Metabolic Transformations of Cortisol-4-[^{14}C] in Human Skin*

S. L. Hsia and Yu-Lee Hao

ABSTRACT: Cortisol-4-[^{14}C] was found actively metabolized by slices of human skin. By paper chromatography, isotopic dilution, and the preparation of acetates, the following metabolites were identified: cortisone, Δ^4-pregnene-11β,17α,20β,21-tetraol-3-one (Reichstein's substances E), Δ^4-pregnene-11β-17α,20α,21-tetraol-3-one (epi-E), Δ^4-pregnene-17α-20β,21-triol-3,11-dione (U), and Δ^4-pregnene-17α,20α,21-triol-3,11-dione (epi-U), allodihydrocortisol, and allotetrahydrocortisol. The latter two steroids were found only after incubation of cortisol-4-[^{14}C] with foreskin and not with skin of other anatomical sites. The two general pathways of metabolism of cortisol in human skin appear to be oxidation of the 11β-ol and reduction of the 20-one while saturation of the Δ^4-double bond and reduction of the 3-one appear limited to the foreskin.

A previous communication from this laboratory presented evidence of active metabolism of cortisol-4-[^{14}C] by slices of human skin (Hsia *et al.*, 1964). Several metabolites were detected by paper chromatography; it was also noticed that one of the peaks of radioactivity in the paper chromatogram was characteristic of the metabolism of foreskin and was not detected after incubation with skin of other anatomical sites. That the metabolism is linked to the pyridine nucleotides was demonstrated in a subsequent study (Hsia *et al.*, 1965) in which skin specimens obtained at autopsy were found to have lost their ability to metabolize cortisol, but the metabolism could be restored by the addition of TPNH[1] or DPNH to the incubation medium. In the same study it was found that both the dermis and epidermis are capable of metabolizing cortisol.

In a continuation of these studies, several metabolites have now been identified, including cortisone and Reichstein's substances E, epi-E, U, and epi-U. Allodihydrocortisol and allotetrahydrocortisol have been found, so far, only after incubation of cortisol with foreskin; their formation after incubation with skin of other anatomical sites could not be established. The evidence for the identification of these metabolites is reported in this paper.

Experimental Section and Results

Materials. Cortisol-4-[^{14}C] with specific activity of 22.5 mc/mmole was purchased from Nuclear Chicago. The material was purified before use by paper chromatography in the system of methanol–benzene–water–ethyl acetate (1:1:1:0.1) as described by Fukushima *et al.* (1960). Tetrahydrocortisol and tetrahydrocortisone were gifts of the Merck Institute of Therapeutic Research and allotetrahydrocortisol, a gift of G. D. Searle and Co. The other steroids used were purchased from Mann Research Laboratories, Inc. Foreskin specimens were collected at circumcision and specimens from other anatomical sites were obtained at surgery or at autopsy 16–20 hr postmortem.

Incubation and Analysis. Details of the procedures were reported previously (Hsia *et al.*, 1964, 1965). The skin specimens were cut free hand to small pieces weighing 25 mg or less, and *ca.* 400–500 mg were incubated in 5 or 7 ml of Krebs–Ringer phosphate buffer (pH 7.4) with cortisol-4-[^{14}C] containing 10^6 dpm of ^{14}C dissolved in 0.1 ml of ethanol. In experiments with cadaver skin a TPNH-generating system was added to the medium, consisting of TPN$^+$ (1 μmole), glucose 6-phosphate (5 μmoles), and glucose 6-phosphate dehydrogenase (1 Kornberg unit). After 4–5 hr of incubation at 37°, the radioactive materials were extracted with methylene chloride with 94–98% recovery and analyzed by paper chromatography in the

* From the Department of Biochemistry and Department of Dermatology, University of Miami School of Medicine, Miami, Florida. *Received November 22, 1965; revised February 2, 1966.* A preliminary report of this work was presented at the meeting of the Federation of American Societies of Experimental Biology in Atlantic City, N. J., April 1965. This investigation was supported by Career Development Award 1-K3-AM-28,095, grants (AM-09497 and AM-07138) from the National Institute of Arthritis and Metabolic Diseases of the National Institutes of Health, U. S. Public Health Service, and by funds of the Dermatology Foundation of Miami.

[1] The following abbreviations and trivial names are used: TPN$^+$ and TPNH, oxidized and reduced triphosphopyridine nucleotide; DPNH, reduced diphosphopyridine nucleotide; cortisol, Δ^4-pregnene-11β,17α,21-triol-3,20-dione; cortisone, Δ^4-pregnene-17α,21-diol-3,11,20-trione; allodihydrocortisol, 5α-pregnane-11β,17α,21-triol-3,20-dione; allotetrahydrocortisol, 5α-pregnane-3α,11β,17α,21-tetraol-20-one; dihydrocortisol, 5β-pregnane-11β,17α,21-triol-3,20-dione; tetrahydrocortisol (THF), 5β-pregnane-3α,11β,17α,21-tetraol-20-one; tetrahydrocortisone (THE), 5β-pregnane-3α,17α,21-triol-11,20-dione; Reichstein's substances E, Δ^4-pregnene-11β,17α,20β,21-tetraol-3-one; epi-E, Δ^4-pregnene-11β,17α,20α,21-tetraol-3-one; U, Δ^4-pregnene-17α,-20β,21-triol-3,11-dione; epi-U, Δ^4-pregnene-17α,20α,21-triol-3,11-dione.

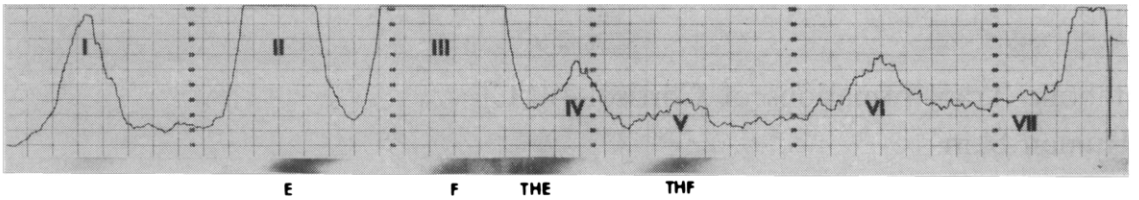

FIGURE 1: Chromatographic behaviors of radioactive metabolites. The radioactive metabolites obtained after incubation of cortisol-4-[^{14}C] with human foreskin were chromatographed together with four reference steroids: cortisone (E), cortisol (F), tetrahydrocortisone (THE), and tetrahydrocortisol (THF). The distribution of ^{14}C was traced by an autoscanner and the reference steroids were visualized by staining with blue tetrazolium.

system of methanol–benzene–water–ethyl acetate (1:1:1:0.1) (Fukushima *et al.*, 1960). For determinations of specific activity, between 300 and 700 μg of the crystals was weighed on a Cahn gram electrobalance and the radioactivity of the sample assayed with a Tri-Carb liquid scintillation counter, Model 314EX. The efficiency for counting ^{14}C was 43%.

Chromatographic Behaviors of Metabolites. In an exploratory experiment for possible identification of the metabolites, *ca.* 200 μg each of cortisone, cortisol, tetrahydrocortisone, and tetrahydrocortisol were chromatographed together with the radioactive metabolites obtained after incubation of cortisol-4-[^{14}C] with foreskin. The distribution of radioactivity was traced with a Vanguard autoscanner 880. The locations of the four reference steroids were visualized by staining with a 0.2% solution of blue tetrazolium in 10% NaOH. Figure 1 is a photograph of the results. The tracing exhibits seven distinct peaks which are designated with Roman numerals in order of decreasing mobility. Peak I, which is immediately behind the solvent front, probably indicates decomposition products of cortisol-4-[^{14}C]; in experiments with freshly purified cortisol-4-[^{14}C], peak I was found to diminish in size. A faint stain with blue tetrazolium is also observable in this area, indicating decomposition products or impurities of the reference steroids. Peak II is similar to the mobility of cortisone (E), peak III to that of cortisol (F), and peak V to that of tetrahydrocortisol (THF). Tetrahydrocortisone (THE) has chromatographic mobility between peaks III and IV. Peak VI indicates a metabolite which is more hydrophilic than tetrahydrocortisol. The significance of peak VII is not clear as its size varies from experiment to experiment.

The tracing shown in Figure 1 is typical of the results obtained after incubation with foreskin. Of the many specimens of foreskin examined, including more than 20 from the newborn, three from prepubertal boys (ages 9, 11, and 13), all produced similar chromatograms in which peak IV was obvious. In experiments with skin from other anatomical sites, including the thigh, the arm, the neck, the abdominal wall, and the sole from both sexes of ages from 5 months to 92 years, peak IV was not apparent while the other peaks were comparable to those in Figure 1. Exemplifying chromatograms were published previously (Hsia *et al.*, 1964, 1965).

Identification of Unmetabolized Cortisol-4-[^{14}C]. The radioactive material from the center of peak III was eluted off the paper and an aliquot containing 2.95×10^5 dpm was diluted with 23.7 mg of carrier cortisol to give a calculated specific activity of 451 dpm/μmole. The mixture was dissolved in *ca.* 2 ml of a mixture of methanol and methylene chloride; crystallization was brought about by the gradual addition of hexane. After the crop of crystals ceased to increase, the mother liquor was carefully decanted and the crystals were washed with hexane. The crystallization was repeated and the crystals were then dried to constant weight under vacuum at 60°. A small sample of the dried crystals (408 μg) was weighed and assayed for ^{14}C and the specific activity was found to be 451 dpm/μmole. After two subsequent crystallizations from a mixture of acetone, benzene, and hexane, the specific activity was 444 dpm/μmole. The crystalline cortisol (12.4 mg) was then acetylated with a mixture of 0.5 ml of pyridine and 0.5 ml of acetic anhydride. After two crystallizations from a mixture of methylene chloride, acetone, and hexane and two more crystallizations from a mixture of acetone, methanol, and hexane, the specific activity of cortisol acetate remained constant at 448 dpm/μmole. Comparison of the calculated specific activity with the observed values indicates that practically all the radioactive material examined could be unmetabolized cortisol-4-[^{14}C].

In more than 50 experiments, the amount of ^{14}C in peak III was 85–90% of the chromatographed radioactivity, indicating 10–15% of the labeled steroid could have been metabolized and were distributed among the other peaks.

Identification of Metabolites. The amounts of ^{14}C in the other peaks were estimated by a Vanguard autoscanner 880 with automatic data system and expressed in percentage of the chromatographed radioactivity. Results of the many experiments were in the following ranges: peak II, 3–8%; IV, 1–1.5% (with foreskin only); V, 1–1.5%; and VI, 1–2%.

The radioactivity in each peak was eluted with methanol. The eluates of the same peak from several paper strips were pooled and chromatographed once

more in the above described system. Aliquots of the rechromatographed radioactive materials were diluted with carrier steroids. The specific activities were determined and the acetates prepared in the same manner as in the identification of the unmetabolized cortisol.

PEAK II. In the examination of the ^{14}C in peak II obtained from the incubations with foreskin it was found that 50.6% of the ^{14}C could be in cortisone. In addition, 46% of the ^{14}C could be in allodihydrocortisol. The details of determination of the specific activities of these two compounds and their acetates are shown in Tables I and II.

In an experiment with ^{14}C of peak II from the incubation with skin of the abdominal wall, the amount of ^{14}C in cortisone was found to be 73%. Although the data in Table II established the formation of allodihydrocortisol as a metabolite of the foreskin, formation of this compound in skin of other anatomical sites could not be demonstrated. In one experiment, allodihydrocortisol (31.2 mg) was added to an aliquot of the ^{14}C (3.54×10^4 dpm) from peak II of a chromatogram obtained from a study with skin of an amputated leg. The calculated specific activity was 414 dpm/μmole. The specific activity decreased after repeated crystallizations and further decreased after the preparation of the acetate. A value of 15 dpm/μmole was obtained after the last crystallization. The experiment was repeated with 22.3 mg of allodihydrocortisol and 5870 dpm of ^{14}C from peak II obtained in an experiment with skin of the abdominal wall. The calculated specific activity was 96 dpm/μmole. The observed specific activity declined after several crystallizations. It declined to 10 dpm/μmole after the preparation of the acetate and then to 7 dpm/μmole after the last crystallization.

The 5β-isomer, dihydrocortisol, also has chromatographic mobility corresponding to peak II. In repeated experiments with skin from several sites, its presence as a metabolite could not be demonstrated.

PEAK IV. The identification of allotetrahydrocortisol with the radioactive material in peak IV is supported by data present in Table III. The data indicate that 78% of the ^{14}C examined could be in tetrahydrocortisol.

PEAK VI. An aliquot containing 4.10×10^4 dpm from peak VI in 10 ml of methanol was treated with 25 mg of

TABLE I: Identification of Cortisone.[a]

Solvents	No. of Crystn	Wt of Crystals (mg)	Sp Activ (dpm/μmole)
Cortisone			
Methanol–hexane	2	18.4	270
Acetone–hexane	2	16.3	270
Acetate			
Methylene chloride–hexane	4	10.5	174
Acetone–hexane	2	7.5	155
	2	4.0	159

[a] Cortisone (21.7 mg) was added to an aliquot containing 1.88×10^4 dpm of ^{14}C from the area of peak II after the incubation of cortisol-4-[^{14}C] with foreskin. The calculated specific activity was 312 dpm/μmole. These data indicate that 50.6% of the ^{14}C examined could be in cortisone.

TABLE II: Identification of Allodihydrocortisol.[a]

Solvents	No. of Crystn	Wt of Crystals (mg)	Sp Activ (dpm/μmole)
Allodihydrocortisol			
Methylene chloride–methanol–hexane	1	29.7	366
	2	22.5	334
Acetone–methanol–hexane	2	16.5	305
Methylene chloride–methanol–hexane	2	15.6	299
Acetone–methanol–hexane	1	8.3	304
Acetate			
Acetone–methanol–hexane	1	3.7	295
	1	2.0	300

[a] Allodihydrocortisol (63.4 mg) was added to an aliquot containing 1.14×10^5 dpm of ^{14}C from the area of peak II after the incubation of cortisol-4-[^{14}C] with foreskin. The calculated specific activity was 652 dpm/μmole. These data indicate that 46% of the ^{14}C examined could be in allodihydrocortisol.

TABLE III: Identification of Allotetrahydrocortisol.[a]

Solvents	No. of Crystn	Wt of Crystals (mg)	Sp Activ (dpm/μmole)
Allotetrahydrocortisol			
Methanol–hexane	1	17.0	261
Acetone–hexane	1	13.3	253
Methanol–hexane	1	9.4	251
Diacetate			
Benzene–hexane	2	5.6	246
	1	4.2	249

[a] Allotetrahydrocortisol (25.6 mg) was added to an aliquot containing 2.22×10^4 dpm of ^{14}C from the area of peak IV after the incubation of cortisol-4-[^{14}C] with foreskin. The calculated specific activity was 318 dpm/μmole. These data indicate that 78% of the ^{14}C examined could be in allotetrahydrocortisol.

$HIO_4 \cdot 2H_2O$ in 1 ml of 0.1 N H_2SO_4. The mixture was kept at room temperature overnight and was then diluted with water and extracted with ether. The extracted ^{14}C (3.80×10^4 dpm) was mixed with 50 μg of Δ^4-androsten-11β-ol-3,17-dione and subjected to paper chromatography. The majority of the ^{14}C was found in the area of peak I together with the reference steroid which was visualized under ultraviolet light. After elution from the paper, 3.04×10^4 dpm was diluted with 26.8 mg of Δ^4-androsten-11β-ol-3,17-dione. The specific activity remained constant at 281 dpm/μmole after repeated crystallizations. The data indicated that 83% of the ^{14}C examined could be in Δ^4-androsten-11β-ol-3,17-dione.

The radioactive metabolite apparently was oxidized by periodic acid to form Δ^4-androstene-11β-ol-3,17-dione by scision of the side chain, giving evidence that the metabolite has the same nucleus as cortisol, and that the metabolic change was in the side chain. Reduction of the 20-one to form the 20-ols (Reichstein's substances E and epi-E) was a distinct possibility.

Consequently aliquots from peak VI were diluted with Reichstein's substances E and epi-E. Tables IV and V show the specific activities of the two compounds after repeated crystallizations and after the formation of their acetates. The data indicate that 70% of the ^{14}C examined could be in substance E and 26% could be in epi-E.

TABLE IV: Identification of Reichstein's E.

Solvents	No. of Crystn	Wt of Crystals (mg)	Sp Activ (dpm/μmole)
Reichstein's E			
Acetone–hexane	4	21.8	278
	1	19.9	265
Diacetate			
Benzene–hexane	2	9.6	234
Acetone–hexane	2	7.7	231
	2	6.0	227

[a] Reichstein's Substance E (24 mg) was added to an aliquot containing 2.12×10^4 dpm of ^{14}C from the area of peak VI after the incubation of cortisol-4-[^{14}C] with skin of the abdominal wall. The calculated specific activity was 323 dpm/μmole. These data indicate that 70% of the ^{14}C examined could be in Reichstein's substance E.

TABLE V: Identification of Reichstein's epi-E.[a]

Solvents	No. of Crystn	Wt of Crystals (mg)	Sp Activ (dpm/μmole)
Reichstein's epi-E			
Methanol–acetone–hexane	2	18.5	514
	2	16.5	519
Diacetate			
Acetone–hexane	2	10.8	264
	2	10.0	252
Methylene chloride–methanol–hexane	2	9.2	235
Acetone–hexane	4	6.8	237

[a] Reichstein's epi-E (19.7 mg) was added to an aliquot containing 4.98×10^4 dpm of ^{14}C from the area of peak VI after the incubation of cortisol-4-[^{14}C] with skin of the abdominal wall. The calculated specific activity was 921 dpm/μmole. These data indicate that 26% of the ^{14}C examined could be in Reichstein's substance epi-E.

PEAK V. Tetrahydrocortisol which is a major urinary metabolite of cortisol has chromatographic mobility similar to that of peak V (Figure 1). Its presence in peak V was examined. An aliquot containing 1.59×10^4 dpm from peak V was diluted with 28.2 mg of tetrahydrocortisol to give a calculated specific activity of 207 dpm/μmole. The material was crystallized repeatedly and the specific activity declined. After the preparation of the acetate, the specific activity further declined and a value of 8.8 dpm/μmole was obtained after the last crystallization. Thus the presence of tetrahydrocortisol in peak V could not be established.

TABLE VI: Identification of Reichstein's U.[a]

Solvents	No. of Crystn	Wt of Crystals (mg)	Sp Activ (dpm/μmole)
Reichstein's U			
Ether–methanol–acetone–benzene–hexane	2	19.6	152
Benzene–methanol–ether–hexane	2	17.3	153
Diacetate			
Acetone–methanol–benzene–hexane	2	8.3	69.2
Methylene chloride–hexane	2	5.2	62.3
Acetone–hexane	2	4.4	62.0

[a] Substance U (23.2 mg) was added to an aliquot containing 1.59×10^4 dpm of ^{14}C from the area of peak V after the incubation of cortisol-4-[^{14}C] with skin of abdominal wall. The calculated specific activity was 249 dpm/μmole. These data indicate that 25% of the ^{14}C examined could be in Reichstein's substance U.

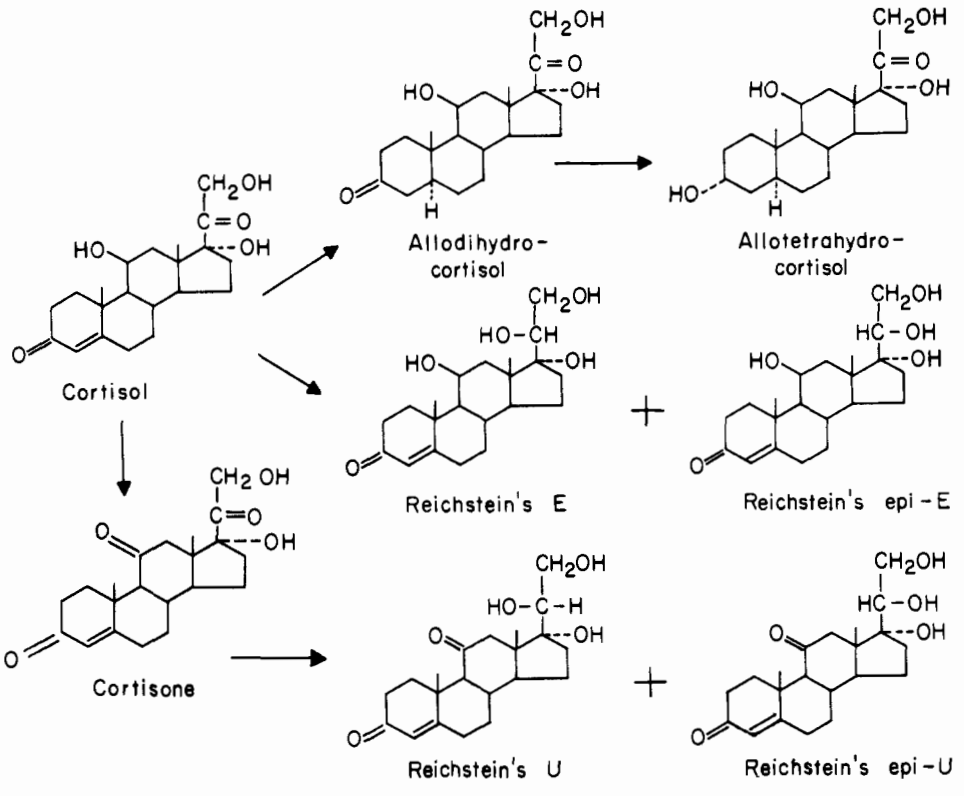

FIGURE 2: Metabolic transformations of cortisol in human skin. Formation of allodihydrocortisol and allotetrahydrocortisol has been confirmed only in foreskin. Oxidation of 11β-ol and reduction of 20-one are common to skin of all anatomical sites studied.

Since Reichstein's E and epi-E were identified as metabolites in peak VI, the possibility of their 11-keto homologs, namely Reichstein's substances U and epi-U, being in peak V was tested by isotopic dilution. Tables VI and VII present the results which indicate that 26% of the ^{14}C examined could be in substance U and 14% could be in substance epi-U.

Discussion

The identification of cortisone as a major metabolite of cortisol in human skin confirms an earlier observation by Malkinson *et al.* (1959), who reported the formation of a compound with chromatographic mobility similar to that of cortisone after incubation of cortisol with human skin. Oxidation of the 11β-ol of cortisol to form cortisone seems to be a common pathway in the metabolism of cortisol. It has been demonstrated in rat liver (Schneider and Horstmann, 1952; Hurlock and Talalay, 1959), in rat kidney, and other tissues (Hurlock and Talalay, 1959; Mahesh and Ulrich, 1960; Berliner *et al.*, 1958; Ganis *et al.*, 1956), and in human placenta (Osinski, 1960). Cortisone has also been identified as a minor urinary metabolite of cortisol in man (Fukushima *et al.*, 1960; Burstein *et al.*, 1953).

The other metabolites identified in this study are the 20-ols: Reichstein's substances E, epi-E, U, and epi-U. The two 20β-ols, E and U, were previously reported as metabolites of cortisol in eviscerated rats (Berliner *et al.*, 1958) and in cultured human fibroblasts (Sweat *et al.*, 1958). The 20α-ol epi-E was identified as a metabolite of cortisol in loose connective tissue of the rat (Berliner and Dougherty, 1958). In the present study the two 20β-steroids were found in greater amounts than their 20α-epimers. Whether one or more enzymes are responsible for their formation awaits further investigation.

It is of interest that allodihydro- and allotetrahydrocortisol are metabolites of the foreskin but their formation could not be established after incubation of cortisol with skin of other anatomical sites. This metabolic difference is an example of the biochemical heterogeneity of the skin.

The results of this study may be compared to those of a study with eviscerated rats (Berliner *et al.*, 1958), in which cortisol was converted to a number of metabolites including cortisone, Reichstein's E and U, Δ^4-androsten-11β-ol-3,17-dione, and 5β-dihydrocortisol. In the present study no 5β-steroid was detected among the metabolites, in contrast to the results of the studies with eviscerated rats (Berliner *et al.*, 1958) and rat liver (Gold and Garren, 1964). The lack of 5β-reductase in the skin is also in marked contrast with systemic metabolism where 5β-steroids constitute the major urinary metabolites of cortisol in man (Fukushima *et al.*, 1960; Peterson *et al.*, 1955; Nadel, 1956).

TABLE VII: Identification of Reichstein's epi-U.[a]

Solvents	No. of Crystn	Wt of Crystals (mg)	Sp Activ (dpm/μmole)
Reichstein's epi-U			
Acetone–benzene–methanol–hexane	3	22.0	40.6
	7	16.1	41.3
Diacetate			
Acetone–hexane	2	14.9	42.0
	2	10.3	39.5
Methylene chloride–hexane	2	9.2	35.9
Acetone–hexane	2	8.4	34.1

[a] Reichstein's epi-U (24.2 mg) was added to an aliquot containing 1.59×10^4 dpm of ^{14}C from the area of peak V after the incubation of cortisol-4-[^{14}C] with skin of abdominal wall. The calculated specific activity was 239 dpm/μmole. These data indicate that 14% of the ^{14}C examined could be in Reichstein's substance epi-U.

Since less than half of the ^{14}C in the area of peak V could be accounted for by Reichstein's U and epi-U, and the significance of peak I and peak VII has not been investigated, other metabolites of cortisol in the skin yet remain to be identified in further studies. The results of the present study, nonetheless, have established the major metabolites and suggest that oxidation of the 11β-ol and reduction of the 20-one constitute the two general pathways of metabolism of cortisol in human skin. A third pathway which so far has been found only in the foreskin is the formation of allodihydro- and allotetrahydrocortisol. These transformations may be summarized as in Figure 2.

Since skin is one of the largest organs of the body, its contribution to systemic metabolism may be significant. Transport of cortisol to and from the body surface through the skin has been demonstrated. After topical application of cortisol-4-[^{14}C] to human subjects, small amounts of ^{14}C were detectable in the urine (Malkinson and Ferguson, 1955), and ^{14}C was found in skin surface lipids following intravenous administration of cortisol-4-[^{14}C] (Cook and Spector, 1964). Whether cortisol is transported through the skin unchanged, partially, or totally metabolized has not been studied, but it is likely that cortisol as well as the skin metabolites can enter the general metabolic pool. Since Reichstein's E, epi-E, U, and epi-U have not been found in the urine, these skin metabolites may well be further metabolized to form the cortols and cortolones which are excreted in the urine.

Acknowledgments

We wish to express our thanks to Dr. Robert Silber of Merck Institute for Therapeutic Research for making available to us samples of tetrahydrocortisol and tetrahydrocortisone and to Dr. James Ahlberg of G. D. Searle and Co. for a sample of allotetrahydrocortisol. We are indebted to Mr. Roger Richard for his able technical assistance throughout this work.

References

Berliner, D. L., and Dougherty, T. F. (1958), *Proc. Soc. Exptl. Biol. Med. 98*, 3.

Berliner, D. L., Grosser, B. I., and Dougherty, T. F. (1958), *Arch. Biochem. Biophys. 77*, 81.

Burstein, S., Savard, K., and Dorfman, R. I. (1953), *Endocrinology 53*, 88.

Cook, T. J., and Spector, A. R. (1964), *J. Invest. Dermatol. 43*, 413.

Fukushima, D. K., Bradlow, H. L., Hellman, L., Zumoff, B., and Gallagher, T. F. (1960), *J. Biol. Chem. 235*, 2246.

Ganis, F. M., Axelrod, L. R., and Miller, L. L. (1956), *J. Biol. Chem. 218*, 841.

Gold, N. I., and Garren, L. D. (1964), *J. Biol. Chem. 239*, 2796.

Hsia, S. L., Mussallem, A. J., and Witten, V. H. (1965), *J. Invest. Dermatol.* (in press).

Hsia, S. L., Witten, V. H., and Hao, Y. L. (1964), *J. Invest. Dermatol. 43*, 407.

Hurlock, B., and Talalay, P. (1959), *Arch. Biochem. Biophys. 80*, 468.

Mahesh, V. B., and Ulrich, F. (1960), *J. Biol. Chem. 235*, 356.

Malkinson, F. D., and Ferguson, E. H. (1955), *J. Invest. Dermatol. 25*, 281.

Malkinson, F. D., Lee, M. W., and Cutukovic, I. (1959), *J. Invest. Dermatol. 32*, 101.

Nadel, E. M. (1956), *Arch. Biochem. Biophys. 61*, 144.

Osinski, P. A. (1960), *Nature 187*, 777.

Peterson, R. E., Wyngaarden, J. B., Guerra, S. L., Brodie, B. B., and Bunim, J. J. (1955), *J. Clin. Invest. 34*, 1779.

Schneider, J. J., and Horstmann, P. M. (1952), *J. Biol. Chem. 196*, 629.

Sweat, M. L., Berliner, D. L., Swim, H. E., Nabors, C. J., Jr., and Dougherty, T. F. (1958), *Biochim. Biophys. Acta 28*, 591.