Stimulation of epidermal protein synthesis *in vivo* by topical triamcinolone acetonide

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The rate of epidermal protein synthesis *in vivo* was determined in the hairless mouse by a method in which a large dose of [³H]phenylalanine (150 μ mol/100 g body wt.) is administered via the tail vein. The epidermal free phenylalanine specific radioactivity rapidly rose to a plateau value which by 10 min approached that of plasma, after which it declined. This dose of phenylalanine did not of itself alter protein synthesis rates, since incorporation of co-injected tracer doses of [³H]lysine and [¹⁴C]threonine was unaffected. The fractional rate of protein synthesis obtained for epidermis was 61.6%/day, whereas values for liver and gastrocnemius muscle in the same group of mice were 44%/day and 4.8%/day respectively. When expressed on the basis of RNA content, the value for epidermis (18.6 mg of protein/day per mg of RNA) was approx. 3-fold higher than those for liver and gastrocnemius muscle. Topical administration of 0.1% triamcinolone acetonide increased the epidermal fractional protein synthesis rate by 33% after 1 day and by 69% after 7 days, compared with vehicle-treated controls. These effects were entirely accounted for by the increase in protein synthesis rates per mg of RNA. RNA/protein ratios were unaffected by this treatment.

INTRODUCTION

Mammalian epidermis consists of a number of distinct cell layers, which represent steps in the keratinocyte differentiation process terminating in cornification. The innermost layer, adjacent to the dermis, consists of basal cells which constitute the proliferative population of keratinocytes. Differentiation of these cells produces the next identifiable stratum of spinous keratinocytes; these non-proliferating cells constitute the major portion of the epidermis, and contain an abundance of well-organized tonofilaments. The next stage of morphological differentiation is the granular cell, characterized by the presence of basophilic keratohyaline granules. Terminal differentiation of these cells forms the stratum corneum, consisting of many tightly packed layers of non-viable squamous corneocytes (for a review, see Odland, 1983).

The keratins are the most abundant structural proteins of the epidermis, and are present as insoluble 8.0 nm filaments in the cytoskeleton. Qualitative studies of epidermal protein synthesis have largely focused on these proteins, which consist of a number of polypeptides of M_r range 40 000–67 000 (Tezuka & Freedberg, 1972; Baden *et al.*, 1973; Steinert & Idler, 1975; Fuchs & Green, 1978). It has been shown that during the course of differentiation of normal human epidermis there is a profound change in the pattern of keratins synthesized (Fuchs & Green, 1980). Furthermore, this change occurs at the level of transcription in both human (Fuchs & Green, 1979) and murine (Roop *et al.*, 1983) epidermis.

In contrast with these qualitative studies of epidermal protein synthesis, the regulation of the rate of epidermal protein synthesis remains poorly understood, in large part because appropriate methods have not been developed for this tissue. In particular, it is a requirement of any radiochemical method to be used for the

measurement of absolute rates of protein synthesis *in vivo* that the specific radioactivity of the precursor pool of amino acid be known over the period of incorporation. It has been shown for a variety of tissues that the use of tracer doses of radiolabelled amino acid results in a wide disparity between amino acid specific radioactivities in the more accessible compartments (plasma, total intracellular pool), and between these compartments and aminoacyl-tRNA (Waterlow *et al.*, 1978). These differences arise from the dilution of the exogenous labelled amino acid by endogenous amino acid in these compartments. As a consequence, the rate of incorporation of labelled amino acid *in vivo* in such tracer-dose studies will depend not only on the rate of protein synthesis but also on the dilution of administered amino acid at the site of synthesis. Since the contribution of the different amino acid pools to total tissue protein synthesis is not known with certainty, methods involving the use of tracer doses of radioactivity do not permit calculation of absolute rates of protein synthesis *in vivo*. This difficulty cannot be avoided by expressing values as the specific radioactivity of incorporated amino acid, i.e. as relative rates of synthesis, since in this case it must be assumed that the amino acid precursor pool(s) are not affected by the experimental conditions.

In previous studies of epidermal protein synthesis *in vivo*, tracer doses of amino acid have been used to determine relative rates of epidermal protein synthesis from tissue protein specific radioactivity (Freedberg & Baden, 1962) and from grain counts obtained from autoradiographs (Fukuyama & Epstein, 1966, 1975). In the present paper we show that the injection of a large dose of labelled phenylalanine results in the 'flooding' of epidermal amino acid pools, allowing for calculation of the absolute rate of epidermal protein synthesis. In addition, we have investigated the effect on epidermal protein synthesis of topical administration of triamcinol-

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one acetonide, a potent synthetic glucocorticoid, in an effort to understand better the mechanisms by which steroids act on this tissue.

EXPERIMENTAL

Materials

L-[4-³H]Phenylalanine, L-[4,5-³H]lysine and L-[U-¹⁴C]-threonine were obtained from Amersham International. Ninhydrin, L-leucyl-L-alanine, L-tyrosine decarboxylase, pyridoxal 5'-phosphate, *Escherichia coli* tRNA and *E. coli* aminoacyl-tRNA synthetase were purchased from Sigma Chemical Co. Fluorescamine was obtained from Aldrich Chemical Co. Triamcinolone acetonide (0.1%) in a hydrophilic cream vehicle (Aristocort A) and cream vehicle alone (Aquatain) were kindly provided by Lederle Laboratories, Pearl River, NY, U.S.A. The water-based cream contained stearyl alcohol, isopropyl palmitate, glycerol, sorbitol, lactic acid and 2% benzyl alcohol.

Experimental animals

Male hairless mice (hr/hr Balb/c; Temple University) were maintained on a standard diet and weighed 25–30 g when experiments were performed. Approx. 0.2 g of 0.1% triamcinolone acetonide cream was applied twice a day (09:00–10:00 h and 17:00–18:00 h) to two groups of six mice, for either 1 day or 7 days. This was sufficient material to cover the trunk with little or no excess. A third, control, group of six mice was not treated, and all animals were killed on the same day. In a separate experiment, a group of five mice was treated with vehicle alone twice a day for 7 days as described above, and a control group of five mice was not treated.

Protein synthesis measurement

The experimental protocol for protein synthesis determination *in vivo* was a modification of that described by Garlick *et al.* (1980). [4-³H]Phenylalanine was evaporated to dryness and dissolved in 150 mM unlabelled phenylalanine to approx. 100 μ Ci/ml. Mice were restrained in a plastic cylinder and injected via a lateral tail vein with 1.0 ml of the [³H]phenylalanine solution/100 g body wt. In tracer-dose experiments, solutions were prepared containing 50 μ Ci of [³H]lysine/ml plus 15 μ Ci of [¹⁴C]-threonine/ml in either 0.9% (w/v) NaCl (control) or 150 mM unlabelled phenylalanine. At the appropriate time after injection, mice were killed by cervical dislocation, and epidermis was removed from both dorsal and ventral surfaces with a Castraviejo keratome (Storz Microinstrument Co., St. Louis, MO, U.S.A.) set to cut at a depth of 0.1 mm. Although some dermis was always present in these samples, microscopic examination of frozen sections showed that the entire epidermis was excised and that at least 80% of the cells obtained were epidermal. The tissue (50–100 mg) was then immediately frozen in liquid N₂, approx. 45 s after cervical dislocation, and 20–50 μ l of blood was transferred from the open chest to a heparinized micro-centrifuge tube on ice. The blood samples were spun in an Eppendorf micro-centrifuge for 5 min, and the supernatant was transferred to 10 vol. of cold 2% (v/v) HClO₄. The tubes were vortex-mixed, centrifuged for 10 min, and HClO₄ was removed from the supernatant by addition of $\frac{1}{2}$ vol. of saturated potassium citrate and centrifuging down the

KClO₄ precipitate. The resulting plasma phenylalanine sample, pH $\sim$ 6.0, was stored at -20°C .

In some experiments, gastrocnemius muscle and liver were obtained in addition to epidermis. Hindlimbs were excised immediately after cervical dislocation and placed in ice/water for about 45 s. Gastrocnemius muscles were dissected free and frozen in liquid N₂. The liver was removed immediately after the hindlimbs, frozen in liquid N₂, and then epidermis and blood samples were obtained as described above. All tissue samples were stored at -80°C .

Frozen epidermis was ground to a powder in a pestle and mortar under liquid N₂, transferred to 2 ml of 2% ice-cold HClO₄, weighed, and homogenized in a Polytron homogenizer. The Polytron head was washed with 0.5 ml of 2% HClO₄, and the combined homogenate and wash was centrifuged at 2000 g for 10 min. The supernatant, containing free (unincorporated) phenylalanine, was adjusted to pH 6–6.5 by addition of $\frac{1}{2}$ vol. of saturated potassium citrate and, after centrifugation (2000 g for 20 min), the supernatant was stored at -20°C . This fraction was used to determine free phenylalanine specific radioactivity. The protein pellet was resuspended in 5 ml of 0.3 M-NaOH, vortex-mixed for 40 s and incubated at 37 $^{\circ}\text{C}$ for 1 h. The alkali-treated material was centrifuged at 2000 g for 10 min, resulting in an alkali-soluble supernatant fraction and an alkali-insoluble pellet. Supernatant (4 ml) was transferred to 2 ml of 20% HClO₄ on ice, mixed, and centrifuged at 1000 g for 10 min. The protein pellet was washed with 3 $\times$ 5 ml of 2% HClO₄ and hydrolysed for 24 h in 6 M-HCl at 110 $^{\circ}\text{C}$. The resulting hydrolysate was evaporated, dissolved in 5 ml of water, re-evaporated to remove HCl and dissolved in 1.2 ml of water. A sample (0.2 ml) was then taken for determination of total amino acid content by the fluorescamine method (Udenfriend *et al.*, 1972), with glycine as standard. The remainder of the hydrolysate was adjusted to pH 6–6.5 with $\frac{1}{2}$ vol. of saturated potassium citrate, and used to determine the specific radioactivity of phenylalanine incorporated into protein (see below).

A 1 ml portion of the remaining 0.3 M-NaOH-digestion supernatant was added to 0.4 ml of 20% HClO₄ and the mixture centrifuged for 20 min at 2000 g. The supernatant was then used for RNA determination by the method of Munro & Fleck (1969), assuming that 32.5 A₂₆₀ units are equivalent to 1 mg of RNA. A correction for peptide absorption at 260 nm in the HClO₄ supernatant was applied (Munro & Fleck, 1969), based on peptide content measured by the method of Lowry *et al.* (1951), with bovine serum albumin as standard. The alkali-soluble protein pellet was redissolved in 1 ml of 0.3 M-NaOH and the protein content determined by the method of Lowry *et al.* (1951), with albumin as standard. The alkali-insoluble epidermal protein fraction was washed with 5 $\times$ 5 ml of 0.3 M-NaOH, hydrolysed in 6 M-HCl and then treated as described above for the alkali-soluble protein fraction.

A similar procedure was used for extraction and analysis of liver and muscle, except that Polytron homogenization was omitted and liver RNA was estimated from absorption of HClO₄ supernatants at 260 nm and 232 nm as described by Fleck & Begg (1965).

The specific radioactivity of [³H]phenylalanine in samples obtained from plasma, tissue HClO₄-soluble and

protein fractions was determined after decarboxylation of phenylalanine to phenethylamine and extraction into heptane and 10 mM- H_2SO_4 , as described by Garlick *et al.* (1980). Radioactivity in 1.0 ml portions of H_2SO_4 extracts was measured by scintillation counting, and the remainder was used for phenethylamine assay by a method based on that of Suzuki & Yagi (1976). Samples (0.1–0.5 ml) were incubated for 1 h at 60 °C in a reaction mixture containing 250 mM-potassium phosphate, 10 mM-ninhydrin and 0.2 mM-leucylalanine. Appropriate standards of phenylethylamine in 10 mM- H_2SO_4 were included. After incubation, tubes were brought to room temperature and fluorescence was determined with an Aminco-Bowman SPF fluorimeter modified to accept a ratio photometer (excitation 390 nm, emission 495 nm).

RESULTS

Fig. 1 shows that the intravenous administration of a large dose (150 $\mu\text{mol}/100\text{ g}$ body wt.) of [^3H]-phenylalanine resulted in a steady decline in plasma specific radioactivity over 30 min. In contrast, the specific radioactivity of epidermal free phenylalanine rose to a constant value within 1.5 min and declined significantly after 10.75 min, when the epidermal specific radioactivity was approx. 80 % of that in plasma.

During the epidermal extraction procedure, a fraction was obtained which did not dissolve in 0.3 M-NaOH after 1 h at 37 °C. In a preliminary experiment using epidermis obtained 10 min after injection of labelled phenylalanine, this alkali-insoluble fraction was hydrolysed in 6 M-HCl, and protein content and radioactivity were determined as described in the Experimental section. No radioactivity was detectable, and the percentage of total epidermal protein content in this fraction was very small ($0.56 \pm 0.08\%$; $n = 6$). As a result of this negligible contribution by alkali-insoluble protein, only alkali-soluble protein was hydrolysed in subsequent experiments.

Fig. 1. Time course of plasma and epidermal free phenylalanine specific radioactivities after injection of a large dose of L-[4- ^3H]phenylalanine into hairless mice

L-[4- ^3H]Phenylalanine [150 μmol (100 μCi)/100 g body wt.] was injected via the tail vein into 25–30 g mice. The specific radioactivities of free [^3H]phenylalanine in plasma (○) and epidermis (●) were determined at various times after injection, as described in the Experimental section. Each point represents the mean $\pm$ S.E.M. for a group of five or six mice.

The fractional rate of protein synthesis, K_{si} , was calculated from the specific radioactivity of phenylalanine incorporated into protein (S_{p}) and the average specific radioactivity of free phenylalanine in the tissue over 10 min (S_{i}), according to the equation given by McNurlan *et al.* (1979):

$$K_{\text{si}} = \frac{S_{\text{p}} \times 100}{S_{\text{i}} \times t} (\%/ \text{day})$$

where t = incorporation time in days.

Since extracellular amino acid derived from plasma may contribute directly to the protein synthesis precursor pool (Waterlow *et al.*, 1978), fractional synthesis rates were also calculated by using the mean plasma phenylalanine specific radioactivity (S_{p}). The resulting values for fractional synthesis rate (K_{sp}) were lower than those calculated from tissue specific radioactivity (K_{si}) because flooding of intracellular amino acid was not complete (i.e. $S_{\text{i}} < S_{\text{p}}$). It would clearly be preferable to calculate protein synthesis rates from the mean tissue aminoacyl-tRNA specific radioactivity over the incorporation period, thereby avoiding uncertainties in the amino acid precursor-pool values. However, difficulties in the determination of the phenylalanyl-tRNA specific radioactivity in the small epidermal samples obtained here (500–100 mg) preclude this approach. It has been shown that approx. 80 % of tissue RNA is ribosomal (Henshaw *et al.*, 1971), so that protein synthesis rates expressed on the basis of RNA content provide a measure of the synthetic efficiency of epidermal ribosomes. The RNA content of the tissue is given by the RNA/protein ratio.

The incorporation of [^3H]phenylalanine into epidermal protein, expressed as the percentage of epidermal protein synthesized by using S_{p} values, was found to proceed linearly with time up to 30 min after intravenous injection (results not shown). The data given in Table 1 indicate that the intravenous administration of the dose of phenylalanine employed for the determination of epidermal protein synthesis rates (150 $\mu\text{mol}/100\text{ g}$ body wt.) did not affect the incorporation of tracer doses of co-injected [^3H]lysine and [^{14}C]threonine over 10 min.

The results of estimations of protein synthesis in epidermis, gastrocnemius muscle and liver from the same group of mice are given in Table 2. Tissues were analysed after a 10 min incorporation period *in vivo* with [^3H]phenylalanine. Epidermal K_{si} values were somewhat higher than those from liver, whereas K_{sp} values were comparable; both tissues showed markedly higher values than those obtained for gastrocnemius muscle. Epidermal protein synthesis rates expressed on an RNA basis were approx. 3-fold higher than those of liver and muscle when calculated from K_{si} and RNA/protein ratios as shown, and were twice those of muscle and liver when K_{sp} values were used.

Table 3 shows that topical administration of 0.1 % triamcinolone acetonide cream significantly increased epidermal fractional synthesis rates after 1 day and that this stimulation was more marked after 7 days of treatment. Similar increases in protein synthesis rates were obtained when calculated on the basis of RNA content, and this is implied by the finding that RNA/protein ratios were unaffected by 1 or 7 days of steroid treatment. Table 3 also shows that the application of cream vehicle alone to the skin for 7 days did not affect either fractional protein synthesis rates or RNA/protein ratios.

Table 1. Effect of administration of a large dose of unlabelled phenylalanine on the incorporation of tracer doses of co-injected L-[³H]lysine and L-[¹⁴C]threonine

Hairless mice (25–30 g) were injected (per 100 g body wt.) with 1.0 ml of a solution of [¹⁴C]threonine and [³H]lysine in either 0.9 % NaCl (control) or 150 mM unlabelled phenylalanine. The animals were killed 10 min thereafter, and radioactivity incorporated into epidermal protein was measured as described in the Experimental section. The differences between values obtained for control and phenylalanine-treated groups were not significant.

	[³ H]Lysine		[¹⁴ C]Threonine	
	(d.p.m./μg of RNA)	(d.p.m./mg wet wt.)	(d.p.m./μg of RNA)	(d.p.m./mg wet wt.)
0.9 % NaCl	57.9 ± 4.0	148 ± 13	8.14 ± 0.57	20.9 ± 2.2
150 mM-Phenylalanine	58.8 ± 4.2	125 ± 16	8.45 ± 0.85	17.8 ± 2.1

Table 2. Rates of protein synthesis *in vivo* in mouse epidermis, liver and gastrocnemius muscle

Six hairless mice (25–30 g) were injected intravenously with a large dose of [³H]phenylalanine and killed after a 10 min incorporation period *in vivo*. Preparation and analysis of tissue were as described in the Experimental section. Values given are means ± S.E.M.

	Fractional synthesis rate (%/day)		Protein synthesis (mg/day per mg of RNA)	RNA/protein ratio (mg/g)
	<i>K_{st}</i>	<i>K_{sp}</i>		
Epidermis	61.6 ± 4.5	35.8 ± 1.9	18.6 ± 2.8	35.5 ± 3.9
Liver	44.0 ± 2.7	41.9 ± 2.5	5.7 ± 0.3	77.9 ± 3.9
Gastrocnemius muscle	4.8 ± 0.5	4.1 ± 0.5	6.2 ± 0.5	7.7 ± 0.4

Table 3. Effect of topical application of 0.1 % triamcinolone acetonide cream, and of cream alone, on mouse epidermal protein synthesis *in vivo*

Groups of six hairless mice (25–30 g) were treated topically with 0.1 % triamcinolone acetonide in cream vehicle for 1 or 7 days, or were left untreated for 7 days (Expt. 1). In a separate experiment, mice were treated with vehicle for 7 days or left untreated (Expt. 2). Protein synthesis rates were then determined after a 10 min period *in vivo* of incorporation of [³H]phenylalanine as described in the Experimental section; tissue free phenylalanine specific radioactivity was used to calculate protein synthesis/RNA. Results are means ± S.E.M. Significance of differences from appropriate control: **P* < 0.05, ***P* < 0.01, ****P* < 0.001. Values in parentheses are percentages of normal controls.

	<i>K_{st}</i> (%/day)	<i>K_{sp}</i> (%/day)	Protein synthesis/RNA (μg/μg of RNA)	RNA/protein (μg/mg)
Expt. 1				
Normal	77 ± 7 (100)	48.8 ± 1.2 (100)	17.0 ± 2.2 (100)	46.7 ± 2.5 (100)
1-Day steroid	103 ± 10* (133)	55.6 ± 2.8* (114)	23.4 ± 2.0* (138)	43.9 ± 1.8 (94)
7-Day steroid	131 ± 14** (169)	67.2 ± 3.1*** (138)	29.5 ± 3.3** (174)	44.9 ± 1.7 (96)
Expt. 2				
Normal	68.1 ± 4.0 (100)	43.1 ± 3.0 (100)	22.4 ± 1.5 (100)	30.6 ± 1.7 (100)
7-Day cream vehicle	73.4 ± 5.8 (108)	44.3 ± 3.5 (103)	24.2 ± 2.9 (108)	31.0 ± 2.8 (101)

DISCUSSION

In this study we have shown that epidermal protein synthesis *in vivo* may be measured from the incorporation of a large dose of radioactively labelled phenylalanine into tissue protein over a 10 min incorporation period. In comparison with a labelling period of many hours, which has been employed in some studies of protein synthesis in

whole skin (Simon *et al.*, 1978; Davis *et al.*, 1981) and other tissues (for review see Waterlow *et al.*, 1978), the use of this relatively short period has the advantage that underestimation of protein synthesis owing to protein turnover during the incorporation period is minimized, i.e. synthesis of both short- and long-half-life proteins is measured. The finding that incorporation of labelled phenylalanine into epidermal protein proceeded linearly

for 30 min after intravenous administration suggests that there was no significant degradation of newly synthesized protein over the 10 min incorporation period of the standard assay, and thus that synthesis of total epidermal protein is measured by this method.

The administration of a large dose of amino acid is intended to flood all possible precursor pools of amino acid in the tissue, bringing their specific radioactivities to a similar value which can be determined over the period of incorporation (Henshaw *et al.*, 1971; Dunlop *et al.*, 1975). In contrast, the use of a tracer dose of labelled amino acid may result in a marked disparity between plasma and tissue free amino acid specific radioactivities, resulting in uncertainty in the precursor specific radioactivity at the site of synthesis and hence in fractional synthesis rates (Waterlow *et al.*, 1978). Furthermore, the single injection of a tracer dose of labelled amino acid results in a complex time course of plasma specific radioactivity, rendering calculation of protein synthesis rates very difficult (for discussion, see Garlick *et al.*, 1980). In the present study, we have shown that injection of 150 μmol of [^3H]phenylalanine/100 g body wt. results in a constant epidermal phenylalanine specific radioactivity over the 10 min incorporation period; at longer periods the values begin to fall, in response to the constantly diminishing plasma specific radioactivity. It is therefore possible to calculate fractional synthesis rates from both epidermal and plasma phenylalanine specific radioactivities (K_{st} and K_{sp} respectively). The greater disparity between K_{st} and K_{sp} values for epidermis as compared with muscle and liver (Table 2) is a consequence of lower tissue/plasma mean phenylalanine specific radioactivity ratios over the incorporation period. Possible explanations for this finding include (1) the presence of a larger epidermal phenylalanine pool and (2) differences in kinetics of amino acid flux into the tissue from plasma after administration.

The epidermis differs from most other tissues for which protein synthesis rates have been determined, including liver and muscle, in that it is not directly served by the vasculature, but obtains nutrients from the underlying dermal vascular bed. For this reason, and because a short incorporation time is employed for protein synthesis measurement, it is most important to demonstrate that maximal amino acid specific radioactivity is achieved rapidly after intravenous administration. The epidermal phenylalanine specific radioactivity rose to a maximum within 90 s of injection and remained at a plateau for approx. 10 min further (Fig. 1). Thus the assumption used in the calculation of protein synthesis rates, that the tissue amino acid specific radioactivity obtained 10 min after injection remains constant throughout the incorporation period, appears to be valid.

Since tissue concentrations of amino acid are of necessity elevated above physiological in this 'flooding dose' method, it is important to show that the procedure does not of itself affect rates of protein synthesis *in vivo*. This is implied by the finding that injection of 150 μmol of phenylalanine/100 g did not affect incorporation of either [^3H]lysine or [^{14}C]threonine; these values are taken as measures of relative rates of protein synthesis only, and cannot be used to calculate fractional synthesis rates, since the precursor-pool specific radioactivities were not known. These amino acids were chosen because their transport into epidermis is unlikely to be affected by elevated phenylalanine concentrations (Christensen,

1969); altered transport kinetics might affect incorporation of radioactivity into protein independently of changes in translation rate, since injection of a tracer dose of labelled amino acid results in a transient rise in plasma and tissue specific radioactivities (Henshaw *et al.*, 1971).

It has been suggested that protein synthesis rates expressed on an RNA basis are similar in all tissues (Millward *et al.*, 1981), i.e. that ribosomes from different tissues have similar synthetic efficiencies. This view implies that differences in protein synthesis rate among tissues simply reflect different ribosome contents. The data presented here clearly do not support this view; indeed, the relatively high epidermal fractional protein synthesis rate can in part be attributed to the fact that epidermal protein synthesis per mg of RNA is approx. 3-fold higher than that for liver and muscle (Table 2). Such differences in ribosomal efficiency have been shown by others. Thus protein synthesis per mg of RNA for whole skin in the rat was approx. 2-fold higher than that for liver and muscle (Preedy *et al.*, 1983), and Henshaw *et al.* (1971) showed that such values for liver greatly exceeded those for brain and testis. The assumption underlying the identity of protein synthesis rates expressed on an RNA basis and ribosomal efficiency is that almost all tissue RNA is ribosomal. Although not tested here for epidermis, this assumption has been validated for a variety of tissues, including liver (Henshaw *et al.*, 1971) and muscle (Young, 1970).

The data presented here show that the epidermis is a highly active tissue with respect to protein synthesis, with a fractional synthesis rate in the range 60–80 %/day in the hairless mouse. This is perhaps not a surprising finding, when it is considered that the epidermis is a tissue undergoing constant regeneration; the non-viable squamous cells of the superficial stratum corneum are continuously sloughed off (desquamation), to be replaced through terminal differentiation of the underlying viable keratinocytes. These cells in turn are constantly replenished by differentiation of the proliferative basal keratinocytes. Another continuously regenerating tissue, the small intestine, has also been shown to have a relatively high fractional synthesis rate of 87 %/day in the rat (McNurlan *et al.*, 1979).

It is of interest to consider epidermal protein turnover in the light of present knowledge of the process of differentiation in this tissue. The keratins, taken together as a class, are the most abundant protein constituent of the epidermis, representing approximately two-thirds of the total dry weight of bovine (Steinert & Idler, 1975) and human (Sun & Green, 1978) tissue. It is now well established that the complement of specific keratins expressed alters during keratinocyte differentiation *in vivo*, as a result of changes in the amounts of specific mRNA species (Fuchs & Green, 1979). It is evident therefore that keratinocyte differentiation requires the synthesis and degradation of a large portion of the constituent protein, i.e. substantial protein turnover must accompany tissue differentiation. Furthermore, Iversen *et al.* (1968) have shown that the time required for the complete differentiation of keratinocytes in hairless mouse epidermis, i.e. for conversion of basal cells into squamous cells, is approx. 3.5 days. Although the epidermal protein synthesis rate reported here is sufficient to account for this rate of keratinocyte differentiation, the finding that protein synthesis is not

markedly in excess suggests that protein metabolism may have a role in the regulation of keratinocyte differentiation. In particular, a marked increase in the rate of epidermal differentiation would be expected to be accompanied by an increase in epidermal protein synthesis.

The possibility that epidermal differentiation may be linked to protein turnover is further supported by the observation reported here that topically applied steroid stimulates epidermal protein synthesis *in vivo*. It has long been known that glucocorticoid administration (topical or oral) results in a decrease in epidermal thickness, or epidermal 'atrophy' (Winter & Wilson, 1976). Indeed, this side-effect limits the clinical utility of this class of drugs in dermatology. This phenomenon has most often been ascribed to anti-mitotic activity, since many studies have shown that glucocorticoids inhibit both mitosis and DNA synthesis in the epidermis (Hennings & Elgio, 1971; Marks & Williams, 1976). Anti-mitotic activity has been related to therapeutic efficacy in hyperproliferative skin diseases such as psoriasis (Fisher & Maibach, 1971). However, Laurence & Christophers (1976) have presented evidence from quantitative histological analysis that steroids act primarily to increase the rate of cell differentiation, with no effect on proliferation. This alternative mechanism is supported by morphological studies in which steroids have been found to enhance skin keratinization (Weismann & Fell, 1962; Spearman, 1964; Sugimoto *et al.*, 1974). Furthermore, the epidermis in psoriatic lesions is characterized by abnormal keratinization (parakeratosis), and clearance of the lesions in response to steroid treatment is accompanied by a return to normal keratinization, or orthokeratosis (Komisaruk *et al.*, 1962; McKenzie, 1963). Our finding that triamcinolone acetonide markedly increased epidermal protein synthesis rates is consistent with the view that steroids enhance epidermal differentiation, and provides further evidence that protein metabolism may play a role in the regulation of differentiation in this tissue. In contrast, our results are not consistent with the view that inhibition of cell division is the sole mode of action of steroids in normal epidermis.

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