THE JOURNAL OF INVESTIGATIVE DERMATOLOGY, 66:222-229, 1976

Vol. 66, No. 4
Printed in U.S.A.

SELECTIVE ACTION OF HYDROCORTISONE ON POSTMITOTIC EPIDERMAL CELLS IN VIVO

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Hydrocortisone administered systemically for 3 weeks has no effect on any phase of epidermal cell proliferation as measured by autoradiographic methods. However, the speed of cell differentiation (maturation) is increased, resulting in a thinning of the living epidermis due to the shorter epidermal cell life.

Comparison of the epidermis from two body sites (ear and sole of foot) in mice receiving 2.4 μg per gm body weight per day of hydrocortisone in drinking water for 3 weeks revealed no change in the labeling with [^{3}H]thymidine, the mitotic indices, or the lengths of the cell cycle phases. Quantitation of the epidermal cell compartments showed that thinning of the epidermis with hydrocortisone was due to the loss of an identical number of differentiating epidermal cells per unit surface from both body sites. In both sites there was the same increased rate of maturation of postmitotic cells while the proliferative cell-pool remained unresponsive to the hormone.

The alteration of the speed of cell maturation is the principal action of hydrocortisone in epidermis. The results indicate that the epidermal cellular concentration of, and the susceptibility to, the hormone were identical in ear and sole of foot despite the differing speeds of turnover of the two tissues.

In a biologic system such as epidermis, the entire cell population consists of spatially interdependent proliferating (keratoblasts, Kb), differentiating (keratocytes, Kc), and functioning (corneocytes, C) cells [1], each type differing in its function, anatomic position, and histologic appearance. Experimental interference by superficial wounding, stripping, dermal inflammation, or other damage results in alterations in the entire system and until now it has been almost impossible to disrupt the relationship between epidermal cell proliferation and differentiation and function. Mainly because of the availability of sophisticated methods for measuring cell proliferation (e.g., monitoring mitosis or quantitating isotopically labeled cells), the dividing cell has been assumed to be the principal site of action for the regulation of epidermal thickness and growth [2]. On the other hand, techniques for the study of epidermal differentiation, maturation, and function are less readily available or have yet to be developed. Thus, the therapeutic effects of corticosteroids which cause thinning of the epidermis have been elegantly explained in terms of cell proliferation, although the precise mode of action has remained obscure [3].

In this present study the whole spectrum of tissue renewal, as distinct from cell renewal in the basal layer, is considered after long-term systemic administration of hydrocortisone. Interpretation of the results has necessitated a change in emphasis from cell proliferation toward the rate of cell maturation. It was found that hydrocortisone selectively affects differentiating epidermal cells, thus disrupting the relationship between epidermal proliferation and differentiation.

MATERIALS AND METHODS

Animals

A total of 750 4-month-old male Swiss S mice (Animal Suppliers, London) was used. For 4 weeks prior to experimentation they were housed 5 per box, under constant conditions of 12 hr of light, 20°C, with food and water ad libitum, while cleaning and disturbance of the animals were confined to the hours of 9:30 to 11:00. They were killed by neck dislocation. Pieces of ear and whole hind foot were removed and fixed immediately.

Administration of Hydrocortisone

Hydrocortisone (Organon Laboratories) was administered orally in the drinking water. Thirty milligrams was dissolved in a minimal quantity (approximately 1.5 ml) of ethanol and diluted with tap water to 1 liter. No precipitate formed. This solution was used as drinking water. The volumes consumed each day were recorded so that the total amount administered to each mouse could

Manuscript received July 29, 1975; in revised form November 17, 1975; accepted for publication November 19, 1975.

Supported in part by grants from the Wellcome Trust and the Deutsche Forschungsgemeinschaft.

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be calculated. The average concentration of hydrocortisone administered in this way was 2.4 ± 0.33 μg per day per gm body weight of mouse.

Histologic Procedures

For routine histologic and autoradiographic examination the ears were fixed in alcoholic Bouin and the whole of the hind foot was fixed in aqueous Bouin. Twenty-four hours later the epidermis together with the superficial dermis of the sole of the foot located behind the pads was removed carefully with a sharp scalpel before processing. Specimens were embedded in paraffin wax, sectioned at 7 μ, and stained in Ehrlich's hematoxylin and eosin for ordinary histology; for autoradiography they were sectioned at 3 μ and subsequently stained in Mayer's hemalum. Autoradiographs were prepared with Kodak AR stripping film; exposure time was 3 weeks at −15°C. A nucleus was recorded as being labeled when there were at least 5 overlying grains above background.

Flat sheets of epidermis were prepared from 12 control and 16 treated mice by placing the ears in 0.8% solution of tetrasodium ethylene diaminotetraacetic acid (Na-EDTA) saline at 37°C for 4 hr. Sheets of epidermis together with the pilary organ were then stripped from the underlying dermis. These strips were mounted basal layer uppermost on filter paper and fixed in Bouin's solution. They were subsequently removed from the paper, stained in Ehrlich's hematoxylin, and mounted basal layer uppermost on slides.

From 10 experimental and 10 treated mice, single cornified cells were prepared from both body sites by direct application of the outer surface of the epidermis to adhesive-coated slides (technique used by Goldschmidt and Kligman [4]).

Cryostat sections (5 μ thick) were prepared from fresh specimens and stained in fluorescein isothiocyanate (FITC) followed by acetic acid swelling [5]. For observations on the stratum corneum a Zeiss fluorescent microscope (Filter combination 12/50 KP 500) was used.

Quantitation of Epidermal Compartments

Thickness of epidermis. From 13 control and 18 experimental animals, the thickness of epidermis was determined using photographs of 7-μ sections which had been magnified 1370 times. Thickness was calculated from measurements of area made with a planimeter and length of the epidermis made with a map measurer.

Cell number in each compartment. From the photographs the number of basal cells and superficial nuclei were counted. These methods are known to be subject to error when different tissues are compared. Therefore the Abercrombie formula was used as a correction factor [6], that is:

$$\text{average number of nuclei} = \text{nuclear count} \times \frac{\text{thickness of section}}{\text{length of nucleus} + \text{section thickness}}$$

Length of nucleus, parallel to the basement membrane, was measured directly from the photographs. The number of basal cells per corneocyte was determined from photographs of flat sheets of ear epidermis taken at different levels of focus. By superimposition of the photographs the number of basal cell nuclei per cornified cell was determined.

For the sole of foot the epidermis was too thick to use this method successfully. Therefore the number of basal cells per cornified cell was determined by extrapolation from the data on the ear epidermis and corrected for cell size differences [7]. This is summarized by the following formula:

$$\text{Kbs} = \text{as} \times \frac{\text{Kbe} \times \text{lbs}}{\text{ae} \times \text{lbe}}$$

where:

- Kbs = number of basal cells per corneocyte in sole epidermis;
- Kbe = number of basal cells per corneocyte in ear epidermis;
- as = area of sole corneocyte;
- ae = area of ear corneocyte;
- lbs = number of basal cells per mm surface length of sole epidermis;
- lbe = number of basal cells per mm surface length of ear epidermis.

Determination of the cell numbers in the stratum corneum was made directly from FITC-stained frozen sections.

Experimental Procedure for Determining Kinetic Data

Labeling index. For determination of the number of cells synthesizing DNA (labeling index), 25 μCi [^{3}H]thymidine ([^{3}H]TdR; sp act 22 Ci/mmole; Radiochemical Centre, Amersham, England) was injected into 5 control and 5 experimental mice at each hour over a 24-hr period. A total of 240 mice was used. The animals were killed 45 min after administration of the [^{3}H]TdR.

S-phase length. For determination of the length of S-phase the pulse-chase method was used, and the percentage of labeled mitoses (PLM) after a single injection of [^{3}H]TdR was recorded. Three hundred mice (150 control and 150 experimental) were injected each with 25 μCi [^{3}H]TdR at 11:30 hours. At each hour for 30 continuous hr, 5 control and 5 experimental animals were killed.

Mitotic index. From the results obtained for the PLM curves the mitotic index was determined over the first 24 hr of the 30-hr period.

Mitotic durations. For determination of the mitotic duration the Colcemid method was used over the period from 11:30 to 15:30 hr when the animals were asleep. Ten experimental and 10 control mice were injected each with 100 μg Colcemid (Ciba) at 11:30 hr and the animals were killed 4 hr later. Ten control and 10 hydrocortisone-treated mice which had not received Colcemid were killed each hour throughout that 4 hr.

Kinetic Data

Labeling index. This was determined by recording the number of labeled cells per 10-mm surface length of epidermis [7]. The average number per hour and the standard error were determined.

Median length of DNA synthesis (S-phase). S-phase was determined from the percentage of labeled mitosis curves obtained by counting the number of labeled and unlabeled mitoses per 10-mm length of surface epidermis. The average and standard error for each hour were determined. The results were graphed and the median value (i.e., the time between the ascending and descending lines of the graph at the 50% values [8]) for the length of S was measured.

Mitotic index. The total number of mitoses recorded each hour in determining the PLM curve formed the mitotic index. This figure is probably uniformly lower than expected due to the difficulty of recognizing labeled prophase cells under these conditions.

Duration of mitosis (M-phase). M-phase was recorded

by determining the number of mitoses per 10-mm length of surface epidermis (M_1–M_4) at each hour without the use of Colcemid and determining the number of mitoses per 10-mm surface length arrested by Colcemid over that 4-hr period (M_c). The M-phase length (T_m) was calculated from the formula:

$$T_m = \frac{\text{Sum of } M_1 + M_2 + M_3 + M_4}{M_c}$$

In all quantitative results the arithmetic means and standard error were recorded.

RESULTS

Changes in Cell Compartments and Cell Dimensions

A comparative histologic description and the methods used for quantitation of compartment size of ear and sole of foot epidermis in mouse have been given previously [7]. The changes which take place in these compartments after hydrocortisone treatment are shown in Tables I and II.

In Table I the histologic parameters (nuclear length, cell number, corneocyte size) of the different cell compartments of the two epithelia are compared. It can be seen that in the treated animals there is a reduction in the number of cells in the two living compartments, i.e., keratoblasts (Kb) and keratocytes (Kc). This reduction in the number of living epidermal cells is found by counting epidermal cells per unit surface length as well as by the three-dimensional analysis expressed per corneocyte. After hydrocortisone treatment the percentage reduction is much smaller in epidermis from the sole of the foot than from the ear. This reduced cell number may be contrasted with an increased nuclear length, particularly in the Kb (Tab. I) in both body sites. Since the nuclear length is measured parallel to the basement membrane, this change is apparently a reflection of the flattening of the cells as seen in Figure 1. Furthermore, in both body sites the overall size of the corneocytes was slightly de-

TABLE I. *Quantification of histologic parameters of epidermis from two different body sites after 3 weeks of hydrocortisone treatment*

		Keratoblasts (K_b)					Keratocytes (K_c)					Corneocytes (C)				
		Control	N	Treated	N	% Change	Control	N	Treated	N	% Change	Control	N	Treated	N	% Change
Nuclear length (μ)	Ear	5.6 ±0.15	30	6.6 ±0.15	20	+18 $p < 0.01$	7.9 ±0.10	30	8.7 ±0.17	20	+10 $p < 0.01$	—		—		—
	Sole	5.2 ±0.23	30	6.7 ±0.17	20	+29 $p < 0.01$	9.0 ±0.16	30	8.5 ±0.17	20	−4 n.s.	—		—		—
Cell size (μ^2)	Ear	—		—		—	—		—		—	685.7 ±9.77	56	600.3 ±3.76	76	−12 n.s.
	Sole	—		—		—	—		—		—	866.5 ±7.37	157	781.5 ±4.48	154	−10 n.s.
Cell number/mm surface length	Ear	74.6 ±1.84	13	59.3 ±3.34	18	−21 $p < 0.01$	39.8 ±2.26	13	28.6 ±2.14	18	−28 $p < 0.01$	—		—		—
	Sole	86.1 ±3.04	13	79.5 ±4.09	18	−8 n.s.	145.9 ±7.15	13	125.4 ±5.29	18	−14 $p < 0.05$	—		—		—
Cell number beneath each corneocyte	Ear	9.5 ±0.84	12	7.1 ±0.22	16	−24 $p < 0.01$	5.1[a]		3.4[a]		−33	9–11	7	12–18	5	+50
	Sole	13.4[a]		12.4[a]		−8	22.7[a]		19.5[a]		−14	35–41	5	38–44	4	+8
Cell loss after hydrocortisone/mm surface length	Ear			15.3					11.2							
	Sole			6.6					20.4							
Cell loss/corneocyte after hydrocortisone treatment	Ear			2.4					1.7							
	Sole			1.0					3.2							

[a] Calculated

TABLE II. *Epidermal thickness and cell number in two different body sites after 3 weeks of systemic hydrocortisone treatment*

		Control N = 13	Treated N = 18	% Change
Thickness of epidermis	Ear	13.4 ± 0.63	10.8 ± 0.50	−19 $p < 0.01$
	Sole	37.4 ± 1.46	30.6 ± 0.89	−18 $p < 0.01$
Ratio basal lamina: surface length	Ear	1.00:1	1.00:1	0
	Sole	1.22:1	1.03:1	−16
Total number of living cells/mm surface length[a]	Ear	114.4	87.9	−23
	Sole	232.0	204.9	−12
Total number of living cells/corneocyte[a]	Ear	14.6	10.5	−28
	Sole	36.1	31.9	−12
Total cell loss/mm surface length[a]	Ear	—	26.5	—
	Sole	—	27.0	
Total cell loss/corneocyte[a]	Ear	—	4.1	—
	Sole	—	4.2	

[a] Taken from Table I

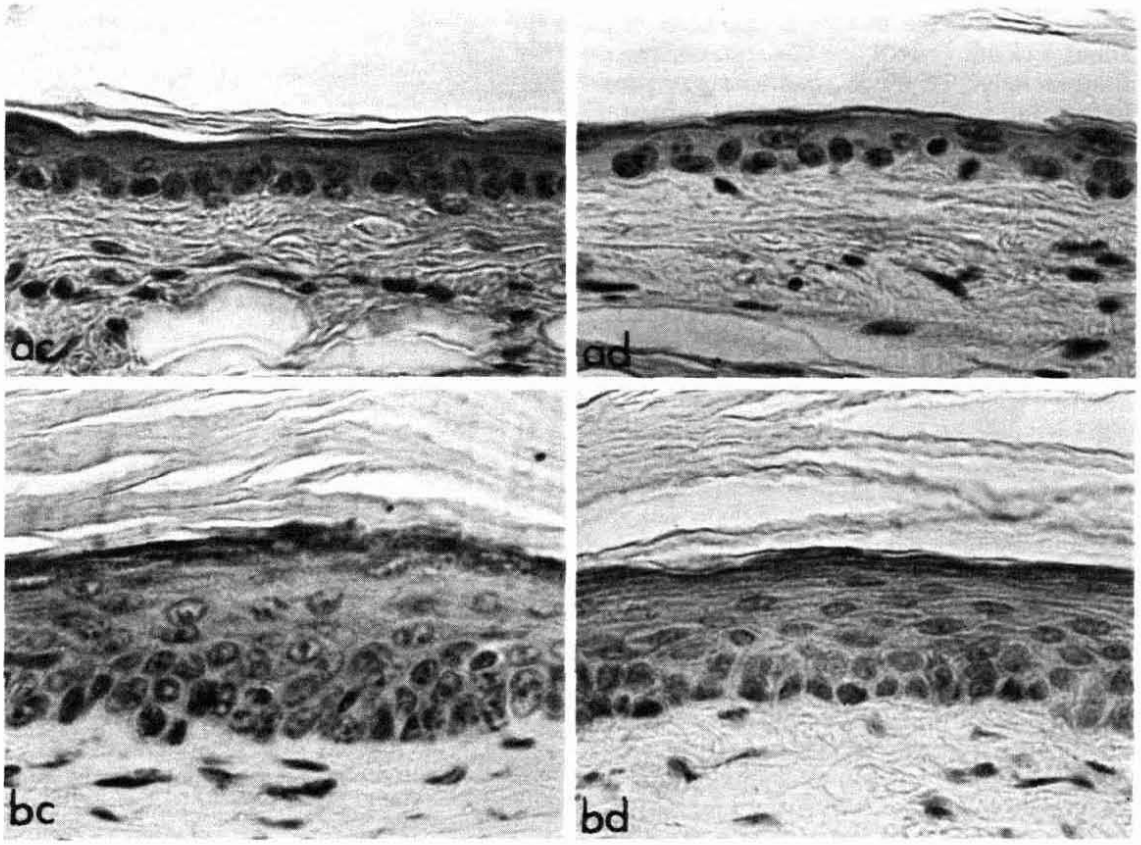

FIG. 1. Photomicrographs of sections of ear (*a*) and sole (*b*) epidermis of control animals (*c*) and those treated (*d*) with hydrocortisone for 3 weeks to show the thinning in the epidermis and reduction in cell number (× 550).

creased with treatment (Tab. I), while only in the ears did a significant increase in the number of cornified cells occur. The slight decrease in the corneocyte size probably represented a decrease in the amount these cells overlapped each other, rather than a reduction in the number per surface length of epidermis. Expressing the results per unit surface length as well as per corneocyte indicates that there was an actual loss of cells from the living compartment.

As a consequence of these changes the thickness of the living epidermis in both body sites decreased (Tab. II). The extent of this thinning was found to be the same in both tissues although the epidermis of the sole is approximately 3 times thicker than that of the ear (see Fig. 1). In the ears the ratio between the length of the basal lamina and the surface is 1:1 (which means the epidermis is flat); the change in the ratio in the sole epidermis reflects a reduction in the small undulations of the basal layer with hydrocortisone treatment (Fig. 1). The striking feature of the cell loss from the nonkeratinized compartments, i.e., Kb + Kc, is shown in Table II. It can be seen that the total number of living cells lost during treatment is the same for both epithelia although the proportion varies for each tissue (23% for ears and 12% for feet).

Cell Cycle Parameters

Since the mice were kept entirely free from stress and also because the treatment was applied in the drinking water, extraneous factors interfering with the kinetics of cell growth and their measurement could be excluded. As much kinetic data as possible were obtained using conventional methods of measurement and autoradiography. The determinations of labeling index (Fig. 2), median S-phase length from PLM curves (Figs. 3 A, B), mitotic index (Figs. 3 A,B), and mitotic duration (Tab. III) are summarized in Table IV.

In the two epithelia studied, cell proliferation occurs in the basal layer only, i.e., the proliferative pool is contained in the basal layer, although in ears not all the cells in this layer belong to the the proliferative pool [5,9]. It is therefore important to take this fact into consideration when determining labeling index, etc., otherwise misinterpretation of data follows, as shown in Table V. If expressed per number of basal cells, the labeling index is actually increased in the ear epidermis after hydrocortisone treatment (18%) and minimally increased in the sole of foot (5%) (Tab. V). This is also true if it is expressed per number of living cells although the differences are not so pronounced. Since the formation of one corneocyte is the ultimate fate of one basal cell produced from the cell division process, expressing all the kinetic results per corneocyte is obviously the best method to adopt. Thus, by taking measurements per unit surface length, any further inhomogeneity in the basal layer caused by the treatment was accommodated as the growth

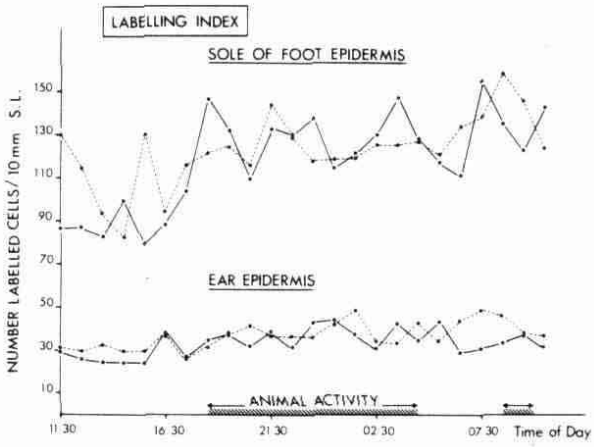

FIG. 2. Labeling index as expressed per 10-mm surface length throughout 24 hr of the experiment. Each point represents the average of 5 animals but the standard errors, which were never greater than ± 20%, have been omitted for clarity. ·····-····· = Control; ·—·—· = hydrocortisone-treated animals.

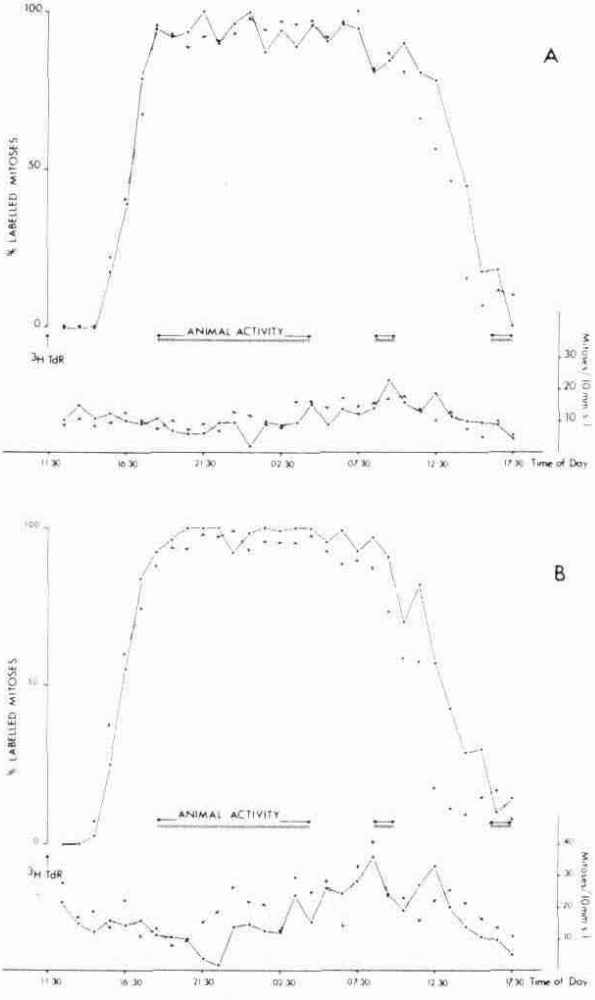

FIG. 3. *A*: Ear. *B*: Sole of foot. PLM curves and number of mitoses in each hour during the 30 hr of the experiment. Each point represents the average of 5 animals but the standard errors have been omitted for clarity. ·····-····· = Control; ·—·—· = hydrocortisone-treated animals.

TABLE III. *Number of mitoses with and without Colcemid during the 4-hr sleep period 11:30–15:30 after 3 weeks of systemic hydrocortisone treatment*

		Time of day		Control	N	Treated	N
Number of mitoses/10-mm surface length (no Colcemid)	Ear	12:30	M_1	8.4 ± 0.61	10	8.6 ± 0.55	10
		13:30	M_2	8.5 ± 0.34	10	5.9 ±0.22	10
		14:30	M_3	8.4 ± 0.30	10	7.4 ± 0.55	10
		15:30	M_4	8.4 ± 0.20	10	7.6 ± 0.49	10
	Sole	12:30	M_1	43.6 ± 1.40	10	33.2 ± 1.73	10
		13:30	M_2	34.5 ± 3.36	10	31.1 ± 1.02	10
		14:30	M_3	30.7 ± 2.10	10	31.5 ± 1.62	10
		15:30	M_4	39.9 ± 1.78	10	33.5 ± 1.93	10
Number of mitoses arrested by Colcemid/10-mm surface length during the hours 11:30–15:30	Ear	11:30–15:30	M_c	10.4 ± 0.72	10	9.2 ± 0.91	10
	Sole		M_c	37.9 ± 2.60	10	37.1 ± 2.09	10
Duration of mitoses during time 11:30–15:30 $\frac{M_1 + M_2 + M_3 + M_4}{M_c}$	Ear	11:30–15:30		3.2 hr		3.2 hr	
	Sole			3.9 hr		3.5 hr	

TABLE IV. *Cell kinetic parameters of the epidermis from two different body sites after 3 weeks of treatment with systemic hydrocortisone*

		Control	N	Treated	N	% Change
Median length of S-phase (hr)	Ear	20.2		21.5		0
	Sole	19.7		20.7		0
Number of labeled cells/10-mm surface length	Ear	36.6 ± 0.82	118	34.6 ± 0.75	111	−5[a]
	Sole	122.0 ± 1.97	118	118.6 ± 2.47	111	−3[a]
Duration of mitosis over 4-hr sleep period (hr)	Ear	3.2		3.2		0
	Sole	3.9		3.5		0
Number of mitoses/10-mm surface length	Ear	11.7 ± 0.48	111	11.6 ± 0.60	99	−1[a]
	Sole	17.4 ± 0.91	111	17.8 ± 0.94	99	+2[a]

[a] Not significant statistically

TABLE V. *Comparison of results using various methods for estimating labeling and mitotic index using data in Tables I, II, and IV*

		% Change after 3 weeks of systemic hydrocortisone treatment	
		Ears	Soles
Per 100 basal cells	Labeling index	+18	+5
	Mitotic index	+33	+11
Per 100 living cells	Labeling index	+21	+9
	Mitotic index	+30	+29
Per corneocyte	Labeling index	−11	−3
	Mitotic index	−7	+16
Per 10-mm surface length	Labeling index	−4	−3
	Mitotic index	−1	+1

fraction remained constant despite changes in basal layer length and cellular content. This has been discussed previously [10] but is particularly relevant here. Table V shows the consistency in results obtained from mitotic index and labeling index using this method. (Change in labeling index is −4% and in mitotic index is −1% for ear epidermis after treatment, and −3% and +1%, respectively, for sole of foot—all of which are insignificant differences.)

Labeling and mitotic indices were taken at hourly intervals throughout the 24 hr so that the diurnal rhythm could be accommodated [11]. It was conceivable that this rhythm could have been affected by the treatment [12] or the rhythm could have caused a temporary reduction in the uptake of [^{3}H]TdR into the cells, causing artifactual results [13]. Indeed, Figures 2, 3, and 4 show that "spot checks" on both labeling and mitotic indices taken at appropriate times of the day could have given a significant stimulus or depression as a result of the treatment.

It may be noted that the overall shape of the PLM curves was the same in the normal and the treated animals as well as being the same for the two tissues (slight increase in the length recorded in experimentally treated animals is within the range of differences expected for control animals). The duration of mitosis was of the same order for both tissues (Tab. III). It has always been assumed that a large number of labeled cells is accompanied by short individual phases of the cell cycle. In sole epidermis the labeling index is nearly 3½ times higher than in ear epidermis. Therefore the results recorded here show that the length of S-phase and mitosis cannot be correlated directly with labeling index and mitotic index but is indicative of a larger proliferative pool in the sole of foot epidermis.

The PLM method of estimation of S-phase length is properly applicable to asynchronous populations of cells. In a semisynchronous population such as epidermis, any method of estimating length of S is subject to controversy. Thus values appear in the literature ranging from 5.5 to 30 hr for epidermis [14]. Whatever the method used, the length of S-phase for ear and sole of foot is comparatively long [15,16]. In this study the [^{3}H]TdR was injected at a time when maximum length of S would be recorded [11,17]. Also, S-phase length using direct measurement from the 50% level on the PLM curves may be an overestimation due to the fact that the unincorporated [^{3}H]TdR "pool" is available in the epidermis for 2 hr after injection and therefore is not strictly "flash-labeling" (unpublished observations). Nevertheless, these criticisms apply to both experimental and control animals so that the comparison is still valid. Furthermore, [^{3}H]TdR was always administered during the sleep period. In consequence, the PLM curve approximated to 100% on the plateau, indicating no restriction in the uptake of [^{3}H]TdR caused by either the diurnal rhythm in the control animals or by the treatment in the experimental mice. If the PLM curve did not plateau at approximately 100% (as previously recorded where topical application of corticosteroids have been used [18]), it could indicate that, at the time of injection, the [^{3}H]TdR was impeded from entering the cells and a low value would be recorded in the labeling index. From this it may be realized that the labeling index recorded in these experiments is a true value.

The outstanding and unexpected conclusion that can be drawn from the results is that none of the measured kinetic parameters in either body site has been altered by the treatment whereas the same number of cells per unit has been lost from both epithelia.

DISCUSSION

There is general agreement that one of the main effects of glucocorticoids is a reduction in epidermal thickness [19]. The precise mechanism, however, has not been adequately determined. It is well known that glucocorticoids inhibit cell proliferation in lymphocytes and fibroblasts [20], and in epidermis similar effects have been assumed to take place [21–24]. Fluorinated steroids have been shown to decrease epidermal mitotic activity [3] or [^{3}H]TdR incorporation [18] although hydrocortisone had no effect when applied topically. Epidermal cell proliferation has recently been found to be insensitive to the action of hydrocortisone in culture ([18], unpublished observations), but there are reports that small amounts of the hormone caused enhancement of keratinization in cultured embryonic chick skin [25]. Regional variation in response has also been reported, e.g., fluocinolone acetonide applied under occlusive dressing for 3 weeks caused a decrease in the number of [^{3}H]-TdR-labeled cells in the human back epidermis while in the forehead no such effects were observed [26].

A complete correlation of tissue growth and differentiation with prolonged corticosteroid treatment has not been attempted before. Also, in this respect, a direct comparison has not been made between two vastly differing epidermal body sites in the same animals. It came as a surprising finding that in the sole epidermis, which is nearly 3 times thicker and has a rather short turnover rate [7], the actual lengths of S, G_2, and M-phases of the cell cycle are the same as in ear epidermis (Tab. IV). It is obvious that kinetically the only difference between the two tissues consists in a greater number of cells participating in growth in the soles (growth fraction). The reasons for this difference remain unknown.

In this study it is shown that the rather high dose of hydrocortisone administered orally for 3 weeks did not change the kinetic parameters of cell proliferation. Neither the length of S, nor mitosis, nor the absolute number of cells engaged in DNA synthesis (labeling index) was affected by the administered hormone which in adult man corresponds to 170 mg per day. Also the length of G_1

phase appears not be be influenced. These conclusions are further substantiated by the comparison of the two body sites where naturally occurring extremes of epidermal thickness and proliferative activity are found (Tabs. II, IV).

On the other hand, in both body sites the absolute number of living epidermal cells per unit is reduced to the same extent after treatment, namely, 26.5 cells in the ear and 27.0 cells in the sole epidermis, calculated per mm epidermal surface length (Tab. II). When calculated per corneocyte, 4.1 cells have disappeared from the epidermal, living cell population compared to 4.2 cells in the sole epidermis. This implies that, regardless of the body site, the effect of treatment is the same in both tissues. This indicates that the susceptibility of differentiating epidermal cells to the hormone is similar in both body sites. By this action the absolute number of cells affected by the hormone is the same regardless of the total number of cells located in a particular body site.

Our observations show that hydrocortisone under the conditions of these experiments causes a considerable change in the rate of epidermal maturation (differentiation). In epidermis this seems to be one of the main actions of the drug. The method of counting [^{3}H]TdR labeled cells or mitoses in order to ascertain specific drug effects in epidermis obviously is of restricted value. In the case of corticosteroids the inhibitory action upon epidermal cell replication appears to be nonspecific. Thus, a more detailed analysis of the mode of corticosteroid action on epidermis should include measurements on the rate of differentiation. At present, quantitation of the tissue compartments seems to be one adequate method. The results reported here show that it is a mistake to disregard the rate of epidermal cell maturation.

The excellent technical assistance of Mrs. S. Knibb and Frl. B. Klahre is gratefully acknowledged.

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