Disposition of Intra-articularly Injected Cortisone and Hydrocortisone

By RALPH E. PETERSON, ROBERT L. BLACK AND JOSEPH J. BUNIM

Cortisone and hydrocortisone labeled with C^{14} were found to disappear from the synovial cavity rapidly and at the same rate when injected intra-articularly. Only a small portion of injected steroid was metabolized locally. Transformation of cortisone to hydrocortisone by synovial tissue was not observed.

Esseva constatate que cortisona e hydrocortisona, marcate con C^{14}, dispare rapidemente ab le cavitate synovial. Illos dispare con le mesme rapiditate como post lor injection intra-articular. Solmente un micre portion del injicite steroides esseva metabolisate localmente. Le transformation de cortisona in hydrocortisona per histos synovial non esseva observate.

THE INTRA-ARTICULAR injection of hydrocortisone and hydrocortisone acetate is followed by a decrease or disappearance of pain and swelling of the affected joint and reduction in intra-articular temperature in most patients with rheumatoid arthritis.[1-3] Conversely, cortisone acetate and cortisone administered intra-articularly are ineffective as local anti-inflammatory agents. Differences in solubility probably do not account for this disparity, since both the acetate derivatives of hydrocortisone and cortisone are less soluble in aqueous fluids than either of the parent steroids.[4] Nor do differences in the rate of disappearance (diffusion) plus metabolism of the two steroids from the synovial cavity explain the divergence in anti-inflammatory activity, since studies have revealed no significant difference in this parameter.[5]

Cortisone and its acetate, when administered systemically, are effective anti-inflammatory agents, being approximately two-thirds as active as hydrocortisone on a weight basis.[6,7] However, when so administered, cortisone and its acetate are both very rapidly transformed to hydrocortisone, presumably in the liver.[8] The possibility exists that cortisone is biologically inactive until transformed to hydrocortisone, and that the synovium is unable to effect such a transformation. In an earlier study using chromatographic methods, hydrocortisone was found in the synovial fluid of one of two patients following the intra-articular injection of cortisone.[9,10] The present study was undertaken to re-examine this question with the use of isotopic technics.

METHODS

Two patients with active rheumatoid arthritis and moderate effusions in the knees were used for these studies. In one subject (G. M. I, fig. 1), 0.1 mg. cortisone-4-C^{14} (specific activity 2,800 counts per minute per μg.) dissolved in 5 ml. of 5 per cent ethanol in sterile normal saline was injected into one knee, which was then flexed and massaged repeatedly to effect mixing of the steroid with the synovial fluid. Samples of synovial fluid (1 to 3

From the National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Bethesda, Md.

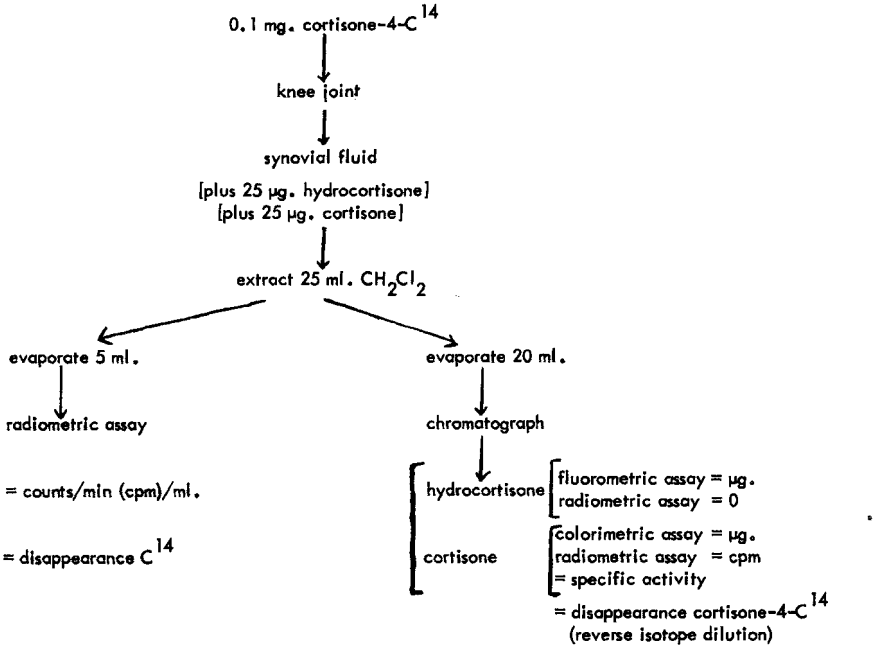

Fig. 1.—Flow sheet for cortisone and hydrocortisone assay in synovial cavity in patient G. M. I.

ml.) were withdrawn at various time intervals up to 4 hours following injection, and added to heparin. Twenty-five µg. hydrocortisone and 25 µg. cortisone were added to each of the samples of fluid, which were then diluted to a volume of 5 ml. with water and extracted with 25 ml. of dichloromethane. Five ml. aliquots of the solvent extracts were evaporated to dryness and counted in the Tri-Carb liquid scintillation spectrometer. The remaining solvent was evaporated to dryness and the dried extracts placed on paper and chromatographed in a modified Bush-type[11] solvent system (benzene 4: methanol 2: water 1) for 16 hours at room temperature. In this system cortisone moves about 35 cm. and hydrocortisone 20 cm. in 16 hours. The hydrocortisone was eluted ,and one aliquot assayed for hydrocortisone by the fluorescence procedure,[12] and another subjected to assay for radioactivity. From the cortisone eluate, one aliquot was taken for phenylhydrazine colorimetric assay[13] and another for assay of radioactivity. From the specific activities of the cortisone, the concentration of cortisone-4-C^{14} in each sample was determined using standard reverse isotope dilution calculations.

This same patient (G. M. II) and an additional patient (R. E.) were then studied in the following manner (fig. 2). One hundredth mg. hydrocortisone-4-C^{14} (6,200 counts/min./µg.) plus 1.0 mg. carrier hydrocortisone were mixed with 5.0 mg. cortisone in 5 per cent ethanol in sterile normal saline in a volume of 10 ml. This was injected into the knee joint space and, after mixing, samples of synovial fluid were withdrawn at various time intervals up to 3 hours (G. M. II) and 4 hours (R. E.). The samples of fluid (1 to 3 ml.) were diluted to 5 ml. with water, and cortisone-4-C^{14} (0.4 µg.) added to each sample. The samples were extracted with 25 ml. dichloromethane and divided into two portions. To a 5 ml. aliquot of this extract, 25 µg. hydrocortisone were added and the sample evaporated to dryness. After paper chromatographic purification, the hydrocortisone was eluted from the paper. One fraction of the eluates of each sample was assayed for hydrocortisone by the fluorescence procedure, and another for radioactivity. From the specific activities of the hydrocortisone, the concentration of hydrocortisone-4-C^{14} in each sample was determined using standard reverse isotope dilution calculations.

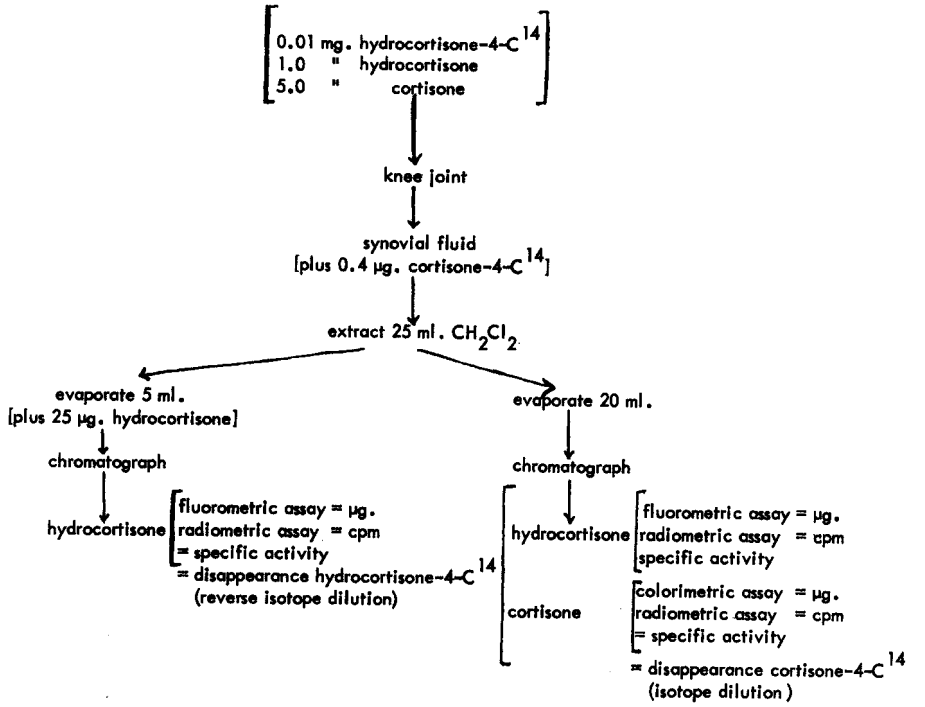

Fig. 2.—Flow sheet for cortisone and hydrocortisone assay in synovial cavity in patients G. M. II and R. E.

The remaining portion of the dichloromethane extract was evaporated to dryness and chromatographed on paper. After chromatographic purification, the hydrocortisone and cortisone fractions were eluted from the paper. Aliquots of these eluates were taken for radioactive assay. From the cortisone eluate, an aliquot was also taken for phenylhydrazine colorimetric assay, and from the hydrocortisone eluates, an aliquot was taken for fluorometric assay. From the specific activities of cortisone in the eluates, the concentration of cortisone in each sample was determined using standard isotope dilution calculations.

Results

Table 1 shows the disappearance of cortisone from the joint cavity in patient G. M. I, given cortisone-4-C^{14}. After adding carrier hydrocortisone to these samples of synovial fluid and subjecting them to paper chromatographic purification, it was not possible to detect any C^{14} in the hydrocortisone fraction. The injected cortisone-4-C^{14} in this subject disappeared from the knee joint at such a rate that one-half had diffused out in 60 minutes. Of the radioactivity present in the knee joint space at 4 hours, 87 per cent remained as cortisone. This indicated that the decline in concentration of steroid in the joint cavity was primarily the result of a diffusion of the cortisone into the tissues, that only a small fraction was metabolized by the synovium, and that no detectable quantity of hydrocortisone appeared in the synovial fluid.

Figure 3 shows the results of the studies on patients G. M. II, and R. E., given hydrocortisone-4-C^{14} plus carrier hydrocortisone and cortisone. The

Table 1.—*Disappearance of Cortisone-4-C^{14} from Synovial Cavity in Patient G. M. II*

Collection Time (hr.)	CPM/ml.* Synovial Fluid	% total counts as: Cortisone (%)	Hydrocortisone (%)
0.5	1400	95	0
1.0	1000	94	0
2.0	500	90	0
3.0	255	91	0
4.0	140	87	0

*Counts per minute per milliliter.

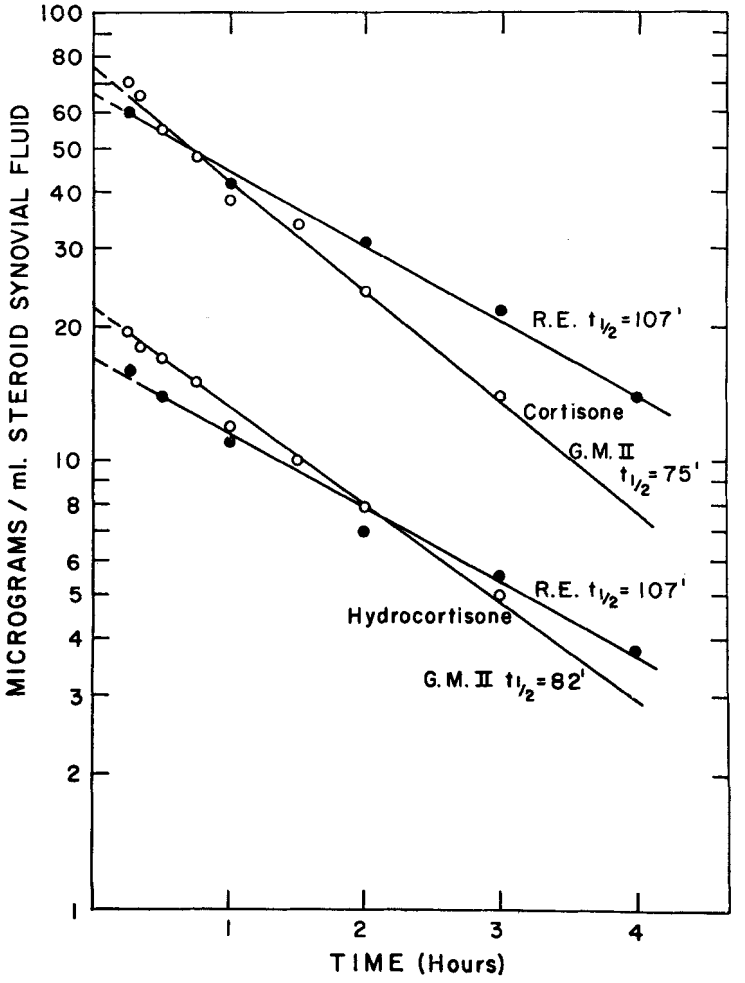

Fig. 3.—Disappearance of cortisone and hydrocortisone from knee joint cavity.

hydrocortisone-4-C^{14} as determined by reverse isotope dilution, disappeared exponentially with half-times of 107 minutes (R. E.) and 82 minutes (G. M.). Cortisone, as determined by isotope dilution, also disappeared exponentially with half-times of 107 minutes (R. E.) and 75 minutes (G. M.).

The results in both patients (G. M. II, R. E.) given intra-articular hydro-

Table 2.—*Specific Activity of Hydrocortisone after Injection of Hydrocortisone-4-C^{14} and Cortisone into Synovial Cavity*

Collection Time (hr.)	Specific Activity (CPM/μg.)* of Hydrocortisone G. M., II	R. E.
0†	62	62
0.25	65	66
0.5	64	61
1.0	63	65
2.0	61	60
3.0	62	61
4.0	—	60

*Counts per minute per microgram.

†Specific activity at zero time determined on the mixture of hydrocortisone-4-C^{14} and cortisone before injection, and after isolation of the hydrocortisone by paper chromatography.

cortisone-4-C^{14} plus carrier cortisone failed to demonstrate the conversion to hydrocortisone (table 2). The specific activity of the injected hydrocortisone was 62 counts per minute per microgram, and up to 4 hours after injection, the specific activity of the hydrocortisone showed no significant change.

Discussion

The results of this study demonstrated that cortisone and hydrocortisone disappeared from the synovial cavity at approximately the same rate. This observation had previously been made by other investigators.[5,9] Our data in one patient indicated that only a small fraction of the injected cortisone was metabolized by the synovium, and most of the steroid disappeared unchanged from the joint space, probably by a simple process of diffusion. Thus, the differences in the therapeutic effectiveness cannot be ascribed to a difference in their rates of absorption or diffusion from the synovial cavity.

Previous investigators[10] have reported that cortisone was converted to hydrocortisone by the synovium in one of two patients but not in the other. Under the conditions of our experiments we were not able to demonstrate this. These differences in results may represent individual variations, or perhaps a difference in the degree of inflammation. It must also be emphasized that the experimental design was different. In the present study 0.1 to 5 mg. of steroid were injected and aliquots of the fluid removed for analysis. In the previous work[10] 100 mg. of steroid in suspension was injected, the entire fluid removed, and the knee cavity twice "washed" out. Also, in the present study quantitative isotope technics were used to study the metabolism of the steroids, whereas chemical isolation and identification was the method used in the previous investigation.

It is well established that, when administered parenterally or orally, cortisone is rapidly converted to hydrocortisone, presumably via the liver.[8,14] Following oral or parenteral administration of cortisone or cortisone acetate, the plasma levels of hydrocortisone are about two-thirds those obtained following the oral administration of a similar quantity of hydrocortisone. By several criteria, it has been demonstrated that cortisone is about two-thirds as active as hydrocortisone when both are administered orally. Thus, most of

the physiologic activity of the administered cortisone may reside in that fraction that is converted to hydrocortisone.

Our studies show that, in contrast to the liver, the synovium may often be unable to transform cortisone to hydrocortisone, and thus this may explain the lack of biologic effectiveness (anti-inflammatory) of cortisone in the synovium. This may also be the explanation for the ineffectiveness of locally applied cortisone in inflammatory conditions of the skin.[15] Cortisone, when applied locally to the eye, has been reported to be nearly as effective as hydrocortisone;[16] however, the ocular structures are apparently able to transform cortisone to hydrocortisone.[17,18] Therefore, the steroid glucocorticoid properties (anti-inflammatory activity, pituitary-adrenal suppression, glycogen deposition) may require the presence of the 11β-hydroxy function (as in hydrocortisone), and the glucocorticoid properties of the in vivo-administered 11-dehydro steroids (such as cortisone) may depend on the presence of 11β-hydroxy dehydrogenase enzymes such as those present in the microsomal fraction in the liver.[19,20]

Summary

Intra-articularly injected cortisone and hydrocortisone were found to disappear rapidly from the knee joint cavity—$t_{1/2}$ = 60 to 107 min. It was not possible to demonstrate a conversion of the injected cortisone to hydrocortisone in two patients. It has been suggested that the ineffectiveness of cortisone locally in the joint cavity may result from the failure of the synovium to convert cortisone to hydrocortisone.

REFERENCES

1. Hollander, J. L., Brown, E. M., Jr., Jessar, R. A., and Brown, 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.
2. Ziff, M., Scull, E., Ford, D., McEwen, C., and Bunim, J. J.: Effects in rheumatoid arthritis of hydrocortisone and cortisone injected intra-articularly. A.M.A.Arch.Int.Med. *90*:774, 1952.
3. Dixon, A. St. J., and Bywaters, E. G. L.: The effect of intra-articular injection of cortisone acetate and of hydrocortisone acetate in rheumatoid arthritis. Clin.Sc. *12*:15, 1953.
4. Merck Index, ed. 6. 1952.
5. Zacco, M., Richardson, E. M., Crittenden, J. O., Hollander, J. L., and Dohan, F. C.: Disposition of intra-articularly injected hydrocortisone acetate, hydrocortisone and cortisone acetate in arthritis. J.Clin.Endocrinol. *14*:711, 1954.
6. Ward, L. E., Polley, H. F., Slocumb, C. H., and Hench, P. S.: Comparative effects of hydrocortisone, hydrocortisone acetate, and cortisone acetate in rheumatoid arthritis. J.Lab.& Clin.Med. *40*:959, 1952.
7. Boland, E. W.: Anti-rheumatic effects of hydrocortisone (free alcohol), hydrocortisone acetate, and cortisone (free alcohol), as compared with cortisone acetate; results from oral administration in patients with rheumatoid arthritis. Brit.M.J. *1*:559, 1952.
8. Peterson, R. E., Pierce, C. E., Wyngaarden, J. B., Bunim, J. J., and Brodie, B. B.: The physiological disposition and metabolic fate of cortisone in man. J.Clin.Invest. *36*: 1301, 1957.
9. 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.

10. —, Fairbanks, R., Scialabba, D., McEwen, C., and Ziff, M.: Metabolites of hydrocortisone and cortisone in synovial fluid in rheumatoid arthritis. J.Clin.Endocrinol. *16*:86, 1956.
11. Bush, I. E.: Methods of paper chromatography of steroids applicable to the study of steroids in mammalian blood and tissues. Biochem.J. *50*:370, 1952.
13. Peterson, R. E., Karrer, A., and Guerra, S. L.: Evaluation of the Silber-Porter procedure for determination of plasma hydrocortisone. Analyt.Chem. 29:144, 1957.
14. Burstein, S., Savard, K., and Dorfman, R. I.: The *in vivo* metabolism of cortisone. Endocrinology 52:448, 1953.
15. Salzberger, M. B., Witten, V. H., and Smith, C. C.: Hydrocortisone (compound F) acetate ointment in dermatology therapy. J.A.M.A. *151*:468–472, 1953.
16. Steffensen, E. H., Olson, J. A., Morgulis, R. R., Smith, R. W., and Whitney, E. L.: The experimental use of cortisone in inflammatory eye disease. Am. J.Ophthalmol. *33*:1933, 1950.
17. Hamashize, S., and Potts, A. M.: The penetration of cortisone and hydrocortisone into the ocular structures. Am.J.Ophth. *40*:211–215 (no. 5, Pt. II), 1955.
18. Bush, I. E.: Personal communication.
19. —: The 11-oxygen function in steroid metabolism. Experientia *12*:325, 1956.
20. Hurlock, B., and Talalay, P.: Microsomal 3α and 11β-hydroxysteroid dehydrogenases. Arch.Biochem.& Biophy. *80*: 468, 1959.

Ralph E. Peterson, M.D., formerly Senior Investigator, National Institute of Arthritis and Metabolic Diseases, Bethesda, Md.; presently Associate Professor of Medicine, Cornell University Medical College, New York, N. Y.

Roger L. Black, M.D., Investigator, National Institute of Arthritis and Metabolic Diseases, Bethesda, Md.; Clinical Assistant Professor of Medicine, Georgetown University, Washington, D. C.

Joseph J. Bunim, M.D., Clinical Director, National Institute of Arthritis and Metabolic Diseases, Bethesda, Md.; Associate Professor of Medicine, Johns Hopkins University, Baltimore, Md.