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Radiolytic Degradation Scheme for ^{60}Co-Irradiated Corticosteroids

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Abstract □ The cobalt 60 radiolytic degradation products have been identified in the following corticosteroids: cortisone, cortisone acetate, hydrocortisone, hydrocortisone acetate, hydrocortisone sodium succinate, isoflupredone acetate, methylprednisolone, methylprednisolone acetate, prednisolone, prednisolone acetate, and prednisone. Two major types of degradation processes have been identified: loss of the corticoid side chain on the D-ring to produce the C-17 ketone and conversion of the C-11 alcohol, if present, to the C-11 ketone. Minor degradation products derived from other changes affecting the side chain are also identified in several corticosteroids. These compounds are frequently associated in corticosteroids as process impurities or degradation compounds. No new radiolytic compounds unique to ^{60}Co-irradiation have been found. The majority of corticosteroids have been shown to be stable to ^{60}Co-irradiation. The rates of radiolytic degradation ranged from 0.2 to 1.4%/Mrad.

Keyphrases □ Corticosteroids—^{60}Co-irradiated, radiolytic degradation scheme □ Degradation—scheme for ^{60}Co-irradiated corticosteroids □ Irradiation—radiolytic degradation of corticosteroids

The U.S. Food and Drug Administration (FDA) has proposed strict limits on allowable residual quantities of ethylene oxide and its major reaction products in drugs because of possible mutagenic and carcinogenic properties of ethylene oxide (1). Faced with the possibility of increased regulatory pressure, the replacement of ethylene oxide with steam or ^{60}Co-irradiation is a major goal of a sterilization alternative program. One phase of this program is to conduct the experimental work required to demonstrate the feasibility of sterilizing bulk drugs and formulated products with ^{60}Co-irradiation.

Recently, the FDA published a proposal regulating irradiated foods for human consumption (2). The proposal permits irradiation of any food at a dose not >100 Krads without the additional safety data. The proposal also permits irradiation of foods at a dose of ≤5 Mrads if the foods comprise only a minor portion (NMT 0.01%) of the daily diet. The proposal includes guidelines for toxicological testing of other foods irradiated at a dose >100 Krads. The proposal is based on projected daily human consumption of radiolytic degradation compounds (3).

The daily dose of pharmaceutical products is substantially less than the amount of foods consumed. Information determining the rate of degradation and identifying degradation compounds unique to irradiation, therefore, should be sufficient to examine the feasibility of ^{60}Co-irradiation as an alternate method for sterilizing pharmaceutical materials and products. The present report provides such information for corticosteroids.

EXPERIMENTAL

^{60}Co-Irradiation—The irradiation source[1], consisting of several rods containing ^{60}Co-pellets mounted on a plaque, was raised from a pool of water into a stationary position for irradiation. Samples were placed in totes on two tiers surrounding the cobalt 60 source. A shuffle–dwell system averaged the radiation gradient around the source by periodically shifting the tote horizontally and vertically. Other samples were stationed at calibrated points where the radiation intensity was more precisely known.

Absorbed irradiation doses were measured potentiometrically using a ceric-cerous dosimeter[2] (4, 5) and spectrophotometrically using a red perspex dosimeter[3].

High-Performance Liquid Chromatography (HPLC) Appara-

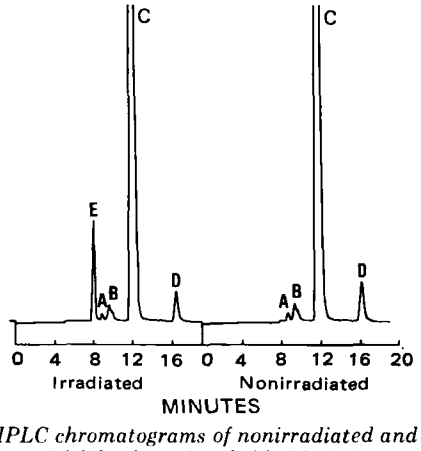

Figure 1—*HPLC chromatograms of nonirradiated and irradiated (6 Mrads) cortisone. Mobile phase: butyl chloride (50% water saturated)–tetrahydrofuran–methanol–glacial acetic acid (950:70:35:30). Column: Brownlee SI-100. Peak identification: (A) cortisone acetate; (B) hydrocortisone acetate; (C) cortisone; (D) hydrocortisone; (E) 4-androstene-3,11,17-trione.*

[1] All irradiations were conducted at a facility of Isomedix Inc., Morton Grove, Il.

[2] Atomic Energy of Canada Limited, Ottawa, Canada.

[3] United Kingdom Atomic Energy Authority, Harwell, Oxon, U.K.

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Table I—Conditions for Isolation of Cobalt 60 Radiolytic Degradation Products

Corticosteroid	Enrichment Solvent	Column[a]	Mobile Phase
Cortisone	Butyl chloride	1	Butyl chloride–tetrahydrofuran–methanol–acetic acid (950:70:35:30)
Cortisone acetate	Butyl chloride	1	Butyl chloride–tetrahydrofuran–methanol–acetic acid (880:25:25:25)
Hydrocortisone	Methyl chloride	1	Butyl chloride–tetrahydrofuran–methanol–acetic acid (950:70:35:30)
Hydrocortisone acetate	Methyl chloride	2	Acetonitrile water (35:65)
Hydrocortisone sodium succinate	Methyl chloride	2	Methanol–water (55:45)
Isoflupredone acetate	Methyl chloride	2	Acetonitrile–water (40:60)
Methylprednisolone	Methyl chloride	1	Butyl chloride–tetrahydrofuran–methanol–acetic acid (950:70:35:30)
Methylprednisolone acetate[b]	Methyl chloride	1	Butyl chloride–tetrahydrofuran–methanol–acetic acid (880:25:25:25)
Prednisolone	Methyl chloride	2	Methanol–water (55:45)
Prednisolone acetate	Methyl chloride	1	Butyl chloride–tetrahydrofuran–methanol–acetic acid (880:25:25:25)
Prednisone	Butyl chloride	2	Acetonitrile–water (40:60)
17α-Hydroxyprogesterone	Butyl chloride	2	Methanol–acetonitrile–water (30:30:40)

[a] Column: (1) Brownlee SI-100 (4.6 × 250 mm) silica gel (2) Brownlee RP-18 (4.6 × 250 mm) C_{18} bonded phase. [b] A minor degradation product was isolated by chromatography on an RP-18 column using a methanol–water (55:45) mobile phase.

Table II—Mass Balance for Methylprednisolone Peak Response[a]

Irradiation Dose, Mrads	Sample No.	Methyl-prednisolone	Methyl-prednisone	6α-Methyl-11β-hydroxy-1,4-androstadiene-3,17-dione	11β,17α-Dihydroxy-6α-methyl 1,4-androstadiene-3-one
0	1	320,060	261	986	112
	2	322,815	760	1128	214
	3	323,134	252	919	401
	$\bar{x}$	322,003	424	1011	292
2.5	1	311,492	1657	3658	805
	2	322,993	1747	3724	889
	3	316,754	1629	3932	1017
	$\bar{x}$	317,080	1681	3771	904
Difference		−4923	+1257	+2760	+662
Relative Molar Absorptivity		1.00	0.88	1.06	1.00
Corrective for Relative Molar Absorptivity[b]		−4923	+1428	+2604	+662
Relative Change, %		−1.5	+0.4	+0.8	+0.1

[a] Methylprednisolone is USP micronized, and peak response is in area per milligram. [b] An equimolar response was assumed for 11β,17α-dihydroxy-6α-methyl-1,4-androstadiene-3-one due to lack of an authentic sample.

tus—The modular liquid chromatographic system consisted of a fixed wavelength UV monitor[4], a high pressure pump[5], and a 20-μl fixed-loop injector[6] or a sample processor[7].

Chromatographic Conditions—The normal-phase chromatographic conditions used for the HPLC analysis of corticosteroids were similar to those described in another report (6). The mobile phase consisted of butyl chloride (50% water saturated)–tetrahydrofuran–methanol–glacial acetic acid (970:70:35:30) for the C-21 alcohols and 880:25:25:25 for the C-21 acetates of corticosteroids. A silica column[8] was used to chromatograph corticosteroids.

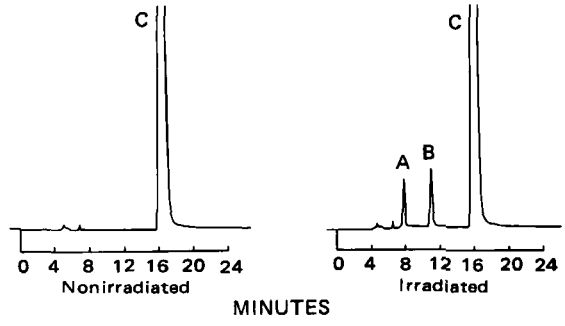


Figure 2—*HPLC chromatograms of nonirradiated and irradiated (2.5 Mrads) hydrocortisone. Mobile phase: butyl chloride (50% water saturated)–tetrahydrofuran–methanol–glacial acetic acid (950:70:35:30). Column: Brownlee SI-100. Peak identification: (A) 11β-hydroxy-4-androstene-3,17-dione; (B) cortisone; (C) hydrocortisone.*

To ensure that all major degradation products were separated, the samples were also analyzed by reversed-phase HPLC using various mixtures of acetonitrile or methanol with water on an octadecylsilane column[9]. Reversed-phase chromatographic methods for analysis of steroids have been reported (7, 8). Normal-phase conditions proved inadequate in only two cases. In hydrocortisone acetate, two degradation compounds eluted at the same retention time, but they were easily separated by reversed-phase chromatography using methanol and water (55:45) as the mobile phase. No degradation product could be eluted by normal phase HPLC for prednisone, but it was detected by reversed-

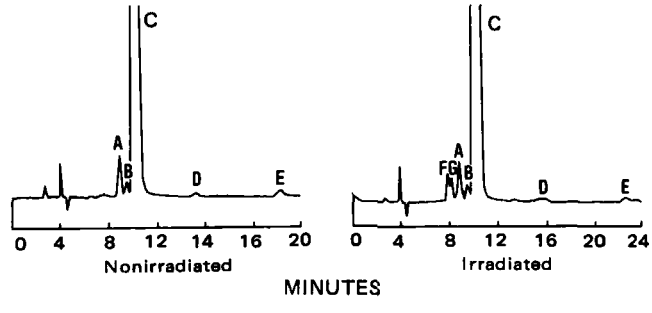


Figure 3—*HPLC chromatograms of nonirradiated and irradiated (6 Mrads) isoflupredone acetate (USP micronized). Mobile phase: butyl chloride (50% water saturated)–tetrahydrofuran–methanol–glacial acetic acid (880:25:25:25). Column: Brownlee SI-100. Peak identification: (A) 9β,11β-epoxy-17α,21-dihydroxy-1,4-pregnadiene-3,20-dione 21-acetate U-6833; (B) 9α-bromoprednisolone acetate U-6429; (C) isoflupredone acetate; (D) isoflupredone; (E) prednisolone; (F) 9α-fluoro-11β-hydroxy-1,4-androstadiene-3,17-dione; (G) 9α-fluoroprednisone acetate.*

[4] 254 nm, model 1203, UV III Monitor, Laboratory Data Control, Riviera Beach, Fla.
[5] Milton Roy Mini Pump, LDC.
[6] Model 7010, Rheodyne, Berkeley, Calif.
[7] Intelligent Sample Processor (WISP) model 710B, Waters Associates, Milford, Mass.
[8] SI-5A, 5-μm particle size, 250 × 4.6-mm i.d., Brownlee Labs., Santa Clara, Calif.
[9] RP-18, 5-μm particle size, 250 × 4.6-mm i.d., Brownlee Labs, Santa Clara, Calif.

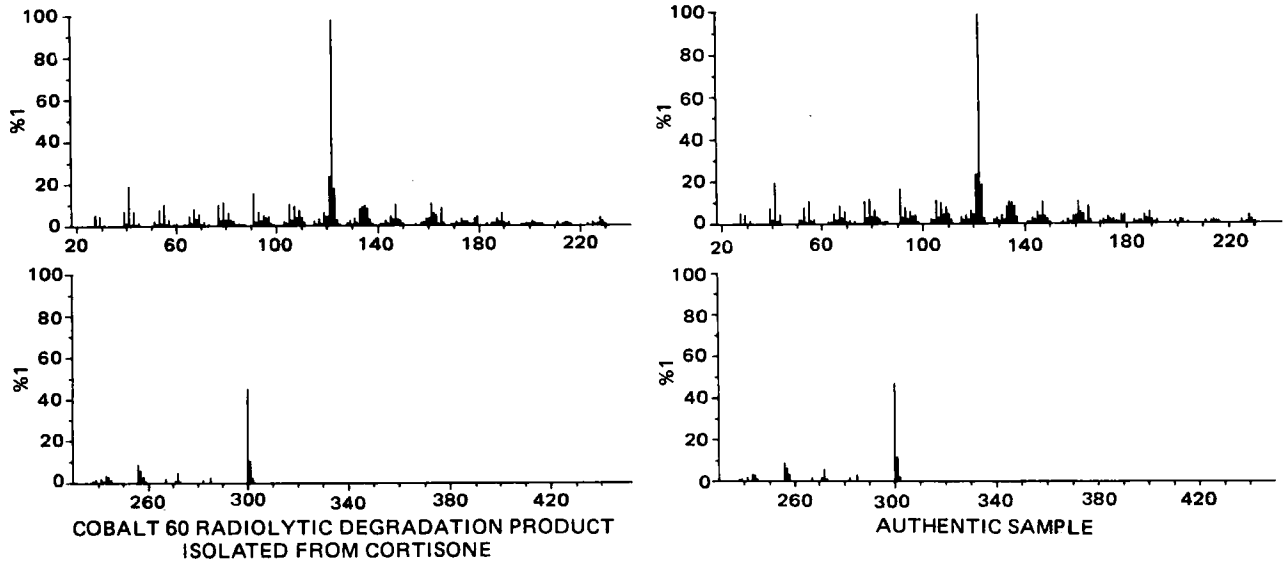


Figure 4—*Mass spectrum of 4-androstene-3,11,17-trione (M^+ m/z 300). (The authentic sample is available from the Upjohn Co.)*

phase chromatography using acetonitrile–water (40:60) as the mobile phase.

The semipreparative scale HPLC methods used to purify the radiolytic degradation compounds for identification are listed in Table I.

Corticosteroids—The following corticosteroids were examined: cortisone, cortisone acetate, hydrocortisone, hydrocortisone acetate, hydrocortisone sodium succinate, isoflupredone acetate, methylprednisolone, methylprednisolone acetate, prednisolone, prednisolone acetate, and prednisone. 17α-Hydroxyprogesterone was also examined.

RESULTS AND DISCUSSION

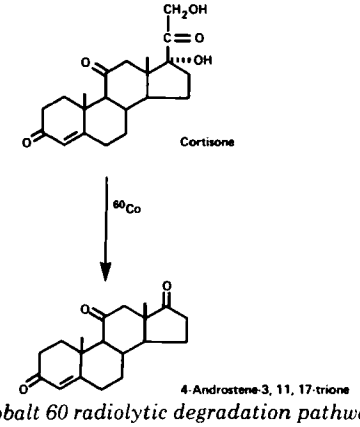
Identification of Radiolytic Degradation Compounds—The identities of the radiolytic degradation products are based in part on the comparison of the HPLC retention times by both normal- and reversed-phase HPLC of the degradation products with the retention times of the reference standards or authentic samples.

The identities of the degradation products were confirmed by comparison of the mass spectra (electron impact at 250°) of samples of the degradation products obtained by preparative HPLC with the mass spectra of the reference standards or authentic samples. The degradation products were isolated by preparative HPLC from enriched samples. In most cases samples could be enriched by refluxing in butyl chloride or methylene chloride. This process is, in principle, the same as recrystallization, where the mother liquor hopefully contains the impurities. For example, the degradation products of prednisolone were enriched by refluxing 2.5 g of irradiated corticosteroid in 10 ml of methylene chloride for 30 min. The methylene chloride was collected by filtration and evaporated to dryness. In the case of hydrocortisone sodium succinate, the sample was first hydrolyzed by treating a solution of 5 g in 10 ml of water with one drop of 40% NaOH. The hydrolysis products, which precipitated within minutes as the reaction proceeded, were collected by filtration and air dried prior to enrichment by refluxing in methylene chloride. The residues obtained after evaporation were then dissolved in ~20 ml of the appropriate mobile phase and chromatographed either by a normal-[7] or reversed-phase column[8]. Between 10 and 20 100-μl injections normally were made. The mobile phase was removed from the collected fractions by freeze drying.

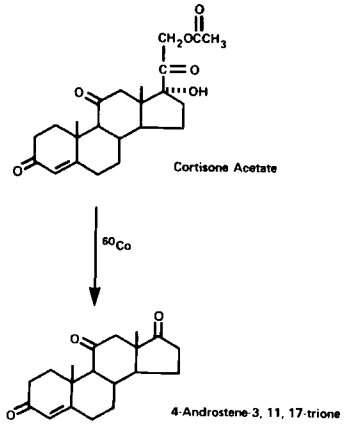
Although this process was successful in enriching the C-11 and C-17 ketone degradation products in methylprednisolone acetate, the process failed to enrich the third minor product. An enriched sample containing this component was obtained from a normal-phase chromatographic column[10] using butyl chloride (50% water saturated)–tetrahydrofuran–methanol–glacial acetic acid (950:70:35:30). A 2-g solution of the irradiated corticosteroid was dissolved in 10 ml of acetonitrile; twelve 280-μl injections were made. Fractions containing the third component of interest were combined and freeze dried. The freeze-dried material was dissolved in 2 ml of mobile phase and rechromatographed.

The choice of whether to use a normal- or reversed-phase for preparative chromatography was made on the basis of which system provided the best separation from existing process impurities. The chromatographic conditions used in the isolation of the degradation products of each corticosteroid are listed in Table I.

Authentic samples were available for HPLC and mass spectra comparison with all degradation products isolated except the C-17 ketone derived from isoflupredone acetate and the C-17α alcohol derived from methylprednisolone. In the former case, the mass spectrum shows the expected molecular parent ion and is sufficiently similar to related compounds to establish the identification. In the latter case, the C-17β epimer was available for comparison purposes. Although the HPLC retention times of the two epimers were clearly distinctive, the mass spectra

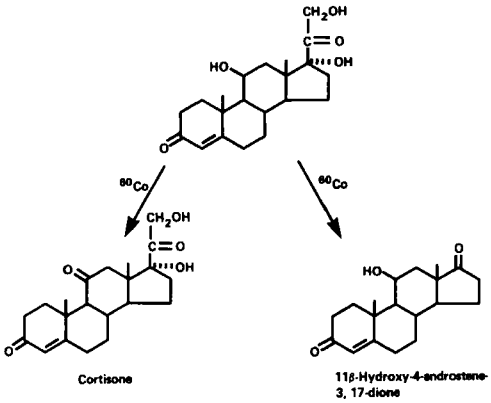


Scheme I—Cobalt 60 radiolytic degradation pathway of cortisone.

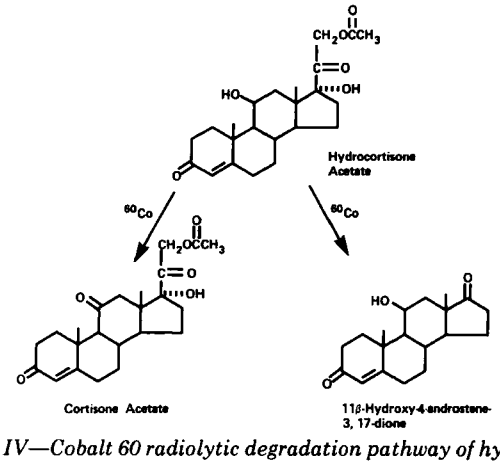


Scheme II—Cobalt 60 radiolytic degradation pathway of cortisone acetate.

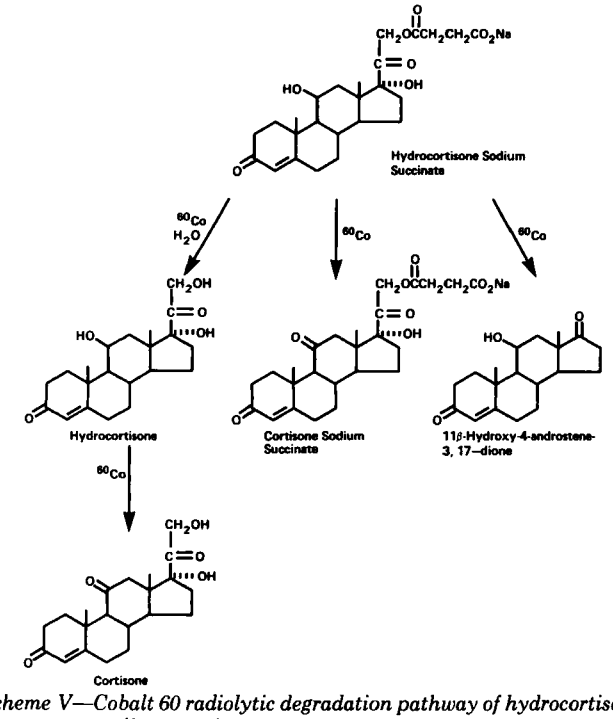
[10] Porasil A 37-75 μm, 7.8 mm × 122 cm, Waters Associates, Milford, Mass.



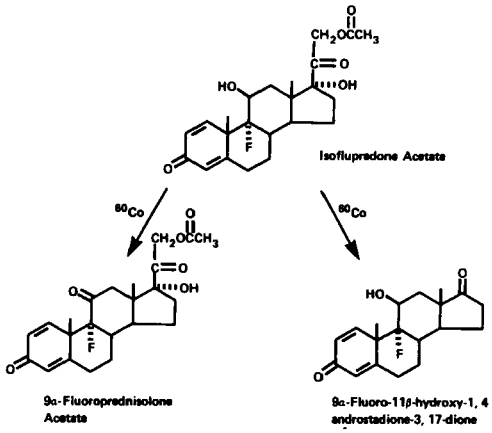
Scheme III—Cobalt 60 radiolytic degradation pathway of hydrocortisone.



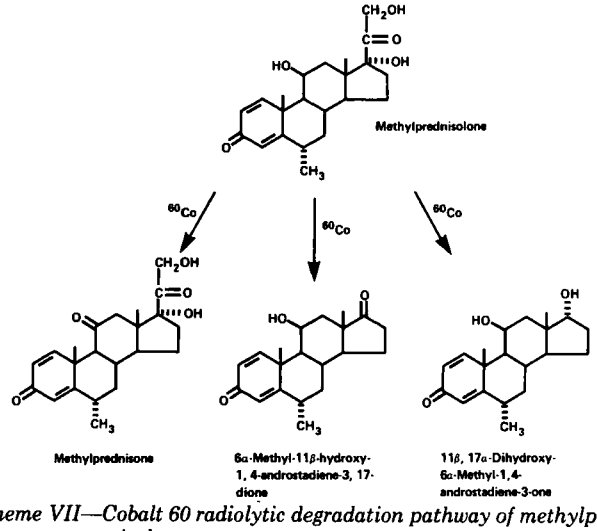
Scheme IV—Cobalt 60 radiolytic degradation pathway of hydrocortisone acetate.



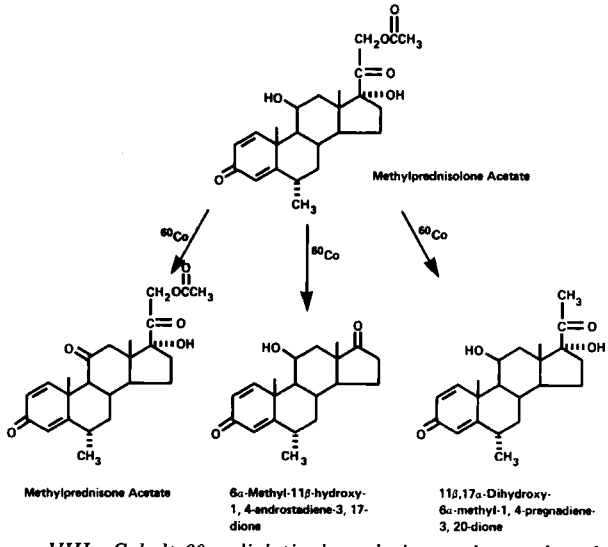
Scheme V—Cobalt 60 radiolytic degradation pathway of hydrocortisone sodium succinate.



Scheme VI—Cobalt 60 radiolytic degradation pathway of isoflupredone acetate.



Scheme VII—Cobalt 60 radiolytic degradation pathway of methylprednisolone.

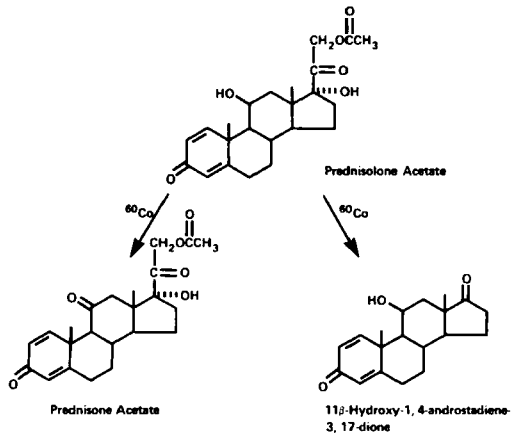


Scheme VIII—Cobalt 60 radiolytic degradation pathway of methylprednisolone acetate.

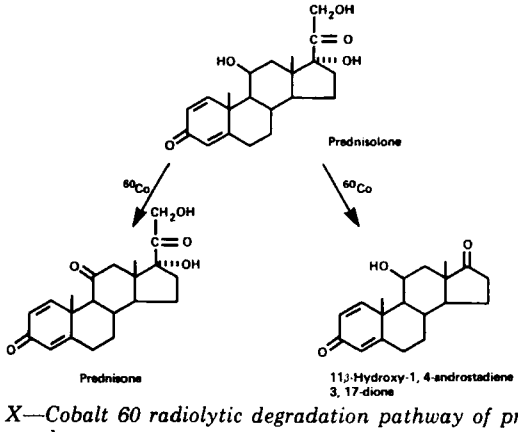
were nearly identical. It is not unusual for stereoisomers, especially epimers, to have indistinguishable mass spectra.

As examples, HPLC chromatograms of irradiated and nonirradiated cortisone, hydrocortisone, and isoflupredone acetate are shown in Figs. 1–3. The mass spectrum of a radiolytic compound in cortisone used to positively identify it as 4-androstene-3,11,17-trione is shown in Fig. 4.

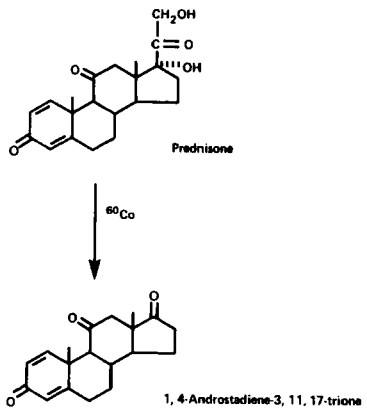
Mass Balances—The mass balances were determined for the majority of the corticosteroids investigated to ascertain how much of the total degradation could be accounted for by the observed degradation process. The mass balances were obtained by comparing samples irradiated at high dose (2.5 or 6 Mrads) with nonirradiated or low dose (0.5 Mrads) samples. Using carefully dried and accurately weighed samples, the dif-



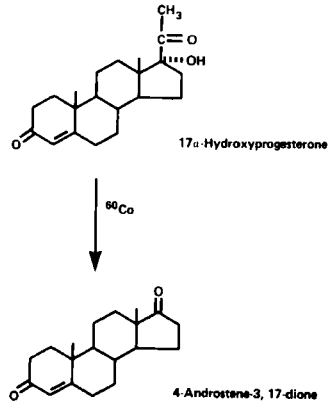
Scheme IX—Cobalt 60 radiolytic degradation pathway of prednisolone acetate.



Scheme X—Cobalt 60 radiolytic degradation pathway of prednisolone.



Scheme XI—Cobalt 60 radiolytic degradation pathway of prednisone.



Scheme XII—Cobalt 60 radiolytic degradation pathway of 17α-Hydroxyprogesterone.

ferences in peak response (are per weight ratio) were determined for the corticosteroid and each degradation product. The differences in peak response for the degradation products were then corrected for differences in relative molar absorptivity and are reported on a percentage basis relative to the response of the corticosteroid in the nonirradiated or low-dose sample. An equimolar response was assumed when an authentic sample was not available for determination of relative molar absorptivity. As an example, the mass balance data for methylprednisolone is shown in Table II. The data support the conclusion that all of the degradation by ^{60}Co-irradiation has been accounted for.

Major Cobalt 60 Radiolytic Degradation Products—The major cobalt 60 radiolytic degradation products were identified in the following corticosteroids: cortisone, cortisone acetate, hydrocortisone, hydrocortisone acetate, hydrocortisone sodium succinate, isoflupredone acetate, methylprednisolone, methylprednisolone acetate, prednisolone, prednisolone acetate, and prednisone. Radiolytic degradation of corticosteroids are shown in Schemes I–XI. A structurally related steroid, 17α-hydroxyprogesterone, was also examined (Scheme XII).

Two major types of degradation processes were found to occur by ^{60}Co-irradiation: loss of the C-17 side chain to produce the C-17 ketone and oxidation of the C-11 alcohol, if present, to the C-11 ketone. Several degradation processes have been reported to occur in corticosteroids without irradiation: they include air oxidation of the C-11 alcohol to the C-11 ketone (9, 10), stepwise air oxidation of the D-ring corticoid side chain to the C-17 ketone, hydrolysis of C-21 esters, acyl migration of C-21 hemisuccinate to C-17 hemisuccinate, and loss of the corticoid side chain to produce the C-17 ketone by thermal treatment (11). Thus, the radiolytic degradation products were found to be identical to existing process impurities or known degradation products. A similar loss of the C-17 acyl side chain in 17α-hydroxyprogesterone to produce the C-17 ketone was also found to occur.

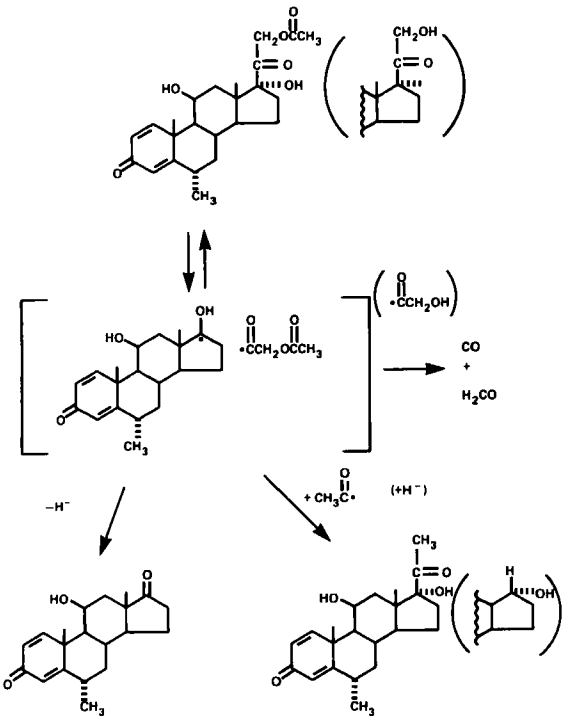
Table III—Rate of Radiolytic Degradation for Corticosteroids

Corticosteroids	Radiolytic Degradation, %/Mrad
Cortisone acetate	0.6
Hydrocortisone	1.0
Hydrocortisone acetate	0.3
Hydrocortisone sodium succinate	1.4
Isoflupredone acetate	0.4
Methylprednisolone	0.7
Methylprednisolone acetate	0.6
Prednisolone	0.7
Prednisolone acetate	0.4
Prednisone	0.2

Minor Cobalt 60 Radiolytic Degradation Products—Additional minor degradation products derived from processes affecting the C-17 side chain were identified in methylprednisolone and methylprednisolone acetate (Schemes VII, VIII). Loss of the C-17 side chain in methylprednisolone was also found to result in formation of the C-17α alcohol (11β,17α-dihydroxy-6α-methyl-1,4-androstadiene-3-one) in addition to the C-17 ketone. The C-21 desoxy derivative (11β,17α-dihydroxy-6α-methyl-1,4-pregnadiene-3,20-dione) was produced in methylprednisolone acetate. Hydrocortisone was identified as an additional degradation product of hydrocortisone sodium succinate (Scheme V). Sufficient water is present in the formulation to allow some hydrolysis to occur during irradiation.

Although similar minor degradation products derived from the C-17 side chain were not observed in the other corticosteroids investigated, the possibility of their formation below the detection limit of HPLC methods cannot be ruled out. The minor degradation products detected in methylprednisolone acetate and methylprednisolone were only present at 0.1 and 0.2%, respectively, at 6 Mrads.

Mechanism of Degradation—Two major types of radiolytic degradation processes for corticosteroids have been identified to occur: loss of the C-17 side chain to form the C-17 ketone and oxidation of the C-11 alcohol to form the C-11 ketone. When considering a mechanism to explain these degradation processes, the fact that the reactions involved occur in the solid state must be considered. Molecular packing within the crystal lattice and the amount of surface area could have an influence on degradation. One possible mechanism that accounts for the formation of C-17 ketones as well as the minor products derived from changes in



Scheme 13—Postulated mechanism for cobalt 60 radiolytic degradation of C-17 side chain of corticosteroids (example: methylprednisolone and methylprednisolone acetate).

the C-17 side chain of methylprednisolone and methylprednisolone acetate is shown in Scheme XIII. By this mechanism, reaction is initiated by homolytic cleavage between C-17 and C-20. Subsequent loss of a proton from the steroidal fragment would lead to the C-17 ketone. Recombination of the initially formed radicals could also occur. Alternatively, further disintegration of the side chain radical could lead to other radicals. Recombination with any of these radicals could also occur. For example, the C-21 acetate substituted side chain in methylprednisolone acetate could produce carbon monoxide, formaldehyde, and an acetyl radical. Recombination would generate the C-21 desoxy derivative. By a similar process, the side chain in methylprednisolone would produce a radical. Recombination in that case would form the C-21α alcohol. Preservation of the original stereochemistry at C-21 in these degradation products is attributed to the crystal lattice structure which would trap the radical intermediates in close proximity. In addition to formation of carbon monoxide and formaldehyde, other possible products derived from the C-17 side chain include methanol, methyl acetate, acetaldehyde, methane, and hydrogen. Formation of C-11 ketones could proceed by a similar mechanism initiated by loss of the C-11 proton with hydrogen produced as the other product.

Rate of Degradation—The rates of radiolytic degradation for corticosteroids were determined by analyzing irradiated and nonirradiated steroid powders using HPLC methods. The rates of degradation, expressed as percentage per megrad, are shown in Table III. The rates of degradation ranged from 0.2 to 1.4%/Mrad, thus, the majority of corticosteroids are stable to ^{60}Co-irradiation.

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Difficulties in Applying the Scatchard Model of Ligand Binding to Proteins—Proposal of New Mathematical Tools—Application to Salicylates

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Abstract □ Ill-considered use of the Scatchard model often leads to unjustified deductions. Since the main difficulty of this model is its number of parameters, new models are proposed that have only two parameters. After checking the models on simulated data, they were applied to real data on the binding of salicylates to albumin.

Keyphrases □ Scatchard model—difficulties in application to ligand binding to proteins, new mathematical tools, application to salicylates □ Salicylates—application of Scatchard model, difficulties in application to ligand binding to proteins, new mathematical tools □ Binding—ligand to proteins, difficulties in applying the Scatchard model, new mathematical tools, application to salicylates □ Proteins—ligand binding, difficulties in applying the Scatchard model, new mathematical tools, application to salicylates

The binding of ligands to proteins is most often analyzed in terms of the model proposed by Scatchard (1), in which proteins are considered to possess binding sites divided into independent classes according to their affinities. From estimations of the free and bound fractions of the ligand, it is in principle possible to determine the number of

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