Absorption and Excretion of 17, 21-Dihydroxy-20-Ketosteroids in Dogs

Robert H. Silber and Evan R. Morgan

Steroid analyses performed after intravenous administration of solutions of hydrocortisone and cortisol to man have established the plasma half-life of hydrocortisone to be 2 hours and that of cortisol 1 hour (1, 2). From isotopic determinations in urine, the half-life of hydrocortisone in the body has been found to be 6 hours and that of cortisol 3.6 hours (3, 4). Thus, cortisol is not only cleared from the plasma more rapidly than hydrocortisone but it is also metabolized and excreted at a faster rate. In rats (5), hydrocortisone acetate has been shown to be very slowly absorbed from injection sites, whereas hydrocortisone is more rapidly absorbed than cortisol, cortisol acetate, or hydrocortisone acetate. In these studies it was shown that biologic activity was dependent upon the rate of absorption of the steroids from the injection sites.

It therefore seemed desirable to compare the absorption, excretion, and half-life in plasma of several steroids including the newer forms, 9α-fluorohydrocortisone, prednisone, and prednisolone. Information regarding the mechanism responsible for the greater biologic activity of these steroids might be obtained in such studies.

EXPERIMENTAL

Adult fasted beagle dogs weighing 12-14 Kg. were used. The analytical procedure was that of Silber and Porter (6) except for the use of a higher concentration of β-glucuronidase as noted elsewhere (7).

For intramuscular administration the steroids were suspended in saline (50 mg./ml.) and for oral administration, they were dissolved in 10-20 ml. of ethanol, diluted with 50 ml. of water, and the stomach tube
was washed with additional water. For intravenous administration, the steroids were dissolved in alcohol (25 mg./5 ml.) and diluted to 125 ml. with saline. The infusion of 25 mg. of steroid was completed in a 15-minute period.

RESULTS

Intramuscular Injection

After intramuscular administration of 100 mg., plasma samples taken after 1, 3 and 5 hours revealed increases in steroid concentration of 8–14 μg./100 ml. after administration of hydrocortisone, cortisone, cortisone acetate, prednisone, and prednisolone without a peak in the 5-hour period. Hydrocortisone acetate and 9α-fluorohydrocortisone injection failed to cause a measurable increase in the plasma steroid concentration.

Oral Administration

After oral administration of 100 mg. striking differences in the plasma concentrations were found (Fig. 1). Prednisolone concentrations were far greater than those observed with the other 6 steroids and the plasma concentrations of the two Δ1 steroids were more prolonged. 9α-Fluorohydro-
drocortisone showed the next highest peak value. Concentrations found after administration of cortisone, cortisone acetate, and hydrocortisone acetate were lowest.

**Intravenous Injection**

After intravenous administration of 25 mg., peak concentrations determined 5 minutes after the infusions ranged from 135–235 µg. per cent. The curves and the half-life times are shown in Fig. 2. 9α-Fluorohydrocortisone and prednisolone had slightly longer half-life times than hydrocortisone, and prednisone had a longer half-life than cortisone, which was the most rapidly cleared of the steroids studied.

**Urinary Excretion**

The urinary excretion data are summarized in Table 1. The steroids are listed in decreasing order, based upon their total excretion after oral administration, with hydrocortisone at the top and 9α-fluorohydrocortisone at the bottom. After intramuscular injection, relatively little steroid was found, with almost negligible amounts excreted after giving all except hydrocortisone and prednisolone. After intravenous infusion, 14 per cent of the dose of hydrocortisone was found, and 10.6 per cent after giving its Δ¹ derivative. From 5 to 7.5 per cent of each of the other three steroids was detected in the urine.

![Fig. 2. Plasma concentrations after 25 mg. intravenously (increase in µg. per cent; averages of 2 dogs).](image-url)
Table 1. Urinary Excretion after Oral, Intramuscular, and Intravenous Administration

<table>
<thead>
<tr>
<th></th>
<th>Oral, 100 mg.</th>
<th>1. M., 100 mg.</th>
<th>I. V., 25 mg.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Free</td>
<td>Conjugated</td>
<td>Free</td>
</tr>
<tr>
<td>Hydrocortisone</td>
<td>2.5a</td>
<td>22.0</td>
<td>1.06</td>
</tr>
<tr>
<td>Prednisolone</td>
<td>6.2</td>
<td>1.9</td>
<td>0.9</td>
</tr>
<tr>
<td>Hydrocortisone acetate</td>
<td>1.9</td>
<td>5.2</td>
<td>0.17</td>
</tr>
<tr>
<td>Cortisone</td>
<td>1.65</td>
<td>5.25</td>
<td>0.75</td>
</tr>
<tr>
<td>Cortisone acetate</td>
<td>2.1</td>
<td>3.1</td>
<td>0.22</td>
</tr>
<tr>
<td>Prednisone</td>
<td>3.5</td>
<td>1.0</td>
<td>0.8</td>
</tr>
<tr>
<td>9α-Fluorohydrocortisone</td>
<td>2.7</td>
<td>1.3</td>
<td>0.33</td>
</tr>
</tbody>
</table>

NOTE: It has been found that tetrahydrocortisone is essentially the sole glucuronide form that is excreted after giving hydrocortisone to dogs, so the values listed in the "conjugated" column have been corrected for the incomplete recovery of this steroid in the procedure used.

* All results are the averages found with 2 dogs, expressed in mg./24 hr.

The total excretion of 17,21-dihydroxy-20-ketosteroids after oral administration of 100 mg. hydrocortisone to 2 dogs (average 24.5 mg.) was not significantly different from the excretion observed after intravenous administration of the same amount (average 25.2 mg.).

Normal dogs excrete very small amounts of 17,21-dihydroxy-20-ketosteroids, about 100-300 µg. in 24 hours, so corrections for the basal excretion have not been made in this paper.

DISCUSSION

The excretion of conjugated forms of prednisone, prednisolone, and 9α-fluorohydrocortisone after oral administration was obviously depressed as a result of the introduction of the Δ1 double bond or the fluorine into the molecule. This indicates that reduction of these forms in the 3 position is inhibited, thereby depressing the rate of conjugation. Since reduction of the A ring is a major inactivation process in the body, it seems likely that retardation of this process is at least partially responsible for the greater biologic activity of these three steroids. Lower excretion of the glucuronide forms of the three steroids was also evident after intravenous or intramuscular administration.

Since it has been found (7) that dogs, like man, excrete a constant fraction of a given oral dose of hydrocortisone, the excretion data in Table 1 should make it possible to estimate the fraction of an intramuscular or oral dose that has been absorbed. Thus, since about 25 percent of the oral hydrocortisone dose was excreted in the urine as 17,21-dihydroxy-20-ketosteroids, excretion of 5 mg. after intramuscular in-
jection of 100 mg. indicates that 20 mg., or 20 per cent, were absorbed in the 24-hour period. Similar calculations reveal that 16–20 per cent of the intramuscular doses of cortisone, prednisolone, and prednisone were absorbed from the injection sites in the same time. Furthermore, if one makes a correction for the basal excretion of 0.2 mg. per dog per day, such calculations indicate that about 1 per cent of the hydrocortisone acetate, 4 per cent of the cortisone acetate, and 10 per cent of the 9α-fluorohydrocortisone were absorbed in the 24 hours after injection.

The plasma half-life of the steroids with increased biologic activity was measurably but not strikingly prolonged after intravenous administration. After oral administration, plasma concentrations were not only higher but also more prolonged than after administration of hydrocortisone or cortisone. These results support the excretion data, which indicated that the metabolic inactivation of prednisone, prednisolone, and 9α-fluorohydrocortisone was depressed.

The plasma half-life times were calculated after plotting the logarithm of the increase in plasma concentration against time. As noted in man after intravenous infusion (1, 2), such a plot yields a straight line. It is of interest that a straight line is also obtained when the values obtained after oral administration are plotted in the same way.

SUMMARY

The plasma concentrations of free 17,21-dihydroxy-20-ketosteroids and urinary excretion of both free and glucuronide forms have been determined after oral, intramuscular, and intravenous administration of cortisone, hydrocortisone, their Δ¹ forms, and 9α-fluorohydrocortisone to dogs.

Excretion of the glucuronide forms of those steroids with a double bond at the 1–2 position or with a fluorine at position 9 was depressed, and the plasma half-life of each of these steroids was longer than that of the parent compound.

REFERENCES