Measurement of protein metabolism in epidermis and dermis

Xiao-Jun Zhang,1,2 David L. Chinkes,1,2 and Robert R. Wolfe1,2,3
1Metabolism Unit, Shriners Hospitals for Children; and Departments of 2Surgery and 3Anesthesiology, University of Texas Medical Branch, Galveston, Texas 77550

Submitted 24 October 2002; accepted in final form 21 February 2003

Zhang, Xiao-Jun, David L. Chinkes, and Robert R. Wolfe. Measurement of protein metabolism in epidermis and dermis. Am J Physiol Endocrinol Metab 284: E1191–E1201, 2003. First published February 25, 2003; 10.1152/ajpendo.00460.2002.—We found that, in the rabbit ear, the dermal protein contains 75.5% of cutaneous phenylalanine and 97.9% of cutaneous proline; the remaining 24.5% of phenylalanine and 2.1% of proline are in the epidermal protein. This finding led us to develop two novel models that use phenylalanine and proline tracers and the rabbit ear to quantify protein kinetics in the epidermis and dermis. The four-pool model calculates the absolute rates of protein kinetics and amino acid transport, and the two-pool model calculates the apparent rates of protein kinetics that are reflected in the blood. The results showed that both epidermis and dermis maintained their protein mass in the postabsorptive state. The rate of epidermal protein synthesis was 93.4 ± 37.6 mg·100 g−1·h−1, which was 10-fold greater than that of the dermal protein (9.3 ± 5.8 mg·100 g−1·h−1). These synthetic rates were in agreement with those measured simultaneously by the tracer incorporation method. Comparison of the four-pool and two-pool models indicated that intracellular cycling of amino acids accounted for 75 and 90% of protein kinetics in the dermis and epidermis, respectively. We conclude that, in the skin, efficient reutilization of amino acids from proteolysis for synthesis enables the maintenance of protein mass in the postabsorptive state.

stable isotopes; gas chromatograph and mass spectrometer; arteriovenous balance; fractional synthesis rate; rabbits

The skin is one of the largest tissues in the body. In normal circumstances, the skin maintains a constant protein mass, whereas skeletal muscle may have a significantly positive or negative protein balance (28). The homeostasis of skin protein mass plays a crucial role in preserving an intact barrier on the body surface. Once the integrity of the skin barrier is destroyed, several problems ensue immediately, such as loss of water and electrolytes, invasion of microorganisms, and impairment of immune functions (11, 19, 20). Thus knowledge of skin protein metabolism is important in understanding the maintenance and repair process of the skin barrier.

In our previous experiments (28–30), we investigated protein metabolism in normal and scalded skin by use of a rabbit ear model. This model has been the only approach to simultaneously quantify the in vivo rates of protein synthesis and breakdown as well as amino acid (AA) transport in the skin. However, this model is limited by the consideration of the skin as a single pool. It is well known that the epidermis and dermis have different structure, function, and turnover rates (12, 17, 25). The dermis serves as a basis for supporting and nourishing the epidermis, whereas the epidermis exerts the barrier function of the skin. It is therefore important to distinguish between the metabolic regulation of the epidermis and that of the dermis under physiological and pathological conditions.

The goal of this study was to establish an approach for quantitation of protein kinetics and AA transport in the epidermis and dermis. This was accomplished by measurement of phenylalanine (Phe) and proline (Pro) kinetics in rabbit ear skin. Because the epidermal and dermal proteins contain different amounts of Phe and Pro, the measured Phe and Pro kinetics are proportional to their contents in the epidermis and dermis. Taking advantage of the different contents of Phe and Pro in epidermal and dermal protein, we have developed models to compute the respective protein and AA kinetics in the epidermis and dermis. In addition, we modified a so-called heat treatment method (14) for the separation of epidermis and dermis, which allowed us to determine AA enrichment in the epidermal and dermal free and protein-bound pools. To estimate the validity of the model-derived values, we measured the fractional synthesis rate (FSR) of protein in the epidermis and dermis by use of the tracer incorporation method.

Glossary

Four-Pool Model

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>CA Phe</td>
<td>Arterial Phe concentration</td>
</tr>
<tr>
<td>CV Phe</td>
<td>Venous Phe concentration</td>
</tr>
<tr>
<td>CA Pro</td>
<td>Arterial Pro concentration</td>
</tr>
<tr>
<td>CV Pro</td>
<td>Venous Pro concentration</td>
</tr>
<tr>
<td>EA Phe</td>
<td>Arterial Phe enrichment</td>
</tr>
<tr>
<td>EV Phe</td>
<td>Venous Phe enrichment</td>
</tr>
<tr>
<td>EA Phe</td>
<td>Dermal free Phe enrichment</td>
</tr>
<tr>
<td>EV Phe</td>
<td>Epidermal free Phe enrichment</td>
</tr>
<tr>
<td>EA Phe</td>
<td>Arterial Pro enrichment</td>
</tr>
<tr>
<td>EV Phe</td>
<td>Venous Pro enrichment</td>
</tr>
<tr>
<td>EA Phe</td>
<td>Dermal free Pro enrichment</td>
</tr>
<tr>
<td>EV Phe</td>
<td>Epidermal free Pro enrichment</td>
</tr>
<tr>
<td>Cg Phe</td>
<td>Dermal protein-bound Phe content</td>
</tr>
<tr>
<td>Cg Phe</td>
<td>Epidermal protein-bound Phe content</td>
</tr>
</tbody>
</table>

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
PROTEIN KINETICS IN EPIDERMIS AND DERMIS

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>CDPro</td>
<td>Dermal protein-bound Pro content</td>
</tr>
<tr>
<td>CEPro</td>
<td>Epidermal protein-bound Pro content</td>
</tr>
<tr>
<td>BF</td>
<td>Blood flow rate in the ear skin</td>
</tr>
<tr>
<td>FInPhe</td>
<td>Arterial delivery of Phe to skin</td>
</tr>
<tr>
<td>FOutPhe</td>
<td>Venous exit of Phe from skin</td>
</tr>
<tr>
<td>NBnPhe</td>
<td>Net balance of Phe across skin</td>
</tr>
<tr>
<td>nBnPhe</td>
<td>Net balance of labeled Phe across skin</td>
</tr>
<tr>
<td>FvA Phe</td>
<td>Physiological shunting of Phe from artery to vein</td>
</tr>
<tr>
<td>FDAPhe</td>
<td>Transport of Phe from artery to dermis</td>
</tr>
<tr>
<td>FVD Phe</td>
<td>Transport of Phe from dermis to artery</td>
</tr>
<tr>
<td>FEDEPhe</td>
<td>Transport of Phe from epidermis to dermis</td>
</tr>
<tr>
<td>FODPhe</td>
<td>Irreversible loss of Phe from dermis (protein synthesis)</td>
</tr>
<tr>
<td>FDO Phe</td>
<td>Production of Phe in dermis (protein breakdown)</td>
</tr>
<tr>
<td>FPEPhe</td>
<td>Irreversible loss of Phe from epidermis (protein synthesis)</td>
</tr>
<tr>
<td>FEOPhe</td>
<td>Production of Phe in epidermis (protein breakdown)</td>
</tr>
<tr>
<td>FinPro</td>
<td>Arterial delivery of Pro to skin</td>
</tr>
<tr>
<td>FOutPro</td>
<td>Venous exit of Pro from skin</td>
</tr>
<tr>
<td>NBPro</td>
<td>Net balance of Pro across skin</td>
</tr>
<tr>
<td>nBPro</td>
<td>Net balance of labeled Pro across skin</td>
</tr>
<tr>
<td>FEDEPro</td>
<td>Transport of Pro from dermis to epidermis</td>
</tr>
<tr>
<td>FODPro</td>
<td>Irreversible loss of Pro from dermis (protein synthesis)</td>
</tr>
<tr>
<td>FDO Pro</td>
<td>Production of Pro in dermis (protein breakdown)</td>
</tr>
<tr>
<td>FPEPro</td>
<td>Irreversible loss of Pro from epidermis (protein synthesis)</td>
</tr>
</tbody>
</table>

Two-Pool Model

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>NBEPhe</td>
<td>Net balance of Phe across epidermis</td>
</tr>
<tr>
<td>NBDPhe</td>
<td>Net balance of Phe across dermis</td>
</tr>
<tr>
<td>NBEPo</td>
<td>Net balance of P (across epidermis)</td>
</tr>
<tr>
<td>NBDPo</td>
<td>Net balance of P (across dermis)</td>
</tr>
<tr>
<td>Rapphe</td>
<td>Rate of appearance of Phe from epidermis to blood</td>
</tr>
<tr>
<td>Rapphe</td>
<td>Rate of appearance of Phe from dermis to blood</td>
</tr>
<tr>
<td>RdisPhe</td>
<td>Rate of disappearance of Phe from blood to epidermis</td>
</tr>
<tr>
<td>RdisPhe</td>
<td>Rate of disappearance of Phe from blood to dermis</td>
</tr>
</tbody>
</table>

METHODS

Animals

We used male New Zealand White rabbits (Myrtle’s Rabbitry, Thompson Station, TN), weighing ~4.5 kg. This study was approved by the Animal Care and Use Committee of The University of Texas Medical Branch at Galveston.

Isotopes

L-[ring-15C]Phe (99% enriched), L-[ring-2H5]Phe (98% enriched), L-[ring-15N]Pro (98% enriched), and L-[ring-2H5]Pro (97–98% enriched) were purchased from Cambridge Isotope Laboratories (Woburn, MA). L-[ring-13C6]Phe and L-[ring-15N]Pro were used as tracers for intravenous infusion. L-[ring-2H5]Phe and L-[ring-2H5]Pro were used to prepare internal standard solutions for calculation of Phe and Pro concentrations in the blood and tissue pools.

Rationale and Equations of the Models

Four-compartment model. The design of the four-pool model was based on the structure and function of the epidermis and dermis. Whereas the dermis has abundant blood circulation, there are no blood vessels in the epidermis; the nutritional supply to the epidermis depends on diffusion from the dermis (17). The keratinocytes in the epidermis are packed together tightly, indicating that the epidermal free AAs can be regarded as an intracellular pool. In contrast, the dermal protein is synthesized in the fibroblasts and deposited as the extracellular matrix (25); thus the dermal free AAs can be divided into intracellular and extracellular pools. When blood moves through the skin circulation, AAs exchange with the interstitial fluid (i.e., extracellular compartment) of the dermis, from which the AAs are either transported into the fibroblasts (i.e., intracellular compartment) or diffuse through the basement membrane to nourish the epidermal cells (keratinocytes). The AAs in the dermal intracellular pool are used for synthesis of dermal protein. The process of dermal protein breakdown releases AAs into the dermal extracellular compartment, from which a portion is reincorporated into dermal protein and the remainder is released into the epidermal extracellular compartment and the venous blood. The proteolysis of epidermal protein releases AAs into the epidermal extracellular compartment, from which they are either used for synthesis or transported into the dermal extracellular compartment. A schematic illustration of the cutaneous protein and AA kinetics is shown in Fig. 1.

In our model, we combined the dermal intra- and extracellular free AA pools into a dermal free AA pool. This simplification ignores the transport of AAs between the dermal intra- and extracellular compartments but does not affect the measurement of protein synthesis and breakdown in the dermis. The advantage of this simplification is that it eliminates the necessity of distinguishing between AAs in the intracellular and extracellular compartments.

![Fig. 1. Schematic illustration of protein and amino acid (AA) kinetics in the epidermis and dermis. This illustration is based on the physiological structure of skin. Arrows indicate directions of AA movements. AAs enter the dermal extracellular pool from arterial blood and leave this pool via venous blood. In the dermal extracellular pool, some of AAs exchange with the dermal intracellular pool. Because there are no blood vessels in the epidermis, the epidermal intracellular pool exchanges AAs with the dermal extracellular pool. The movement of AAs out of the epidermal and dermal extracellular pools (other than into the dermal extracellular pool) indicates incorporation into protein, and movement into the intracellular pools (other than from the dermal extracellular pool) indicates protein breakdown.](http://ajpendo.physiology.org/)
breakdown, which is the sum of appearance from dermal protein (RaD) from proteolysis into disposal in the dermal protein (RdD) and epidermal protein (RdE). FV,O, cellular fluid pool (pool V). Arrows indicate the movement of AA from 1 pool to another. Fouts, rate of AA inflow into the skin from the artery; Fouts, exit from the skin via vein; FD,A, rate of AA delivery from pool A to pool D; FV,D, from pool D to pool V; FV,A, from pool A to pool V; FO,D and FO,E, rate of incorporation of AA into dermal and epidermal protein (i.e., rate of protein synthesis), respectively. The arterial and venous pools are arterial and venous pools (pool A and pool V), dermal fluid pool (pool D), and epidermal intracellular fluid pool (pool E). The 2 pools are arterial (pool A) and venous pool (pool V). Arrows indicate movements of AA from 1 pool to another. FO,A, rate of disposal (Rd) of AA via protein synthesis, which is the sum of disposal in the dermal protein (RdD) and epidermal protein (RdE). Fv,O, rate of appearance (Ra) of AA into the venous blood from protein breakdown, which is the sum of appearance from dermal protein (Rao) and from epidermal protein (Rae).

dermal intra- and extracellular compartments. Figure 2A is an illustration of our four-pool model for skin protein kinetics and AA transport.

Epidermis and dermis had to be separated to measure AA enrichment and concentration in the free and protein-bound pools. This was performed on the ventral skin of the rabbit ear. Anatomically, the skin on the ventral ear has a flat connection between epidermis and dermis (12). When a partial thickness thermal injury is created on the ear, the formation of blisters appears only on the ventral site of the ear skin (29). This observation led us to use the heat treatment method (14) for separation of epidermis and dermis. This method was originally used to separate epidermis from dermis in newborn hairless rats by immersing the skin in 55°C water for 30 s and immediately cooling with ice-cold water. We reduced the heat exposure to 2 s to minimize proteolysis, which would have decreased the isotopic enrichment of the skin fluid. We found that, by submerging the ventral ear skin in 65°C water for 2 s immediately followed by cooling in ice-cold water, the epidermal sheet can be easily peeled off from the dermis. Histologic observation showed that this separation procedure generated pure epidermis and dermis (data not presented here).

Ideally, if one AA is contained only in the epidermis and another AA is only in the dermal protein, we could use these two AAs to reflect protein kinetics in the epidermal and dermal protein, respectively. However, such AAs do not exist. As an alternative, we used Pro and Phe tracers to achieve this goal. This was because we found that the dermal Pro accounted for 97.9% of total skin protein-bound Pro and epidermal Pro accounted for only 2.1%. In contrast, the dermal protein contains 75.5% of total skin protein-bound Phe, whereas the remaining 24.5% is in the epidermis. Thus a Pro tracer reflects dermal protein kinetics. The use of Phe tracer to reflect epidermal protein kinetics was based on the consideration that the epidermal protein has a much faster turnover rate than the dermal protein, so that the 24.5% of epidermal Phe should account for a large majority of Phe kinetics in the skin. The distribution difference of Pro and Phe in the epidermal and dermal protein allowed us to solve the kinetic parameters in Fig. 2A with Pro and Phe tracers.

A glossary of symbols is provided above, and assumptions and derivations of equations are described in the Appendix. The following are equations used to calculate the kinetic parameters in the four-pool model (Fig. 2A). Theoretically, both Phe and Pro kinetic data can be calculated from the four-pool model. Because Phe and Pro kinetic data can be converted to each other according to their respective concentrations in the four pools, we selected the Phe data to represent protein and AA kinetics, and the Pro data were included only when they were necessary for model calculations. The use of Phe kinetics to reflect both epidermal and dermal protein kinetics does not conflict with the notion that Phe kinetics reflect mainly epidermal protein kinetics in the whole skin. This is because, in whole skin, Phe kinetics represents mainly epidermal protein kinetics, but in the dermis Phe kinetics reflect exclusively dermal protein kinetics

\[ F_{o,D}Phe = (C_pPhe \times nbPhe + E_pPhe \times C_pPhe - nbPhe \times E_pPro \times C_pPro) \]

\[ + (E_pPro \times E_pPhe \times C_pPro \times C_pPhe - E_pPhe \times E_pPro \times C_pPro \times C_pPhe) \]
In the equations above, \( F_{in}, F_{out}, F_{A}, F_{D}, F_{E}, F_{D,D}, F_{D,E}, F_{D,O}, \) and \( F_{E,O} \) are defined in the four-pool model (Fig. 2A) and further described in the Glossary. BF stands for blood flow rate in the ear skin. NB and nb stand for net balance of total (labeled + unlabeled) and labeled Phe or Pro, respectively. \( C_{D} \) and \( C_{E} \) represent contents of Phe or Pro in the dermal and epidermal protein. \( F_{D,O} \) and \( F_{E,O} \) are the measures of protein synthesis, which are expressed as the rates of Phe incorporation into dermal and epidermal protein, respectively. \( F_{D,O} \) and \( F_{E,O} \) are the measures of protein breakdown, which are expressed as the rates of Phe release from dermal and epidermal protein, respectively.

Two-compartment model. The two-pool model calculates the apparent rate of disposal (\( R_{d} \)) of AAs in the dermal or epidermal protein by use of the blood-derived AAs. The rate of appearance (\( R_{a} \)) is the rate of AA release, from dermal or epidermal protein breakdown, into the venous blood. The protein kinetics measured by the two-pool model do not include AAs that are released from breakdown and reused for synthesis without ever appearing in the blood. In other words, the kinetic data from the two-pool model underestimate the absolute rates of synthesis and breakdown by the amount of intracellular AA cycling.

The assumptions and derivations of the equations are presented in the Appendix. The principle of the calculations was originally described by Galim et al. (6). Because we used two tracers to measure respective protein kinetics in the epidermis and dermis, new equations are necessary to achieve the goal. The schematic illustration of the two-pool model is presented in Fig. 2B, and the formulas are described below. Phe and Pro kinetics in the epidermis and dermis can be interconverted on the basis of their tissue concentration difference. For clarity, we have presented only the Phe kinetics to represent protein kinetics and used the Pro data only where necessary for model calculations.

\[
F_{D,O} \text{Phe} = (C_{D}\text{Phe} \times F_{D,O} \text{Phe} - C_{D} \text{Pro})/C_{D} \text{Pro}
\]

\[
F_{D,O} \text{Phe} = [C_{D}\text{Phe} \times C_{D}\text{Phe} \times NB\text{Pro} - C_{D} \text{Pro}] \times C_{D}\text{Phe} \times NB\text{Pro} + F_{D,O} \text{Phe} \times (C_{D} \text{Pro} \times C_{D}\text{Phe} - C_{D} \text{Pro} \times C_{D}\text{Phe})/(C_{D} \text{Pro} \times C_{D}\text{Phe} - C_{D} \text{Pro} \times C_{D}\text{Phe})
\]

\[
F_{E,O} \text{Phe} = -NB\text{Phe} + F_{D,O} \text{Phe} - F_{D,O} \text{Phe} + F_{D,O} \text{Phe}
\]

\[
F_{E,E} \text{Phe} = F_{E,O} \text{Phe} \times E\text{Phe}/(E\text{Phe} - E\text{Pro})
\]

\[
F_{D,Z} \text{Phe} = F_{E,O} \text{Phe} + F_{E,Z} \text{Phe} - F_{D,O} \text{Phe}
\]

In the equations above, \( F_{in}, F_{out}, F_{A}, F_{D}, F_{E}, F_{D,D}, F_{D,E}, F_{D,O}, \) and \( F_{E,O} \) are defined in the four-pool model (Fig. 2A) and further described in the Glossary. BF stands for blood flow rate in the ear skin. NB and nb stand for net balance of total (labeled + unlabeled) and labeled Phe or Pro, respectively. \( C_{D} \) and \( C_{E} \) represent contents of Phe or Pro in the dermal and epidermal protein. \( F_{D,O} \) and \( F_{E,O} \) are the measures of protein synthesis, which are expressed as the rates of Phe incorporation into dermal and epidermal protein, respectively. \( F_{D,O} \) and \( F_{E,O} \) are the measures of protein breakdown, which are expressed as the rates of Phe release from dermal and epidermal protein, respectively.

Experimental Design

Five rabbits were studied in the postabsorptive state under general anesthesia. The anesthetic and surgical procedures were described in detail in our previous publications (28, 30). In brief, after an overnight fast with free access to water, the rabbits were anesthetized with ketamine and xylazine. Catheters were inserted into the right femoral artery and vein through a groin incision. The arterial line was used for blood collection and monitoring of blood pressure and heart rate; the venous line was for infusion. A tracheal tube was placed via tracheotomy. The central ear artery was dissected from the base of the left ear, and a flow probe (1RB; Transonic Systems, Ithaca, NY) was placed on the artery to measure blood flow rate on an ultrasonic small animal blood flowmeter (model T106, Transonic Systems).

After collection of a blood sample and a skin specimen from the incision on the groin for background measurements, priming doses of L-[ring-\( ^{13}\text{C} \)]Phe (6 \( \mu \text{mol/kg} \)) and L-[\(^{15}\text{N} \)]Pro (8 \( \mu\text{mol/kg} \)) were injected intravenously, which were followed immediately by a continuous infusion of L-[ring-\( ^{13}\text{C} \)]Phe (0.15 \( \mu\text{molkg}^{-1}\text{min}^{-1} \)) and L-[\(^{15}\text{N} \)]Pro (0.2 \( \mu\text{molkg}^{-1}\text{min}^{-1} \)). Blood samples were collected from the arterial line at 60 and 120 min to check the isotopic enrichment. Between 180 and 240 min, four simultaneous arterial and ear-venous blood samples were collected every 15 min. The blood flow rate in the ear was recorded at each arteriovenous (a-v) sampling. At 240 min, a skin sample was taken from the ventral side of the left ear. At the end of the experiment, the ear was cut off at the skin fold between ear base and auricle to weigh the ear. The weight of the ear was multiplied by 0.78 to get the weight of ear skin; the value of 0.78 was derived from dissection of 10 ears in our previous experiment (28). The measured blood flow rates in the ear in milliliters per minute per ear were then converted to the unit of milliliters per 100 grams per minute.

The blood samples were immediately transferred to tubes containing sulfosalicylic acid and the internal standard solution and kept in an ice-water bath until the end of the infusion study (30). The skin samples were immediately frozen in liquid nitrogen and stored at \(-80^\circ\text{C}\) for later processing.

During the isotope infusion, mean arterial blood pressure, heart rate, rectal temperature, and ear blood flow rate were
monitored continuously and were maintained relatively constant by adjusting the dose of anesthetic, infusion rate of saline, and heating lamps. The surface temperature of the left ear was maintained at 37°C using a heating lamp. The vital signs and ear blood flow rate were recorded every 30 min.

**Blood Preparation**

Immediately after collection of blood samples, 250 μl of blood were transferred to a tube containing 1 ml of 7.5% sulfosalicylic acid and 75 μl of an internal standard solution (28, 30). The internal standard solution contained 30 μmol/l of L-[ring-2H5]Phe and 60 μmol/l of L-[ring-15N]Pro. After deproteinization the supernatant was processed to make the N-acetyl, n-propyl ester (NAP) derivatives for measurement of Phe and Pro enrichment in the blood (26).

**Separation of Epidermis and Dermis**

Skin samples from the ventral side of the experimental ears were cut into strips of −0.5−× 5 cm². Each time, one strip of skin was submerged in 65°C saline for 2 s and then immediately put in ice-cold saline for 2 s. The epidermal sheet was peeled off from the dermis with a fine forceps at 4°C.

**Skin Sample Processing**

The samples of epidermis, dermis, and whole skin of ~30 mg each were homogenized in 10% perchloric acid three times at 4°C. The supernatant was pooled and evenly divided into two parts. One part was processed to make the NAP derivatives for measurement of Pro enrichment, and the other part was processed for t-butyldimethylsilyl (TB-DMS) derivatives for measurement of Phe (5). The protein pellets were washed thoroughly to remove free AAs and lipids (28). The samples were dried in an oven at 80°C overnight and hydrolyzed in 6 N HCl at 110°C for 24 h. The hydrolysate was dried and processed for the NAP derivatives (28). These samples were used for analysis of AA enrichment in the protein-bound pools.

**Contents of Phe and Pro in Skin Proteins**

To determine the contents of Phe and Pro in epidermal and dermal protein, we separated epidermis and dermis from the ventral side of the ear skin with the modified heat treatment method. The samples of epidermis and dermis were cut into small pieces and washed twice in ice-cold distilled water and kept in ice-cold water overnight to eliminate free AAs. After centrifugation, the supernatant was discarded, and the tissue samples were washed in absolute alcohol and ether to remove lipids and were then dried in an oven at 80°C. An internal standard solution was added to the dry tissue at the ratio of 1 μl of the solution to 1 mg of dry tissue; the internal standard solution contained L-[ring-13C6]Phe at 9.21 μmol/ml and L-[15N]Pro at 28.80 μmol/ml (for epidermis) or at 96.61 μmol/ml (for dermis). The samples were hydrolyzed in 6 N HCl at 110°C and processed for the NAP derivatives (26). The dry tissue weight, amount of internal standard solution added, and isotopic enrichment were used to calculate the content of these two tracer AAs in the epidermal and dermal protein.

**Measurement of Isotopic Enrichment**

The isotopic enrichment in the blood and in the skin protein hydrolysate for AA content was measured on a Hewlett-Packard 5985 gas chromatograph-mass spectrometer (GC-MS) (Hewelett-Packard, Palo Alto, CA) with chemical ionization. Ions were selectively monitored at mass-to-charge (m/z) ratios of 250, 251, 255, and 256 for Phe and at m/z ratios of 200, 201, and 207 for Pro. The isotopic enrichment in the skin supernatant prepared as the NAP derivatives was determined on a Hewlett-Packard 5989B GC-MS; ions were selectively monitored at m/z ratios of 200, 201, and 207 for Pro. The isotopic enrichment in the skin supernatant prepared as the TBDMS derivatives was determined on a Fison MD 800 GC-MS (Beverly, MA); ions were selectively monitored at m/z ratios of 234, 235, 239, and 240 for Phe.

L-[ring-13C6]Phe and L-[15N]Pro enrichment in the skin protein hydrolysate was measured on a gas chromatograph-mass spectrometer (Pinnigan). The measured 15CO2 enrichment was converted to Phe enrichment by multiplying 14/6 to account for the dilution of the labeled carbons with the carbons in other positions of the derivatized Phe. The measured 15N enrichment represented the Pro enrichment, because each molecule of L-[15N]Pro has only one nitrogen and that nitrogen was labeled. The enrichment of Phe and Pro was used to calculate the FSR of skin proteins by the tracer incorporation method.

The isotopic enrichment was expressed as mole percent excess (MPE) after correction for the contribution of the abundance of isotopomers of lower weight to the apparent enrichment of isotopomers with larger weight and also a skew correction factor to calculate L-[ring-13C6]Phe enrichment (21).

**Measurement of Hydroxyproline Concentration**

Some of the Pro incorporated into dermal collagen is converted to hydroxyproline (HPro) via posttranslational modification on the collagen peptide (1, 9). Thus the Pro a-v balance should include the release of HPro, and the content of HPro in the skin supernatant prepared as the NAP derivatives was determined on a Hewlett-Packard 5989B GC-MS (Beverly, MA); ions were selectively monitored at m/z ratios of 250, 251, 255, and 256 for Phe and at m/z ratios of 200, 201, and 207 for Pro. The net balance of Pro across the skin. The Pro content in the dermal protein-bound pool also included HPro. To this end, we measured HPro in the a-v blood and in the dermal protein hydrolysate. This was completed by measuring the peak sizes of Pro and HPro in the NAP derivative on a 5890A gas chromatograph (Hewelett-Packard) equipped with an sp-2330 column (Supelco, Bellefonte, PA). The oven temperature was set at 160−400°C at 8.0°C/min with an injection temperature of 250°C. The measured ratio of Pro peak size to HPro peak size from the gas chromatograph was used to calculate the concentration of HPro. Because the concentration of Pro was measurable using the internal standard method, the concentration of HPro equals Pro concentration divided by the peak size ratio of Pro to HPro. The net release of HPro is equal to venous HPro concentration minus arterial HPro concentration. The value of HPro net release was added to the venous Pro concentration to calculate the net balance of Pro across the skin. The Pro content in the dermal protein-bound pool also included HPro.

**Calculations of FSR**

The FSR of skin proteins was calculated for the tracer incorporation method (26). FSR = [(E1 - E0)/E0 (t1 − t0)] × (t1 − t0), where (E1 - E0) is the increment of enrichment in the protein-bound pool between two sampling times, and E0 (t1 − t0) is the average precursor enrichment between time 0 and time 1.

The FSR of skin proteins was also converted from the rate of synthesis measured from the four-pool model. We used FSRc to distinguish it from FSR measured directly by the tracer incorporation method. FSRc = (rate of synthesis in
μmol Phe·100 g⁻¹·h⁻¹/μmol protein-bound Phe in 100 g of tissue).

Statistics

Data are expressed as means ± SD. Student’s t-test was used to test the difference between two variables. A P value < 0.05 was considered statistically significant.

RESULTS

We measured Phe and Pro contents in epidermal and dermal protein. Six pieces of skin were taken from the ventral side of the ear. After separation using the modified heat treatment method, the wet epidermis and dermis accounted for 15 ± 2 and 85 ± 2% of the wet skin. After a thorough washing and complete drying, the dry epidermis and dermis were 2.64 ± 0.53 and 22.86 ± 1.09% of wet skin weight, respectively. By use of the internal standard method, 1 g of dry epidermal protein contained 242 ± 25 μmol of Phe and 262 ± 67 μmol of Pro, 1 g of dermal protein contained 86 ± 5 μmol of Phe and 1,094 ± 15 μmol of Pro. In the dermal protein hydrolysate samples, the ratio of peak area of Pro to HPro was 3.9 ± 0.4, meaning that the content of HPro was 283 ± 30 μmol/g. The total content of Pro and HPro was then 1,379 ± 35 μmol/g. Thus the dermal Pro (including HPro) accounted for 97.9% of total skin protein-bound Pro, and epidermal Pro accounted for only 2.1% of the total. In contrast, the dermal protein contained 75.5% of total skin protein-bound Phe, and the remaining 24.5% was in the epidermis.

The mean arterial blood pressure, heart rate, and rectal temperature were relatively constant in the rabbits (data not presented here). During the 180–240 min of the isotope infusion, isotopic equilibrium was achieved for both Phe and Pro. The recorded blood flow rate in the ear skin was 8.5 ± 0.5 ml·100 g⁻¹·min⁻¹. The isotopic enrichment and concentration values used to calculate the parameters in the four-pool model are presented in Table 1. The HPro concentrations in the arterial and efferent venous blood were 0.0110 ± 0.0020 and 0.0126 ± 0.0032 μmol/ml (P < 0.01 by paired t-test, n = 18), resulting in a net increase of 0.0016 μmol/ml in the venous blood. Thus the Pro concentration in the venous blood was increased by 0.0016 μmol/ml to account for net release of HPro.

The calculated protein kinetics and Phe transport in the epidermis and dermis according to the four-pool model are presented in Fig. 3. Whereas the protein mass of epidermis and dermis was essentially maintained in these rabbits, the epidermis had a much faster turnover rate than the dermis. Expressed as Phe kinetics, the absolute rate of Phe incorporation into epidermal protein was 22.6 ± 9.1 μmol·100 g⁻¹·h⁻¹, which was 28-fold greater than that of the dermal protein (0.8 ± 0.5 μmol·100 g⁻¹·h⁻¹). Because 1 g of dry epidermal protein contains 242 μmol of Phe and 1 g of dry dermal protein contains only 86 μmol of Phe, the protein synthetic rates converted from the Phe incorporation rates were 93 and 9.3 mg·100 g⁻¹·h⁻¹ for epidermis and dermis, respectively. Thus the epidermal protein had a fast synthetic rate that was 10-fold greater than the dermal protein.

The rates of protein synthesis and breakdown in the epidermis and dermis calculated from the two-pool model were lower than the corresponding values calculated from the four-pool model. This was because the data from the two-pool model did not include the rates of intracellular cycling. Table 2 presents the rates of protein synthesis, breakdown, net balance, and intracellular cycling calculated from the four-pool and two-pool models and expressed as Phe kinetics. The rate of cycling was the difference between the rates of synthesis and breakdown calculated by the four-pool and two-pool models.

The enrichment data of Phe and Pro in the skin free and protein-bound pools and the FSR values of epider-
The present experiment successfully measured protein and AA kinetics in the epidermis and dermis. Epidermal protein was shown to have a fast turnover rate, which was 10-fold that of dermal protein. In the postabsorptive state, AA recycling provided over 75% of AAs for protein synthesis in the dermis and epidermis, respectively. The efficiency in reutilization of AAs from proteolysis for synthesis likely plays an important role in the maintenance of skin homeostasis. The rapid AA transport between epidermis and dermis, being comparable with the rate of AA transport between blood and dermis, ensured sufficient nutritional supply to the avascular epidermis.

The validity of the four-pool model for measurement of protein kinetics in the epidermis and dermis is supported not only by the FSR values measured from the tracer incorporation method (Fig. 4) but also by published data. Using the flooding dose method, Harmon and Park (7) reported an FSR of epidermal protein of 61%/day in hairless mice. Their results were subject to underestimation, because the epidermal samples that they harvested with a dermatome contained ~20% of dermis. Thus the epidermal FSR of 85%/day that we obtained from the four-pool model is comparable with their value, both showing very fast turnover rates. For dermal collagen synthesis, El-Harake et al. (4) reported that the immature, detergent-soluble collagen FSR in human skin was 0.038 ± 0.030%/h when skin-free Pro enrichment was used as the precursor. Our model-derived dermal protein FSR value was 1.0 ± 0.7%/day (0.042 ± 0.029%/h), which is slightly greater than the human dermal collagen value but smaller than the collagen FSR (0.17%/h) in rats (15, 24). These small differences are probably due to differences in species and experimental conditions. The rate of protein turnover in rabbits appears to be between the human and rat values. For example, the FSR of muscle protein in rabbits was 0.079 ± 0.095%/h (28), which is greater than the human value of 0.055%/h (averaged from 80 human muscle measurements, unpublished data from this laboratory) and smaller than the value of 0.20%/h in rats (16). In the present experiment, the four-pool model measured the dermal protein (including noncollagen components) in anesthe-

### Table 2. Protein kinetics in the epidermis and dermis

<table>
<thead>
<tr>
<th></th>
<th>Synthesis</th>
<th>Breakdown</th>
<th>Net Balance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Derby</td>
<td>Epidermis</td>
<td>Derby</td>
</tr>
<tr>
<td>4-Pool model</td>
<td>0.8 ± 0.6</td>
<td>22.6 ± 9.1</td>
<td>0.8 ± 0.5</td>
</tr>
<tr>
<td>2-Pool model</td>
<td>0.16 ± 0.09</td>
<td>1.94 ± 1.45</td>
<td>0.14 ± 0.05</td>
</tr>
<tr>
<td>Intracellular</td>
<td>0.6 ± 0.5</td>
<td>20.7 ± 8.7</td>
<td>0.6 ± 0.5</td>
</tr>
</tbody>
</table>

Data are means ± SD in μmol Phe·100 g⁻¹·h⁻¹. Rates of intracellular cycling are the difference between the corresponding values from the 4-pool and 2-pool models.

### Table 3. FSR of dermal and epidermal protein

<table>
<thead>
<tr>
<th></th>
<th>Tissue fluid</th>
<th>Protein bound</th>
<th>Infusion h</th>
<th>FSR, %/day</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Phe tracer</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Derby</td>
<td>0.0445 ± 0.0119</td>
<td>0.000194 ± 0.000038</td>
<td>4.2 ± 0.1</td>
<td>2.6 ± 1.0</td>
</tr>
<tr>
<td>Epidermis</td>
<td>0.0075 ± 0.0037</td>
<td>0.000633 ± 0.000104</td>
<td>4.2 ± 0.1</td>
<td>55 ± 20</td>
</tr>
<tr>
<td>Whole skin</td>
<td>0.0292 ± 0.0054</td>
<td>0.000547 ± 0.000024</td>
<td>4.2 ± 0.1</td>
<td>8.1 ± 0.8</td>
</tr>
<tr>
<td><strong>Pro tracer</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Derby</td>
<td>0.0335 ± 0.0073</td>
<td>0.000028 ± 0.000018</td>
<td>4.2 ± 0.1</td>
<td>0.5 ± 0.4</td>
</tr>
<tr>
<td>Epidermis</td>
<td>0.0056 ± 0.0036</td>
<td>0.001797 ± 0.000139</td>
<td>4.2 ± 0.1</td>
<td>150 ± 44</td>
</tr>
<tr>
<td>Whole skin</td>
<td>0.0213 ± 0.0026</td>
<td>0.000059 ± 0.000036</td>
<td>4.2 ± 0.1</td>
<td>1.6 ± 0.4</td>
</tr>
</tbody>
</table>

Data are means ± SD. FSR, fractional synthesis rate. Isotopic enrichment is expressed as tracer/tracee ratio.
tized rabbits with stress response, which differs from
dermal collagen measured in conscious patients (4) or
rats (15, 24). Thus the dermal protein kinetic data
measured by the four-pool model are considered reason-
able. The comparisons of the synthesis rates be-
tween the four-pool and two-pool models indicate that
the intracellular recycling of AAs in the dermis ac-
counted for 75% of protein synthesis. This is also con-
sistent with the finding that, in fibroblasts, ~90% of
Pro for collagen synthesis is derived from collagen
degradation (see Ref. 13).

In comparison with the four-pool model, the two-pool
model measures the rate of protein synthesis by using
the blood-derived (as opposed to the proteolysis-de-
ved) AAs for protein synthesis and the rate of releas-
ing AAs from proteolysis into venous blood (as opposed
to reuse for protein). If the end points of an experiment
are the efficiency of using blood-derived AAs for syn-
thesis and the net protein balance in the skin, the
two-pool model is sufficient. This is because only an
increased incorporation of blood-derived AAs into pro-
collagen could lead to an anabolic response if the rate of AA
release from proteolysis to the venous blood is not pro-
portionally increased. Any increase in AA recycling
will increase the absolute rate of protein turnover but
will not improve the net balance, since 100% reutili-
zaion would yield a zero balance.

The finding that Phe and Pro have different distri-
butions in epidermal and dermal protein can be a
useful tool in estimating metabolic contributions from
epidermal and dermal proteins. Because 97.9% of skin
Pro is in dermal protein, the whole skin FSR value
measured from Pro tracer represents mainly dermal
protein FSR. In contrast, 75.5% of skin Phe is in the
dermis; the whole skin FSR value measured from Phe
tracer represents dermal protein to a lesser extent
than does the Pro tracer. As the rates of Phe incorpo-
ration into epidermal and dermal protein (Fig. 3) and
the contents of Phe and Pro in the epidermal and
dermal protein are known, the rate of Phe incorpo-
ration into skin proteins could be calculated as
(22.6 × 24.5%) + (0.8 × 75.5%) = 6.141 (mol·100 g⁻¹·h⁻¹).
This means that 90% of the Phe tracer was incorpo-
rated into epidermal protein and only 10% was incor-
porated into dermal protein. In contrast, the rate of Pro
incorporation into skin protein was
(22.6 × 2.1%) + (0.8 × 97.9%) = 1.2578 (mol·100 g⁻¹·h⁻¹).
Thus 38% of Pro tracer was incorporated into epidermal
protein, and 62% was incorporated into dermal protein.
Thus, when the Phe tracer was used to measure whole skin
FSR, the value largely reflected epidermal protein syn-
thesis. Because the rate of Phe incorporation into epi-
dermal protein was found to be 28-fold greater than
that of dermis (Fig. 3), the Phe tracer generated a high
value of whole skin FSR. In contrast, when the Pro
tracer was used for skin FSR, a mixture of epidermal
protein synthesis (38%) and dermal protein synthesis
(62%) was reflected. Consequently, the FSR value was
much lower when the Pro tracer was used. When both
Phe and Pro tracers are used, the measured whole skin
FSR values enable us to estimate the respective FSR
values in the epidermal and dermal protein. Taking
the rabbit ear skin as an example (see Table 3), the
whole skin FSR values measured from the Phe and Pro
tracers were 8.1 and 1.6%/day, respectively. If we use
EFSR and DFSR to represent epidermal and dermal
protein FSR and T Phe and T Pro to represent total skin
Phe and Pro, we will have the following two equations:

(24.5% × T Phe × EFSR) + (75.5% × T Phe × DFSR) =
T Phe × 8.1 (%/day); and (2.1% × T Pro × EFSR) + (97.9% ×
T Pro × DFSR) = 1.6 (%/day). In these two equations,
24.5 and 75.5% are the percentage contents of Phe
in the epidermal and dermal protein, and 2.1 and 97.9%
are the percentage contents of Pro in the epidermal
and dermal protein. T Phe and T Pro can be canceled out
from the equations. Solving these two equations, we
get EFSR = 30% / day and DDFSR = 1%/day. Whereas
these estimated FSR values are lower than the corre-
sponding average FSR values (epidermis 103%/day;
dermis 1.5%/day) that were directly measured from the
tracer incorporation method, the calculation above indi-
cates that the whole skin FSR values measured from
both Phe and Pro tracers provide useful information
with regard to FSR in the epidermis and dermis. Even
if the percentage contents of Phe and Pro in the whole
skin are not known, the changes of whole skin FSR
measured from Phe and Pro tracers allow us to esti-
mate whether the experimental perturbation primarily
affects epidermal or dermal protein synthesis or both.
This provides a reasonable approach when it is not
feasible to distinguish epidermal and dermal synthesis
directly. This could be potentially useful for human
studies in which only a very small biopsy is available.

The measurement of skin protein metabolism has
been hindered by several major limitations. One is the
uncertainty of the true precursor (i.e., aminoacyl-
tRNA) for skin protein synthesis. To our knowledge,
the skin aminoacyl-tRNA enrichment has been measured only by El-Harake et al. (4). They reported that, in human skin, the prolyl-tRNA enrichment was ~50% of the free Pro enrichment. However, they did not distinguish between the prolyl-tRNA enrichments in dermis and epidermis. Cutaneous collagen and keratin are synthesized in fibroblasts and keratinocytes, respectively. Thus it is necessary to obtain their respective aminoacyl-tRNA enrichments for calculations of dermal collagen and epidermal keratin synthesis. This issue remains unresolved. In most published experiments, the skin free enrichment has been used as a surrogate of precursor for skin protein synthesis. This may have underestimated the skin protein turnover rate. Nevertheless, as long as the relationship between skin fluid and tRNA enrichment remains constant, this approach still gives valuable information. When two AA tracers are used for skin protein measurement, as in the present experiment, an additional issue is that, if they have a different relationship between tissue fluid and tRNA enrichment, the use of tissue fluid as precursor enrichment could result in inconsistent FSR values. It is likely that possible compartmentalization of Pro pools (18) explains why the FSR values in epidermal and dermal protein measured from Phe and Pro tracers were different (see Table 3), although both showed that epidermal FSR was much greater than dermal FSR. In the present experiment, we also measured skin protein FSR from a leucine tracer. The average FSR values were 72%/day for epidermis and 2.9%/day for dermis, which were close to the values of 55%/day for epidermis and 2.6%/day for dermis measured by the Phe tracer. Another major limitation is that the skin is a highly heterogeneous tissue. In this experiment, we assumed that the skin is composed of epidermal and dermal proteins. This assumption is still a simplification of the skin structure, because the dermis is composed of different types of collagen and elastin and epidermis is composed of a large family of keratin polypeptides (25). Thus our models and the tracer incorporation method all measure protein kinetics in mixed dermal and epidermal proteins.

In our a-v models, we have assumed that neither Phe nor Pro is synthesized or degraded in the skin. This is well established to be true in the case of Phe. Pro, on the other hand, is traditionally considered to be a nonessential AA because it can be synthesized from ornithine and glutamate. However, our data showed that the rate of net Pro balance in the skin was consistent with that of Phe: neither was significantly different from zero (Table 1). The only way that this result could be obtained if Pro was synthesized and/or metabolized in skin is if the rate of biosynthesis equaled that of degradation. It is more likely that Pro is neither produced nor metabolized in skin. The metabolic pathways of Pro are closely related to \( \Delta^1 \)-pyrroline-5-carboxylate (P5C), the obligate intermediate in the interconversions of Pro, ornithine, and glutamate (1–3, 8–10, 22, 23, 27). The degradation of Pro to P5C is catalyzed by the mitochondrial enzyme Pro oxidase. This enzyme is localized in the liver, kidney, heart, lung, and small intestine but is not detectable in muscle and adipose tissue (8, 27) or in fibroblasts (22). These findings support the notion that Pro is not degraded in the skin, meaning that the possibility of equal rates of Pro biosynthesis and degradation can be excluded. Furthermore, biosynthesis of Pro from its immediate precursor P5C is catalyzed by the cytosolic enzyme P5C reductase, with either glutamate or ornithine as precursor. Although this enzyme may reside in almost all cells (8), the biosynthesis of Pro in the skin has been shown to constitute a minor contribution to collagen synthesis (2, 3, 13). In particular, in fibroblasts, P5C reductase is sensitive to feedback inhibition, with 50% inhibition at a Pro concentration of 200 nmol/ml (23). In the ear skin of rabbits, the concentration of free Pro was 426 ± 75 nmol/ml (350–504 nmol/ml). Therefore, even if P5C reductase exists in the skin, it would have been inhibited by the high Pro concentration. To assess the extent of Pro synthesis in the skin, we infused \( ^{3} \text{H}_2 \text{H}_3 \text{H} \) glutamate into three rabbits. The arterial glutamate enrichment plateau was 2.5–3.5%, but the enrichment of Pro was not detectable in the arterial blood (reflection of whole body Pro biosynthesis) or ear-venous blood (reflection of skin Pro biosynthesis) (unpublished data in this laboratory). The above discussion supports the notion that Pro biosynthesis constitutes no more than a minor contribution to dermal collagen synthesis. Thus, in this animal model, it is acceptable to use a Pro tracer in conjunction with Phe tracer to quantify protein kinetics in the skin.

We conclude that our newly described models provide reasonable values with respect to protein and AA kinetics in the epidermis and dermis. The results indicate that the skin protein maintains a balance between synthesis and breakdown in the postabsorptive state, thereby maintaining skin barrier integrity. Epidermal protein turns over rapidly, which is likely essential for the repair process. There is also rapid AA transport between epidermis and dermis, which suggests that a healthy dermal base is essential for normal protein metabolism in the epidermis. Our new models of skin protein metabolism may be used to study skin protein metabolism in physiological and pathological conditions. Furthermore, our results indicate that it is possible to distinguish between dermal and epidermal protein synthesis even when it is not feasible to physically separate the tissues for direct analysis.

**APPENDIX**

**Four-Compartment Model**

*Assumptions.* To solve the parameters in the four-pool model (Fig. 2), we assume that 1) the values of protein synthesis and breakdown in the dermis and epidermis measured from the Pro and Phe tracers are proportional to the contents of Pro and Phe in the dermal and epidermal protein; 2) both dermis and epidermis are composed of a single free AA pool and a protein-bound AA pool, and free AA in the dermis and epidermis are precursors for protein synthesis; 3) there are no de novo synthesis and degradation of Phe and Pro in the skin, so that their appearance and disappearance in the dermis and epidermis represent protein breakdown

---

**AJP-Endocrinol Metab • VOL 284 • JUNE 2003 • www.ajpendo.org**
and synthesis, respectively; and 4) there is no label recycle from the protein-bound pool to the free pool in either epidermis or dermis.

Calculation of Phe transport between pools. Because we used both Pro and Phe tracers, the protein and AA kinetics could be expressed by either Pro or Phe. To simplify the calculation, we used Phe data to represent protein and AA kinetics; the Pro data were included only when they were necessary to solve the parameters of Phe kinetics

\[ \text{F}_{\text{in-Phe}} = C_{\text{p}} \times \text{BF} \]  
\[ \text{F}_{\text{out-Phe}} = C_{\text{p}} \times \text{BF} \]  
\[ \text{NB}_{\text{Phe}} = \text{F}_{\text{in-Phe}} - \text{F}_{\text{out-Phe}} \]  
\[ \text{F}_{\text{Pro}} = C_{\text{p}} \times \text{BF} \]  
\[ \text{F}_{\text{out-Pro}} = C_{\text{p}} \times \text{BF} \]  
\[ \text{NB}_{\text{Pro}} = \text{F}_{\text{in-Pro}} - \text{F}_{\text{out-Pro}} \]  
\[ \text{F}_{\text{V,Phe}} = \text{F}_{\text{out-Phe}} \times (\text{E}_{\text{Phe}} - \text{E}_{\text{Phe}}) / (\text{E}_{\text{Phe}} - \text{E}_{\text{n-Phe}}) \]  
\[ \text{F}_{\text{V,A Phe}} = \text{F}_{\text{out-Phe}} - \text{F}_{\text{V,Phe}} \]  
\[ \text{F}_{\text{D,A Phe}} = \text{F}_{\text{in-Phe}} - \text{F}_{\text{V,A Phe}} \]

Calculation of protein synthesis. We can calculate net tracer uptake across the tissue bed by multiplying the a-v tracee concentration difference by the tissue blood flow. We will denote net Phe tracer uptake across the tissue bed as \( \text{nb}_{\text{Phe}} \) and net Pro tracer uptake across the tissue bed as \( \text{nb}_{\text{Pro}} \). On the basis of \textit{assumption 3}, the only pathways of irreversible loss of Phe and Pro across skin are incorporation into bound dermis and bound epidermis, i.e.

\[ \text{nb}_{\text{Phe}} = \text{E}_{\text{Phe}} \times \text{F}_{\text{D,D Phe}} + \text{E}_{\text{Phe}} \times \text{F}_{\text{D,O Pro}} \]  
\[ \text{nb}_{\text{Pro}} = \text{E}_{\text{Pro}} \times \text{F}_{\text{D,D Pro}} + \text{E}_{\text{Pro}} \times \text{F}_{\text{D,O Phe}} \]

where \( \text{F}_{\text{D,D Phe}} \) and \( \text{F}_{\text{D,D Pro}} \) are the rates of incorporation of Phe and Pro into dermis bound protein, \( \text{F}_{\text{D,O Phe}} \) and \( \text{F}_{\text{D,O Pro}} \) are the rates of incorporation of Phe and Pro into epidermis bound protein, \( \text{E}_{\text{Phe}} \) and \( \text{E}_{\text{Pro}} \) are the precursor enrichments of dermis bound Phe and Pro, and \( \text{E}_{\text{Phe}} \) and \( \text{E}_{\text{Pro}} \) are the precursor enrichments of epidermis bound Phe and Pro (\textit{assumption 2}).

According to \textit{assumption 1}, the incorporation of Phe and Pro into protein occurs in proportion to their respective contents in the protein, i.e.

\[ \text{F}_{\text{D,O Phe}} / \text{C}_{\text{D Phe}} = \text{F}_{\text{D,O Pro}} / \text{C}_{\text{D Pro}} \]

and

\[ \text{F}_{\text{D,O Phe}} / \text{C}_{\text{E Phe}} = \text{F}_{\text{D,O Pro}} / \text{C}_{\text{E Pro}} \]

where \( \text{C}_{\text{D Phe}} \) and \( \text{C}_{\text{D Pro}} \) are the dermis bound contents of Phe and Pro and \( \text{C}_{\text{E Phe}} \) and \( \text{C}_{\text{E Pro}} \) are the epidermis bound contents of Phe and Pro. If the four equations above are solved for the four unknowns \( \text{F}_{\text{D,O Phe}}, \text{F}_{\text{D,O Pro}}, \text{F}_{\text{D,E Phe}}, \text{and} \text{F}_{\text{D,E Pro}} \), then one obtains

\[ \text{F}_{\text{D,O Phe}} = (\text{C}_{\text{Phe}} \times \text{nb}_{\text{Pro}} \times \text{E}_{\text{Pro}} \times \text{C}_{\text{Pro}} - \text{nb}_{\text{Phe}} \times \text{E}_{\text{Phe}} \times \text{C}_{\text{Phe}}) / (\text{E}_{\text{Pro}} \times \text{E}_{\text{Phe}} \times \text{C}_{\text{D Pro}}) \]  
\[ \times (\text{E}_{\text{Phe}} - \text{E}_{\text{Pro}}) / (\text{E}_{\text{Phe}} - \text{E}_{\text{n-Phe}}) \]  

Calculation of protein breakdown. We can calculate net tracer uptake across the tissue bed by multiplying the a-v tracee concentration difference by the tissue blood flow rate. We will denote net Phe uptake across the tissue bed as \( \text{NB}_{\text{Phe}} \) and net Pro uptake across the tissue bed as \( \text{NB}_{\text{Pro}} \).

According to \textit{assumption 3}, the only pathways of production of Phe and Pro across skin are release from bound dermis and bound epidermis, i.e.

\[ \text{NB}_{\text{Phe}} = \text{F}_{\text{D,D Phe}} - \text{F}_{\text{D,O Phe}} - \text{F}_{\text{D,E Phe}} - \text{F}_{\text{E,O Phe}} \]

and

\[ \text{NB}_{\text{Pro}} = \text{F}_{\text{D,D Pro}} - \text{F}_{\text{D,O Pro}} - \text{F}_{\text{D,E Pro}} - \text{F}_{\text{E,O Pro}} \]

where \( \text{F}_{\text{D,O Phe}} \) and \( \text{F}_{\text{D,O Pro}} \) are the rates of release of Phe and Pro from the dermis protein-bound pool and \( \text{F}_{\text{E,O Phe}} \) and \( \text{F}_{\text{E,O Pro}} \) are the rates of release of Phe and Pro from the epidermis protein-bound pool.

According to \textit{assumption 1}, the release of Phe and Pro from protein breakdown occurs in proportion to their respective contents in protein, i.e., \( \text{F}_{\text{D,O Phe}} / \text{C}_{\text{D Phe}} = \text{F}_{\text{D,O Pro}} / \text{C}_{\text{D Pro}} \), and \( \text{F}_{\text{E,O Phe}} / \text{C}_{\text{E Phe}} = \text{F}_{\text{E,O Pro}} / \text{C}_{\text{E Pro}} \). If the four equations above are solved for the four unknowns \( \text{F}_{\text{D,O Phe}}, \text{F}_{\text{D,O Pro}}, \text{F}_{\text{E,O Phe}}, \text{and} \text{F}_{\text{E,O Pro}} \), then one obtains

\[ \text{F}_{\text{D,O Phe}} = (\text{C}_{\text{Phe}} \times \text{C}_{\text{Pro}} \times \text{F}_{\text{Pro}} - \text{C}_{\text{Phe}} \times \text{C}_{\text{D Phe}} - \text{C}_{\text{Pro}} \times \text{C}_{\text{D Pro}}) / (\text{C}_{\text{E Phe}} \times \text{C}_{\text{E Pro}}) \]

In Eqs. A14-A17, \( \text{C}_{\text{E Phe}}, \text{C}_{\text{D Phe}}, \text{C}_{\text{E Pro}}, \) and \( \text{C}_{\text{D Pro}} \) are constant values, and the values of isotopic enrichment can be measured after separation of epidermis and dermis. Thus all the kinetic values in Fig. 2 can be solved.

Two-Compartment Model

Assumptions. The assumptions of the two-pool model are that 1) the Fick Principle holds; 2) the release of Phe and Pro from dermis and epidermis is proportional to the relative amount of Phe and Pro in dermal and epidermal protein; 3) there is no label recycle from the protein-bound pool to the free pool in either epidermis or dermis. On the basis of \textit{assumption 1}, the total release of Phe or Pro from skin is equal to the sum of release from dermis and epidermis. On the basis of \textit{assumption 2}, the rate of release of Phe from epidermis divided by the rate of release of Pro from dermis is equal to the concentration of Phe in dermal protein divided by the concentration of Pro in dermal protein, and the rate of release of Phe from epidermis divided by the rate of release of Pro from epidermis is equal to the concentration of Phe in epidermal protein divided by the concentration of Pro in epidermal protein. The total rates of release of Phe and Pro from skin are computed using the traditional two-pool model equations. The concentrations of Phe and Pro in dermis and epidermis are constant values. To calculate the rate of release of Phe from dermal protein breakdown, four equations are required. There are four equations and four unknowns, so the unknowns can be determined. The incorporation of blood-derived Phe and Pro into epidermal and dermal protein and the net balance of Phe and Pro across epidermis and dermis can be calculated using the same assumptions as above. The formulas are described below.
Calculations. The calculation of \( F_{\text{Phe}}, F_{\text{outPhe}}, \text{NBPro}, \text{FoutPro}, \) and \( \text{NBPro} \) are described in Eqs. A1–A6 for the four-pool model:

\[
\text{RdPhe} = (E_A \times \text{Phe}) - (E_V \times \text{outPhe})/E_A \tag{A18}
\]

\[
\text{RdPro} = (E_A \times \text{Pro} - E_V \times \text{outPro})/E_A \tag{A19}
\]

\[
\text{RdPhe} = (C_P \times C_{\text{Phe}} \times E \text{Phe}) - (C_P \times C_{\text{Phe}} \times E \text{Phe})/E_A \tag{A20}
\]

\[
\text{RdPro} = (C_P \times C_{\text{Pro}} \times E \text{Pro})/E_A \tag{A21}
\]

We are grateful to Yunxia Lin, Guy Jones, and Zhiping Dong for technical assistance. We also thank the staff at the Electron Microscope Laboratory of Shriners Hospitals for Children for preparation of the histological slides of epidermis and dermis separated by the modified heat treatment method, and the staff at the Animal Resource Center of The University of Texas Medical Branch for professional care of experimental animals.

This work was sponsored by Grants S630 and 8490 from the Shriners Hospital.

REFERENCES