 40 min). Measurements were made on a "Temp" dual-wavelength automatic cytospectrophotometer [1] at wavelengths of 565 and 605 nm (90 × 1.25 objective, oil immersion, objective-condenser 20 × 0.40, calibrated probe larger than the nucleus). In each case at least 50 nuclei from three or four animals were subjected to photometry. The haploid equivalent of the DNA–fuchsine complex (c) was determined by photometry of spermatid nuclei of the same mice in sections through the testes, treated simultaneously with the skin sections. The results were expressed in conventional units, treated statistically, and presented in the form of histograms of distribution of the cells by DNA–fuchsine content in their nuclei (the class interval amounted to 20% of the haploid equivalent value).

EXPERIMENTAL RESULTS

The epidermis of the thin mouse skin is divided into clearly distinguishable stratum basale, str. spinosum, and str. granulare. In the stratum basale, with the stains used, three types of cells were discovered. Type I consisted of basal keratinocytes and melanocytes, containing a large number of pigment granules. They were difficult to differentiate from each other when stained by Feulgen's method. Type II consisted of elongated, nonpigmented epithelial

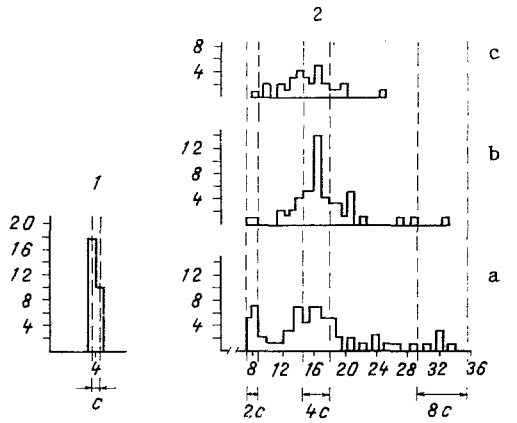

Fig. 2. Distribution of spermatids (1) and epidermal cells from mouse skin in interscapular region (2) by DNA content in their nuclei. Abscissa, DNA content (in conventional units); ordinate, number of cells; a) stratum basale, b) str. spinosum, c) str. granulare.

precursor cells of the keratinoid series. Cells of type III were arranged in groups and contained little or no pigment. These are the cells of Merkel and Langerhans. They are larger than keratinocytes and melanocytes. Sometimes they could be differentiated from one another by the fact that Langerhans' cells have processes. In the stratum basale the cells were arranged in one or, occasionally, in two rows. Up to 6-8 rows of cells could be counted in the stratum spinosum. Spinous keratinocytes were larger than basal, polygonal in shape, with larger nuclei, and easily identified. In the stratum granulare highly differentiated keratinocytes, rhomboid in shape with a large elongated nucleus, firmly packed together, were arranged in one to three rows (Fig. 1).

The autoradiographic data showed that only cells of stratum basale, in which the proliferative pool reached 14.4%, had the ability to incorporate ^{3}H-thymidine. Basal keratinocytes (26.5 ± 0.5%) and melanocytes (18.3 ± 0.6%) were labeled most actively. Precursor cells also incorporated ^{3}H-thymidine actively (21.0 ± 0.8%). Merkel's cells were labeled much less frequently (1.4 ± 0.2%). No Langerhans' cells were labeled. Precursor cells, basal keratinocytes, and melanocytes multiplied actively (mitotic index 5-7%). However, no divisions were recorded among Merkel's and Langerhans' cells.

It will be clear from the histograms that cells of the basal layer were heterogeneous for DNA content (Fig. 2). Some cells here were found to contain from 2 to 8c of DNA in their nuclei. The great majority of them contained 4c DNA, half as many cells contained the diploid amount, and about 12% of the population were hypertetraploid and octaploid cells. Cells of the stratum spinosum were more uniform as regards the DNA content in their nuclei. Here the overwhelming majority of cells were tetraploid (Fig. 2b).

Keratinocytes of the stratum granulare were even more homogeneous for DNA content in their nuclei, and the average DNA content in the nuclei of these cells was less than in cells of the stratum spinosum. This was evidently due to gradual destruction of the nuclei because of conversion of the cells into scales.

The heterogeneity of the stratum basale necessitated DNA to be determined in the nuclei of each of the cell types identified. By DNA content these cell types differed significantly from one another (Fig. 3). For instance, precursor cells of the keratinoid series (type I) contained 2-4c DNA. This, together with the autoradiographic data, indicate that they are an actively multiplying population. The fact that there were more 4c than diploid cells suggests that the process of release of these cells from the cycle begins in the stratum basale actually in the postsynthetic phase. During further progress into the stratum spinosum and with differentiation, these cells preserve their diploid DNA content in the nuclei (Fig. 3, I, II). This is evidence that delay of the cells in the G_2 phase is observed in the early stage of differentiation. These cells evidently do not divide but are displaced into the layer of basal keratinocytes, where they take part in the formation of the 4c-keratinocyte fraction. This last fraction is also replenished by basal keratinocytes emerging from the cycle in the G_2 phase, whereas another part of them continues to multiply. Besides 2c cells, among the

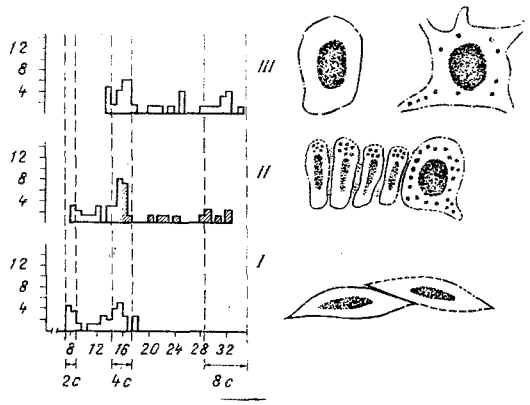

Fig. 3. Distribution of cells of stratum basale of epidermis of mouse skin by DNA content of their nuclei. I) Precursor cells, II) keratinocytes and melanocytes (shaded parts of histograms), III) Merkel's and Langerhans' cells. Remainder of legend as to Fig. 2.

melanocytes there are also H4-8c-cells, but most of them are blocked in the G_2 phase (Fig. 3, II). Since the labeling index of the melanocytes is sufficiently high, it can be postulated that the 4c-cells do not divide but continue to synthesize DNA up to 8c values. Among Merkel's and Langerhans' cells no diploid cells whatever were found: most of these cells were 4c-cells, but they were also H4c- and 8c-DNA-containing cells (Fig. 3, III). Since DNA synthesis was observed only among a few Merkel's cells, and none whatever in the case of Langerhans' cells, this suggests that hyperreplication of DNA in these cells takes place in the earlier stages of development of the mice, and in adult animals they are preserved in a terminally differentiated state. The cytophotometric and autoradiographic data also point to a long duration of the life cycle of these cells, evidently many times longer than the life of cells of the keratinoid series, complete renewal of which in different animals is observed after 20-30 days [7]. On staining by Feulgen's method, the basal keratinocytes and melanocytes can be distinguished only by the shape of their nuclei, but for young cells this can be difficult. Mature melanocytes, on the other hand, can be well identified. The results of the cytophotometric and autoradiographic study of DNA synthesis in these cells suggest also that they consist of a subpopulation of young multiplying and nondividing polyploid cells. It can be postulated that the second subpopulation consists mainly of melanocytes containing 4-8c DNA (the shaded part of the histogram in Fig. 3, II), since nondividing keratinocytes containing 4c DNA are displaced into the stratum spinosum, while the melanocytes remain.

The certain degree of scatter of DNA content around the modal values (2, 4, and 8c) on the histograms may be connected with the fact that the investigation was conducted on sections and not on films or squash preparations. Meanwhile the absence of "fuzziness" of the histogram of distribution of spermatid nuclei by their DNA content, likewise obtained as a result of measurements of nuclei in sections (Fig. 2), may perhaps be evidence that during hyperreplication of DNA of the epidermal cell nuclei, a strict cycle such as is characteristic of polyploidization is not observed. The possibility likewise cannot be ruled out that "intermediate" values of DNA are obtained as a result of incomplete replication of separate regions of the genome.

Thus all the specialized cells of the mouse epidermis are polyploid. Consequently, hyperreplication of DNA, realized by polyploidization, is part of the program of their development. Hyperreplication of DNA in keratinocytes is most probably due to the need for synthesis of specific proteins and their accumulation in the keratinocytes of the stratum spinosum and stratum granulare, in a relatively short time interval: in mice complete renewal of the keratinocyte population takes place after 19 days [7], and the volume of specialized keratinocytes of the stratum spinosum and stratum granulare is about 10 times greater than the volume of the basal keratinocytes. Hyperreplication of DNA in melanocytes also is probably determined by the need for synthesis of melanin, which takes place in keratinocytes [9].

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EFFECT OF HYPOXIA ON PRIMARY CARDIOMYOCYTE CULTURE

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The use of a culture of heart muscle cells as a model of states of hypoxia and ischemia of the myocardium has many advantages [1, 3]. The body responds to creation of ischemia by a multicomponent compensatory reaction.

In the investigation described below a state of hypoxia was modeled on a primary culture of neonatal rat cardiomyocytes in a constant-temperature gas-flow chamber, with the use of two gas mixtures containing 0 and 5% of oxygen respectively, and attempts were made to select conditions at which pathological changes in the cells were reversible in character.

EXPERIMENTAL METHOD

Primary cultures of neonatal rat cardiomyocytes were prepared from the hearts of 3-day-old rats. The hearts were minced, washed to remove blood, and incubated in a 0.1% solution of collagenase ("Sigma") in medium 199 at 37°C for 2.5 h. The material was then dispersed in a small volume of medium 199 by gentle pipetting. A solution of Versene (1:1) was added to the cell suspension thus obtained, and the mixture was centrifuged (800g, 3 min). The residue was resuspended in Eagle's medium with the addition of 10% serum (a mixture of different quantities of embryonic calf serum and bovine serum). The cells were seeded into Carrel's flasks in a concentration of not less than 1×10^6/ml. After incubation for 2 h at 37°C in an atmosphere containing 5% CO_2, about 50% of the cells settled on the glass. These flasks were discarded. The suspension of nonadherent cells was transferred into 12-well panels with coverslips. During the first 3 days the medium was changed daily, and thereafter every other day. Contracting cells, on reaching the state of a confluent monolayer, on the 4th-6th day of culture, were used in the experiments. The fraction of cardiomyocytes in the cultures was estimated in preparations stained for mitochondria by Mallory's method. Hypoxia and anoxia were produced in a constant-temperature gas-flow chamber by the use of two gas mixtures: 100% N_2 and 90% N_2 + 5% CO_2 + 5% O_2. The duration of exposure was 1 and 2 h at 37°C. The medium was changed 2 h before the experiment began. The state of the cells was assessed by light microscopy in preparations stained with Ehrlich's hematoxylin. To assess the relative proportions of the cells in culture, no fewer than 1000 cells were counted in each preparation under a magnification of 400. The average was found for four or five preparations taken from different experiments.

EXPERIMENTAL RESULTS

When obtaining a culture of cardiomyocytes there is the risk of contamination by fibroblast-like cells, which multiply much faster than cardiomyocytes, and during long-term culture they may displace all the other cell types in mixed cultures. Fibroblasts and endothelial

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