CHANGE IN ENZYME ACTIVITY AND RIBONUCLEIC ACID CONCENTRATION WITHIN THE EPIDERMAL CELL OF THE RAT DURING THE GROWTH STAGE OF THE HAIR CYCLE*

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During the hair cycle there occurs a rapid alteration of all structures in the skin of rats and mice (1, 2). In the early growth stage (anagen), the epidermis temporarily increases two to three times in thickness, and there is greater mitotic activity in the basal layer. Simultaneously the external root sheath becomes more active, and the hair germ develops into a bulb which begins to descend through the corium into the subcutaneous fat. When the bulb reaches the lower layer of fat, at about the sixth or seventh day, hair growth begins. The epidermis is then very quickly reduced to its previous thickness. At the end of hair growth the follicle shortens (catagen), coming to rest high in the corium. Hair growth in the rat ceases at about the 25th day (3), in the mouse at the 19th day (1). The resting stage (telogen) lasts several days in the young animal and lengthens progressively to months as the animal matures. Text-fig. 1 is a diagrammatic summary of these phenomena.

The few biochemical and enzyme studies, which have been made on mouse skin during the hair cycle, have not included the epidermis. They have all been qualitative, histochemical investigations and are subject to the usual uncertainties of such studies. They show that alkaline phosphatase is abundant in the dermal papilla of the growing hair follicle but is absent from the papilla of the resting follicle (4). Glycogen accumulates in the external root sheath of growing follicles and disappears abruptly when hair growth ceases (5).

In the course of studies on the respiratory enzymes of rat epidermis, the effect of the hair cycle on enzyme activity in the epidermis became apparent (6). It seemed profitable to investigate this effect further in order to show how the influence of biological factors may be controlled in enzyme experiments and also to make quantitative measurements of enzyme activities in growing

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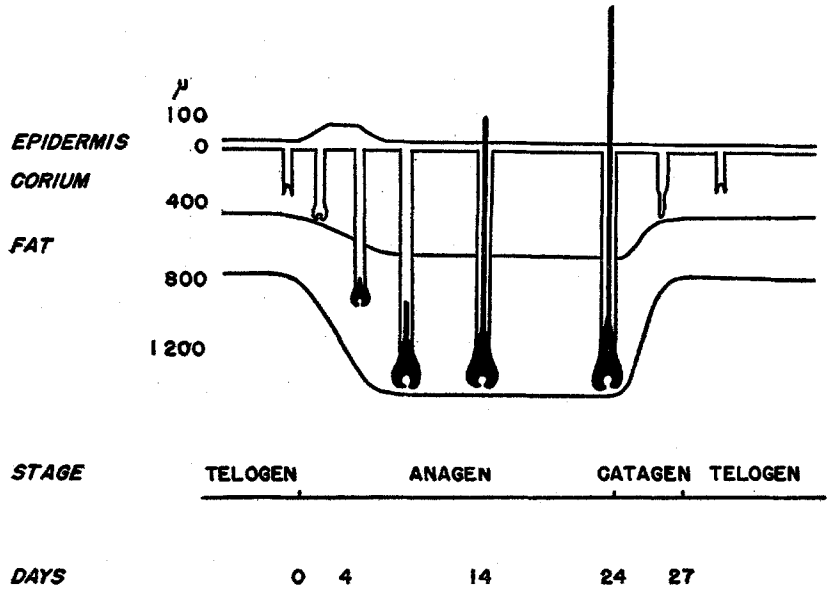
epidermis which might demonstrate a correlation between structural and functional changes and enzyme activity in the epidermal cell. In the present study, rat epidermis, as it proliferates during the hair cycle, was assayed for succinic dehydrogenase, cytochrome oxidase, deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). These enzymes and RNA were found to increase significantly in the epidermal cell during this proliferation.



TEXT-FIG. 1. Anatomical alterations occurring in rat skin during the hair cycle. Compilation based on data from references 1–3, and original observations.

Methods

Synchronization of Hair Cycles.—So that the epidermis of the whole animal could be used for enzyme studies, it was necessary to synchronize the hair cycles on the entire body surface. In the young rat each hair cycle begins simultaneously on the head and on the abdomen. A wave of hair growth passes caudally and dorsally over the body until finally all hairs are growing at the same time (7). At the end of anagen, a resting wave passes over the hair follicles of the rat until eventually all are at rest. Mohn reports that in the rat all follicles are resting at ages 7 to 8 weeks, 12 to 13 weeks, and 4 months (3). In older rats this pattern of growth becomes bizarre, as can be shown by intraperitoneal injection of a dye which becomes concentrated in growing hair follicles (8). A new hair cycle may be initiated at will by plucking the hair from a resting follicle. The ensuing changes in the skin surrounding this follicle are precisely the same as those which occur during a spontaneous cycle and are not the result of trauma (1). No histological signs of inflammation beneath the epidermis are discernible. It is possible to synchronize the hair cycles over the entire body surface by plucking the whole animal when all follicles are at rest. If this is done, all plucked follicles will remain in phase throughout the ensuing cycle. The epidermis of the whole animal is then presumably subject to identical biological factors during each stage of the following cycle.

Preparation of Animals.—White male Wistar rats 12 to 13 weeks of age were obtained from the Charles River Laboratories, Boston. Their diet had consisted of mixed cereals, milk, liver, meat, fish meal, linseed oil meal, and mineral and vitamin supplements. The animals were anesthetized with ether, and the hairs of the back were plucked. In the studies reported here, six animals were sacrificed immediately, and six others after 4 days when anatomical changes in the epidermis were most striking. Histological sections of skin from each animal were prepared to check the stage of the cycle.

In Fig. 1 is shown the increase in thickness of the epidermis during the first 4 days of the cycle. 4th day epidermis (*B*) contains a greater number of layers of viable cells and a more prominent stratum granulosum than 0 day epidermis (*A*).

In preliminary experiments, studies were also made on animals in the latter part of anagen when the epidermis had become thin. The histological appearance and enzyme assay values of 14th day epidermis were equivalent to those of 0 day epidermis. Thereafter, studies were concentrated on the period of proliferation during the first 4 days of the cycle.

Preparation of Homogenates of Epidermis.—The skin of the entire animal was removed and tacked to a slightly convex board. The epidermis was scraped from the dermis by a safety razor. Microscopic examination of these shavings of epidermis showed that they contained a variable amount of collagen, a few sebaceous glands and a few fibrocytes. The shavings were homogenized in a glass homogenizer at 0°C. for 1 minute.

Enzyme Assays.—Succinic dehydrogenase and cytochrome oxidase activities were determined on homogenates of epidermis of each animal by means of Warburg respirometers according to a method developed previously in this laboratory (6). The epidermis from an entire animal is required for a combined assay.

Reference Bases.—The choice of reference bases was made so that tissue enzyme values could be related to the number of viable cells and the enzyme activity per epidermal cell could be determined. Because of variation in the amount of inert material in homogenates of epidermis, such as stratum corneum and collagen, enzyme activities could not be related satisfactorily to wet or dry weight or to nitrogen. A better reference base is DNA content, which reflects the number of viable cells present in a tissue. The amount of DNA per viable cell has been shown to be constant for many tissues under a great variety of conditions, such as regeneration of the liver, starvation, pregnancy, and aging (9). Therefore, basing enzyme activities on the DNA content of the epidermis permitted quantitative determination of enzyme activities in the epidermal cell.

Estimations of the DNA and RNA content of homogenates of epidermis were made by the micro procedure of Scott *et al.* (10). This uses measurement of the optical density of purine and pyrimidine bases at 260 mμ as its quantitative tool. First RNA was extracted with 1 N NaOH at 25°C. for 1 hour. Then DNA was removed by 1.6 N perchloric acid at 60°C. for 7 minutes. To assay DNA and RNA in 1.0 ml. aliquots of homogenates of epidermis, the only modification required was to increase the volumes of all reagents to thirty times the amount used by Scott *et al.* It was then possible to use 3.0 ml. Beckman cuvettes instead of 0.1 ml. microcuvettes.

The Scott method compared favorably with the Schneider method (11) in accuracy: values for DNA and RNA of homogenates of epidermis obtained by the Scott method were 95 per cent of the Schneider values. The Scott method was fifteen times more sensitive than the Schneider method, detecting the DNA and RNA in 3 mg. of wet epidermis.

RESULTS

The results of enzyme studies on epidermis at the 0-day and the 4th day of the hair cycle are listed in Table I. The following determinations were made on 1.0 ml. aliquots of homogenates of the epidermis of each animal: dry weight,

DNA, RNA and succinic dehydrogenase and cytochrome oxidase activity. Enzyme activity was referred to three bases: dry weight (Q_{O_2}), DNA (Q/DNA) and RNA (Q/RNA).

In 4th day epidermis, the activity (Q_{O_2} and Q/DNA) of both succinic dehydrogenase and cytochrome oxidase increased about 30 per cent above that for 0 day. This increase was statistically significant only when the enzyme

TABLE I

Effect of the Hair Cycle on Enzyme Activities and Nucleic Acid Content of Rat Epidermis

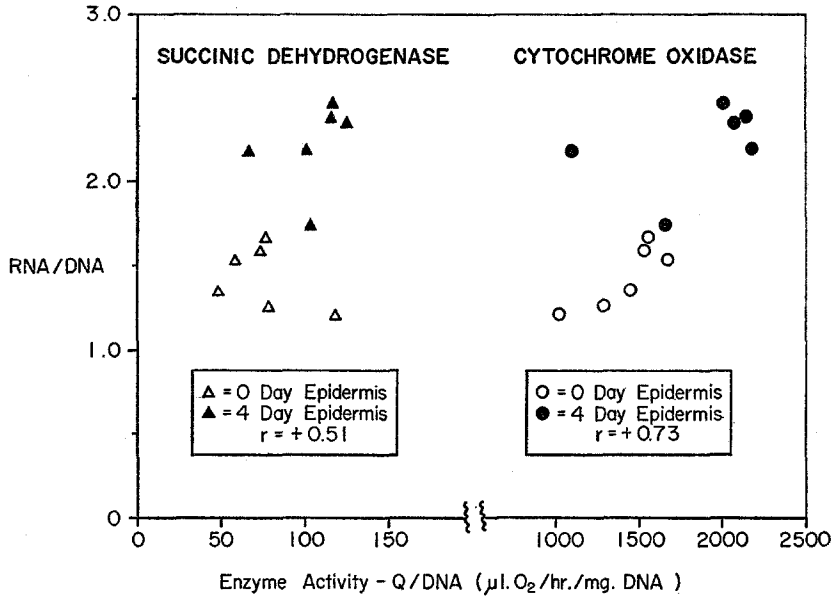
	Day of cycle		Difference (4 − 0)	Per cent difference $\frac{(4-0)}{0} \times 100$	Probability
	0	4			
Succinic dehydrogenase					
Q/mg. dry weight (Q_{O_2})	0.64 ± 0.11	0.84 ± 0.09	+0.20	+31.2	0.2
Q/mg. DNA	75.8 ± 9.8	105.2 ± 8.5	+29.4	+38.8	**0.05**
Q/mg. RNA	54.2 ± 9.6	47.6 ± 3.9	−6.6	−12.2	0.3
Cytochrome oxidase					
Q/mg. dry weight (Q_{O_2})	11.8 ± 0.8	14.8 ± 1.4	+3.0	+25.4	0.1
Q/mg. DNA	1420.0 ± 94.0	1865.0 ± 171.0	+445.0	+31.3	**0.05**
Q/mg. RNA	981.0 ± 39.0	842.0 ± 71.0	−139.0	−14.2	0.15
Nucleic acids					
μg. RNA/mg. dry weight	12.0 ± 0.57	17.5 ± 0.61	+5.5	+45.8	<0.001
μg. DNA/mg. dry weight	8.4 ± 0.45	8.0 ± 0.59	−0.4	−4.7	0.65
RNA/DNA	1.45 ± 0.08	2.22 ± 0.11	+0.77	+53.1	**0.01**

Six animals were studied at both the 0 and the 4th day of the cycle. The values in the first two columns represent the averages for the six animals (±standard error of the mean). Q/mg. = enzyme activity as μl. O_2/hour/mg. of reference base. All enzyme activities and weights were determined on 1.0 ml. aliquots of homogenates of epidermis.

values were based on DNA. (The lack of significance in the difference between enzyme values referred to dry weight was probably due to variation in the amount of inert material in the homogenates; *e.g.*, stratum corneum and collagen.)

The RNA content of 4th day epidermis increased 53 per cent with respect to DNA. An approximately parallel increase in enzyme activity and RNA content in proliferated epidermis is evident upon comparison of Q/DNA with RNA/DNA. Moreover, in Text-fig. 2 the relationship between enzyme activity and RNA content appears to hold for rat epidermis regardless of the stage of the hair cycle. Even though regression curves cannot be satisfactorily con-

structed, the suggestion of a positive correlation between enzyme activity and RNA content remains.



TEXT-FIG. 2. Correlation between RNA concentration and enzyme activity in rat epidermis during the hair cycle. *r*, correlation coefficient.

DISCUSSION

The experiments reported here show that in the rat the increase in epidermal thickness due to multiplication of epidermal cells during the early growth stage of the hair cycle is accompanied by an increase in respiratory enzyme activity and in RNA content in the viable epidermal cell. This type of correlation between the biological state of cells and their enzyme activity is a step on the way toward understanding growth processes.

Change in cellular enzyme activity during growth has been documented by other investigators. Carruthers found an increase in the activity of succinic dehydrogenase and cytochrome oxidase of mouse epidermal carcinoma over that of normal mouse epidermis (12). But in mouse hepatoma the activity of these enzymes was observed to be much lower than that of normal liver (13).

In the development of the author's enzyme assay methods (6), efforts were made to control all factors known to affect the activity of succinic dehydrogenase and cytochrome oxidase. Unless some unknown factor influenced enzyme activity in the experiments reported here, it is reasonable to suppose

that the enzyme activity values measured were proportional to enzyme concentrations and that an increase in enzyme activity was probably indicative of enzyme synthesis.

Many investigators are currently studying the relationship between RNA and protein and enzyme synthesis. It may be pointed out that the observation of a parallel increase in RNA content and in concentration of oxidative enzymes in the proliferating epidermal cell during the rat hair cycle is consistent with the hypothesis that RNA is closely concerned with protein synthesis. For example, it lends support to the opinion of Spiegelman that protein synthesis is possibly dependent on simultaneous synthesis of new RNA (14).

The results of the present study bring to mind the phenomenon of enzyme adaptation, mechanisms of which might explain the alteration in enzyme concentration reported here. Several stimuli lead to adaptive change in enzyme concentration in animal tissues: diet, substrates, hormones, altitude, acidosis, etc. (15). Details of these adaptive processes are not known, but effects are more precise and possibly more nearly primary with hormones than with other stimuli. Cortisone elevates the tryptophan peroxidase content of liver (16). Thyroxine increases succinoxidase and cytochrome oxidase (17) in many tissues. Hormones influence tissue RNA as well as tissue enzyme levels. Administration of thyroxine (18) or growth hormone (19) increases RNA relative to DNA in regenerating liver.

Since hormones are among the stimuli which may initiate the rat hair cycle, *e.g.* plucking of resting hairs, adrenalectomy, and administration of thyroxine (20), it is possible that the anatomical and enzyme changes noted in epidermis in the rat hair cycle arise from the effect of hormonal stimulation. The correlation of enzyme activity with morphologic change in the epidermis during the hair cycle under the influence of thyroxine, thiouracil, cortisone, and adrenalectomy must be investigated to test this hypothesis.

SUMMARY

The rat hair cycle produces rapid anatomical and physiological changes in the entire skin. By the 4th day of the cycle, the epidermis has doubled in thickness.

The present study shows that, during this proliferative period, there is a correlation between the biological state (growth) and the molecular composition (enzyme concentrations) of the epidermal cell. In proliferated 4th day epidermis there has occurred not only an increase in the number of viable cells but also an increase in succinic dehydrogenase and cytochrome oxidase activity and ribonucleic acid (RNA) content in the viable epidermal cell. Moreover, RNA content and enzyme activities appear to be correlated.

The significance of these findings is discussed in the light of recent evidence

supporting the relationship between RNA and enzyme synthesis and the role of hormones in the induction of enzyme adaptation.

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EXPLANATION OF PLATE 137

FIG. 1. Histological changes in rat epidermis during the hair cycle. *A*, 0 day epidermis. *B*, 4th day epidermis. The entire epidermis has become thicker, the number of layers of viable cells has increased, and the stratum granulosum is more prominent by the 4th day. × 520.

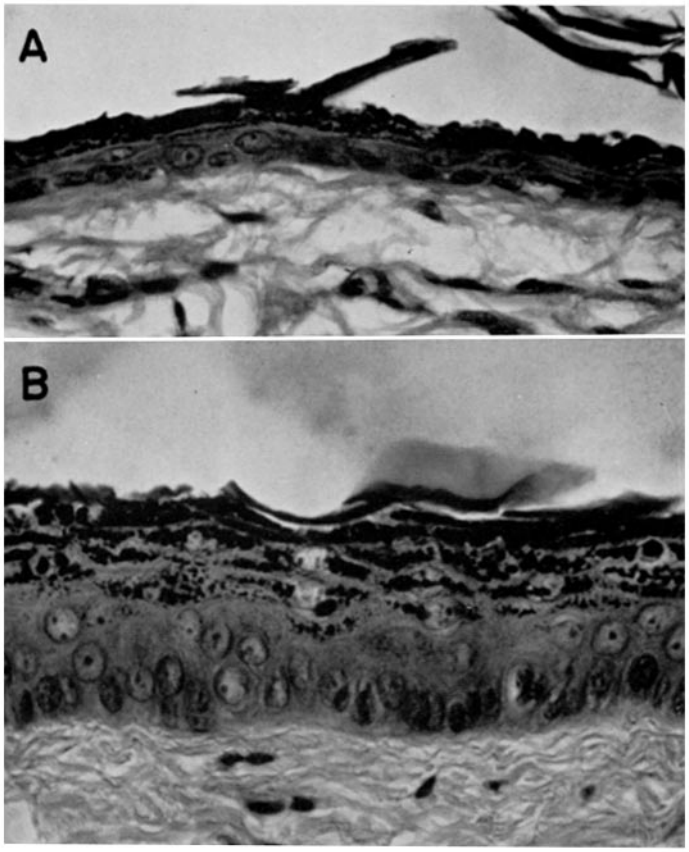

FIG. 1