PERCUTANEOUS ABSORPTION OF CORTISONE -4-C^{14} THROUGH NORMAL HUMAN SKIN*

FREDERICK D. MALKINSON, M.D., EDWARD H. FERGUSON, M.D.† AND MING C. WANG, B.S.

The early use of local cortisone preparations in dermatologic therapy proved quite disappointing (1, 2). Topical hydrocortisone preparations, however, have been fairly effective in the treatment of certain acute inflammatory dermatoses, particularly those in the eczema group (3–6). The striking therapeutic disparity between these two hormones has suggested one of two explanations: that either there are marked differences in percutaneous absorption of these compounds, or that anti-inflammatory effects depend on hydrocortisone or its metabolites, substances which the skin cannot produce from cortisone.

Since the penetration of hydrocortisone-4-C^{14} through normal human skin had been established by a previous study (7) identical with that now being reported for cortisone-4-C^{14}, it appeared that an investigation of the percutaneous absorption of cortisone might add further to our knowledge of the physiology and metabolism of these adrenal steroids in the skin.

METHOD

The studies were performed on three hospitalized patients at bed rest. Subject #1, a fifteen-year old girl with idiopathic thoracolumbar scoliosis and spondylolisthesis, had undergone surgery for spinal fusion. Subject #2 was a sixty-six-year old woman with degenerative arthritis of the right hip recently operated on for hip fusion. Subject #3 was a sixteen-year old girl who, like the first patient, had undergone a spinal fusion procedure for progressive idiopathic thoracolumbar scoliosis. All three individuals were otherwise in good health except for subject #1 who was recovering from homologous serum jaundice. This complication had developed one month following blood tranfusion received at the time of surgery, and the study with cortisone -4-C^{14} ointment was begun 13 days later when her liver function tests were as follows: serum bilirubin direct 0.79 mgm. %; serum bilirubin indirect 0.99 mgm. %; alkaline phosphatase 7.6 units %; thymol flocculation 1+; cephalin flocculation negative. The results of liver function studies for subjects #2 and #3 were normal.

7.5 microcuries of cortisone -4-C^{14} acetate‡ weighing 6.30 mgm. and having a specific activity of 0.49μc/m mole were incorporated with 6.27 mgm. of non-radioactive "carrier" cortisone acetate into 0.5 Gm. of a cholesterolized petro-

* From the Section of Dermatology, University of Chicago, Chicago, Illinois.

† Present address: Monroe Clinic, Monroe, Wisconsin.

Presented at the Seventeenth Annual Meeting of The Society for Investigative Dermatology, Inc., Chicago, Illinois, June 10, 1956.

This study was supported in part by Public Health Grant # RG-4478.

‡ This material was kindly supplied by the National Institutes of Health through Dr. Sam R. Hall.

TABLE 1

Subject no. 1

Urine (24 hours specimens)	#1	#2	#3	#4	#5	#6	#7	#8
Planchet net counts per minute (actual count minus background)	52	31	27	23	*	8	31†	N.S.‡
Calculated total disintegrations per minute (24 hour specimens)	1191	381	384	393	*	193	845†	N.S.‡

* Specimen used for another analysis—see text.

† Specimen collected the 3rd day after removal of dressing and residual ointment.

‡ Not significant. Specimen collected the 7th day after removal of dressing and residual ointment.

TABLE 2

Subject no. 2

Urine (24 hour specimens)	#1	#2	#3	#4	#5	#6	#7	#8	#9
Planchet net counts per minute (actual count minus background)	46	27	18	23	15	19	10*	N.S.†	N.S.‡
Calculated total disintegrations per minute (24 hour specimens)	853	286	303	250	252	227	192*	N.S.†	N.S.‡

* Specimen collected the 2nd day after removal of dressing and residual ointment.

† Not significant. Specimen collected the 4th day after removal of dressing and residual ointment.

‡ Not significant. Specimen collected the 10th day after removal of dressing and residual ointment.

latum base.* Application of this 2.5% cortisone ointment was made to a 39 square centimeter area of normal skin over the flexor aspect of the forearm and the material was rubbed in sufficiently to provide a thin film over the skin surface. The amounts applied were 92 mgm. of ointment containing 1.2 mgm. of cortisone -4-C^{14} acetate to subject #1, 70 mgm. of ointment containing 0.9 mgm. of cortisone -4-C^{14} acetate to subject #2 and 55 mgm. of ointment containing 0.6 mgm. of cortisone -4-C^{14} acetate to subject #3. The sites of application remained covered for 6–7 days with a perforated aluminum eye patch taped firmly to the skin, and daily 24-hour urine specimens were collected throughout this period. The dressings were then removed and the skin thoroughly cleansed of residual ointment with ether soaked cotton pledgets. Collection of 24-hour urine specimens was continued intermittently as shown in tables #1–3.

* This base contains petrolatum, cholesterol, multiwax and mineral oil. It is the standard vehicle for the hydrocortisone ointment preparations of the Upjohn Company, Kalamazoo, Michigan.

TABLE 3

Subject no. 3

Urine 24 (hour specimens)	#1	#2	#3	#4	#5	#6	#7	#8	#9	#10
Planchet net counts per minute (actual count minus background)	52	45	75	*	46	29	25	35†	11‡	N.S.§
Calculated total disintegrations per minute (24 hour specimens)	452	380	764	*	728	485	370	410†	322‡	N.S.§

* Incomplete sample.

† Specimen collected the 2nd day after removal of dressing and residual ointment.

‡ Specimen collected the 3rd day after removal of dressing and residual ointment.

§ Not significant. Specimen collected the 5th day after removal of dressing and residual ointment.

The 17-Ketosteroid fraction of the urine was isolated by the method of Koch (8) as follows:

1) 38% hydrochloric acid was added to each 24-hour urine specimen in an amount equal to 10% of the volume of the specimen. The resultant mixture was boiled for 15 minutes under a reflux condenser and allowed to cool.

2) 500 cc. aliquots of the acidified urine were extracted 3 times for 3 minutes each with 75 cc. of anhydrous absolute ether.

3) The ether extraction series were then combined and washed successively with distilled water, a saturated solution of sodium bicarbonate, a 10% solution of sodium hydroxide, 2N sulfuric acid, and again with distilled water.

4) The ether was distilled off and the residue redissolved in absolute alcohol. After the alcohol was blown off with nitrogen, the remaining extract was weighed, and an aliquot of known weight was transferred to a planchet for measurement of radioactivity in a gas flow counter.

Disintegrations per minute were calculated for each total extract on a weight basis following determinations of net counts per minute for the planchet sample.

RESULTS

Within 24 hours after application of the 4-C^{14} labeled cortisone, significant radioactivity was found in the urine of the 3 individuals tested (see tables #1–3). Subjects #1 and #2, furthermore, showed their peak excretion of radioactive material during this first 24-hour period. Each of the 3 subjects showed continuing but relatively low levels of activity in all urine extracts for the 6–7 period in which the ointment sites remained covered. After removal of the ointment, radioactivity was still observed in urine extracts up to 3 days later, but beyond that time significant activity was not detectable in the urine of any of the subjects tested. Examination by enzymatic hydrolysis (9) of the 5th 24-hour urine specimen from subject #1 revealed that after paper chromatography and elution

tetrahydrocortisone had a specific activity of 0.0233 μc/m mole.* This analysis clearly indicated the presence of radioactivity in a urinary compound which is probably the chief excretion product of cortisone.

In 2 instances (subjects ⁂2 and ⁂3) ether recovery of some of the removed ointment showed high radioactivity, indicating that appreciable amounts of cortisone-4-C^{14} remained on the skin surface throughout the test period.

The moderate impairment in liver function tests noted in the first patient seemed to produce no appreciable effects on the excretion pattern observed. Also of interest in this individual was the suddenly increased activity found in extract ⁂7. This extract was prepared from the first urine specimen obtained 3 days after vigorous removal of the ointment from the skin surface with ether-soaked cotton pledgets. In subject ⁂3 there was also a suggestion of a rise in cortisone excretion after cleansing of the treated skin site with ether. Here extract ⁂8, obtained 48 hours after ointment removal, showed maintenance of previous activity levels where a sharp drop would ordinarily have been expected. No increase in urine activity was seen in subject ⁂2 after removal of the ointment.

The excretion pattern observed in subject ⁂3 varied somewhat from that seen in the other persons studied. There was no distinctly high 24-hour excretion peak, and the period of greatest radioactivity in the urine was found on the 3rd and 5th days rather than during the first 24 hours. In this regard it should be mentioned that the changes in urinary activity coincided with sharp fluctuations in urine volume. The patient's urine output was 600 cc. for each of the first 24-hour periods following self-imposed restriction of fluids because of discomfort in using the bedpan. From the 3rd day fluids were forced and the urine output doubled. It has been stated (10) however, that daily excretion of 17-hydroxy-corticoids is largely independent of urine volume, so the importance of this observation and the significance of the different excretion pattern seen in this patient remain undetermined.

DISCUSSION

The previous study with topically applied hydrocortisone-4-C^{14} (7) showed some urinary radioactivity in the first 24-hour period, peak excretion of active material in the *second* 24 hours, and continued low levels of urinary excretion until the ointment was removed from the skin surface. The urinary excretion pattern of topically applied cortisone -4-C^{14}, then, is similar to hydrocortisone except for peak excretion in the *first* rather than the second 24-hour period. The occurrence of highest urinary activity at different time intervals is consistent with the findings of Sandberg *et al* (10) that after oral administration of these hormones the steroid degradation products of cortisone appeared in the urine at a more rapid rate than the degradation products of hydrocortisone.

The distinct elevation in excretion of both hormones shortly after application, and the rise in cortisone -4-C^{14} excretion after vigorous removal of the ointment probably reflect transfollicular absorption from mechanical introduction of radioactive hormone into the hair follicles. The persistent but lower levels of

* This analysis was carried out by Dr. Harold Werbin, Argonne Cancer Research Hospital.

urine activity throughout the period of ointment contact with the skin indicate continuous transepidermal or transfollicular absorption of hormone. This assumption is supported by the recovery from subjects #2 and #3 of appreciable quantities of cortisone -4-C^{14} still present in the ointment 6–7 days after application. Scott and Kalz (11) have conclusively shown by autoradiography that percutaneous absorption of hydrocortisone-4-C^{14} occurs by both the transepidermal and transfollicular routes, and the present investigation indicates that cortisone is similarly absorbed.

Finally, both the hydrocortisone-4-C^{14} and cortisone-4-C^{14} studies show that only a small fraction of the applied hormones—less than 1% by the described procedure—could be demonstrated in the urine.

Since cortisone and hydrocortisone are now shown to be similarly absorbed qualitatively and quantitatively through the skin, it is apparent that the contrasting therapeutic effectiveness of these hormones is due to differences in their metabolism by the skin. In this regard the work of Wilson and her associates (12, 13) is of great interest. It has been found that hydrocortisone is far more active than cortisone in suppressing inflammation when injected into joints with synovial effusions (14, 15) although both steroids are equally effective in the treatment of rheumatoid arthritis when given systemically. Wilson *et al* (12, 13) demonstrated that the synovial cavity contains enzyme systems capable of producing a variety of steroid metabolites following local injection of either cortisone or hydrocortisone, but that the pattern of the metabolites as indicated by paper chromatography was quite different with these two hormones. Some conversion of cortisone to hydrocortisone occurred in the synovial cavity but, since cortisone does not have anti-inflammatory activity in rheumatoid synovial tissue, the authors concluded that the local effectiveness of hydrocortisone probably involves the low yield production of a metabolite specific for this hormone.

Only comparable studies of the cutaneous metabolism of cortisone and hydrocortisone will provide the answer to the distinctly different topical therapeutic effects obtained with these two hormones. The isolation and identification of the postulated anti-inflammatory steroid metabolites in the skin should be of considerable significance.

SUMMARY

1) Percutaneous absorption of cortisone through normal human skin has been established by detection of radioactivity in the urine following topical application of cortisone-4-C^{14} acetate incorporated into an ointment base. Peak excretion of cortisone occurred in the first 24 hours after application, but lower levels of radioactivity persisted throughout the 6–7 day experimental period.

2) Comparison with previous studies of the percutaneous absorption of hydrocortisone-4-C^{14} indicates that penetration of cortisone and hydrocortisone into the skin are closely similar, qualitatively and quantitatively.

3) From the latter observation the inefficacy of topical cortisone therapy, in contrast to hydrocortisone, implies formation in the skin of one or more potent anti-inflammatory metabolites specific for hydrocortisone.

REFERENCES

1. Goldman, L., Thompson, R. G. and Trice, E. R.: Cortisone acetate in skin disease; local effect in skin from tropical application and local injection. Arch. Dermat. & Syph., **65:** 177, 1952.
2. Sulzberger, M. B. and Baer, R. L.: Present Status of ACTH, Cortisone, and Compound F in Dermatologic Management: A Guide for the General Practitioner. In: Year Book of Dermatology and Syphilology, 1952, p. 7–21. Chicago, The Year Book Publishers, 1953.
3. Sulzberger, M. B. and Witten, V. H.: The effect of topically applied compound F in selected dermatoses. J. Invest. Dermat., **19:** 101, 1952.
4. Sulzberger, M. B., Witten, V. H. and Smith, C. C.: Hydrocortisone (Compound F) acetate ointment in dermatological therapy. J. A. M. A., **151:** 468, 1953.
5. Robinson, Jr., H. M. and Robinson, R. C. V.: Treatment of dermatoses with local application of hydrocortisone acetate. J. A. M. A., **155:** 1213, 1954.
6. Malkinson, F. D. and Wells, G. C.: Clinical experience with hydrocortisone ointment. Brit. J. Dermat., **66:** 300, 1954.
7. Malkinson, F. D. and Ferguson, E. H.: Preliminary and Short Report: Percutaneous absorption of hydrocortisone-4-C^{14} in two human subjects. J. Invest. Dermat., **25:** 281, 1955.
8. Landau, R. L.: Diagnostic significance and laboratory methods in determination of the 17-ketosteroids. Am. J. Clin. Path., **19:** 424, 1949.
9. Glenn, E. M. and Nelson, D. H.: Chemical method for the determination of 17-hydroxycorticosteroids and 17-ketosteroids in urine following hydrolysis with β-glucuronidase. J. Clin. Endocrinol. and Metab., **13:** 911, 1953.
10. Sandberg, A. A., Nelson, D. H., Glenn, M. E., Tyler, F. H. and Samuels, L. T.: 17-hydroxycorticosteroids and 17-ketosteroids in urine of human subjects: Clinical application of a method employing β-glucuronidase hydrolysis. J. Clin. Endocrinol. and Metab., **13:** 1445, 1953.
11. Scott, A. and Kalz, F.: The penetration and distribution of C^{14}-hydrocortisone in human skin after its topical application. J. Invest. Dermat., **26:** 149, 1956.
12. Wilson, H., Glyn, J., Scull, E., McEwen, C. and Ziff, M.: Rate of disappearance and metabolism of hydrocortisone and cortisone in the synovial cavity in rheumatoid arthritis. Proc. Soc. Exper. Biol. & Med., **83:** 648, 1953.
13. Wilson, H., Fairbanks, R., Scialabba, D., McEwen, C. and Ziff, M.: Metabolites of hydrocortisone and cortisone in synovial fluid in rheumatoid arthritis. J. Clin. Endocrinol. and Metab., **16:** 87, 1956.
14. Hollander, J. L., Broun, Jr., E. M., Jessar, R. A. and Broun, C. Y.: Hydrocortisone and cortisone injected into arthritic joints: Comparative effects of and use of hydrocortisone as a local anti-arthritic agent. J. A. M. A., **147:** 1629, 1951.
15. Ziff, M., Scull, E., Ford, D., McEwen, C. and Bunin, J. J.: Effects in rheumatoid arthritis of hydrocortisone and cortisone injected intra-articularly. Arch. Int. Med., **90:** 774, 1952.

DISCUSSION

Dr. Leon Goldman (Cincinnati, Ohio): This is a very interesting and a very important study. It indicates the necessity for continuing studies of this type. There is a question about local injection of cortisone into the guinea pig skin because local injection here does inhibit local inflammatory reaction. We did a few experiments with local injection of cortisone into the skin of guinea pigs and chromatograms in our experiments showed only cortisone. We thought from these studies that the guinea pig skin was unable to metabolize cortisone to hydrocortisone.