SOME ASPECTS OF THE METABOLISM AND DISPOSITION OF BETAMETHASONE

By

D. Murphy, H. F. West and A. M. Bethel

ABSTRACT

Following the administration of 4 mg of tritiated betamethasone, with specific activity 75 μc/mg, the concentration of betamethasone and some of its metabolites were measured repeatedly in saliva, plasma and urine. The finding of particular interest was the metabolism of betamethasone within the salivary glands to a steroid provisionally identified as 11-oxo-betamethasone. The significance of this finding and of the extra-hepatic metabolism of corticosteroids in general were discussed. Also discussed was the bearing of other findings upon the nature of the factors responsible for a cortisol analogues potency.

Cortisol analogues have been used extensively in clinical practise during the last 9 years but very few studies of their metabolism and disposition have been published. In this study of betamethasone (16β-methyl-9α-fluoro-1,2-dehydro-cortisol) metabolites have been measured in saliva, in addition to urine and plasma, as it is the only readily available body fluid that may reveal something of the concentrations of diffusible steroids within cells and provide evidence of their extrahepatic metabolism (Greaves & West 1963).

MATERIAL AND METHODS

4 mg of betamethasone, tritiated in the 16α-position and in the 16β-methyl group and with a specific activity of 75 μc/mg, was enclosed within two gelatine capsules and

* In the Bush B₅ chromatography system 100 % of the radioactivity ran with standard betamethasone.
swallowed by a normal individual. Urine, plasma and mixed saliva were subsequently collected at the times and in the volumes given in Table 1. The saliva flow was stimulated by chewing paraffin wax and the collections made at a steady rate throughout each 2 hour period. The urine specimens, collected without preservative, were stored before extraction at 4° C for periods not exceeding 24 hours. Blood was collected in oxalate bottles and the plasma removed immediately from the first two samples and after storage over-night at 4° C in the case of the third. Aliquots of each native specimen were taken for radioactivity counting.

**Table 1.**
The times at which urine, saliva and plasma were collected and the volumes, in ml, of each collection.

<table>
<thead>
<tr>
<th>Time Hours</th>
<th>Volumes ml</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Urine</td>
<td>Saliva</td>
<td>Plasma</td>
<td></td>
</tr>
<tr>
<td>0 to 1</td>
<td>U₁</td>
<td>S₁</td>
<td>P₁</td>
<td>8 at 2</td>
</tr>
<tr>
<td>1 to 3</td>
<td>U₂</td>
<td>S₂</td>
<td>P₂</td>
<td>10 at 5</td>
</tr>
<tr>
<td>3 to 4</td>
<td>U₃</td>
<td>S₃</td>
<td>P₃</td>
<td>10 at 8</td>
</tr>
<tr>
<td>4 to 6</td>
<td>U₄</td>
<td>S₄</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 to 7</td>
<td>U₅</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7 to 9</td>
<td>U₆</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9 to 10</td>
<td>U₇</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 to 12</td>
<td>U₈</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12 to 24</td>
<td>U₉</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24 to 36</td>
<td>U₁₀</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>36 to 48</td>
<td>U₁₁</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>48 to 60</td>
<td>U₁₂</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>60 to 72</td>
<td>U₁₃</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Extraction**

Only the free steroids were studied. They were extracted 3 times with equal volumes of ethyl acetate and before the extracts were combined they were washed once with 0.1 vol. N-Na₂CO₃, once with 0.1 vol. N-NaOH, once with 0.05 vol. satd. NaCl soln. and once with 0.05 vol. 0.5% glacial acetic acid. After combining the extracts, water was frozen out and they were taken to dryness in a rotary evaporator. The NaOH wash was omitted in the case of the saliva as the extracts were relatively clean. The saliva and plasma extracts were further purified by partition between 4 ml 70% methanol and 1 ml of petroleum ether (100-120). The petroleum ether layer was discarded and the aqueous methanol layer reduced to dryness under nitrogen. Aliquots of each extract were taken for radioactivity counting.

**Paper chromatography**

This was carried out in a constant temperature room at 28° C on Whatman 3MM and No. 2 washed paper. The following systems were used: Bush B₅, benzene/metha-
nol/water, 100:50:50 and Bush 3b, petroleum ether/toluene/methanol/water, 67:33:85:15 (Bush & Mahesh 1959). Smith II, benzene/acetone/water, 2:1:2 and Smith VI, benzene/petroleum ether/methanol/water, 5:5:7:3 (Smith et al. 1959). Steroids were detected on the chromatograms by ultraviolet fluorescence at 257 m\(\mu\), by the blue tetrazolium reaction, by spraying with isonicotinic acid hydrazide (Umberger 1955) and by reference to standards run in parallel. Paper strips for elution were cut into small pieces and soaked in methanol/ethyl acetate (1:2) for one hour and then washed at least 3 times with further quantities of this solvent. Acetylation was effected by dissolving the extracts in 0.2 ml pyridine and 0.1 ml acetic anhydride, incubating for 1\(\frac{1}{2}\) hours at 60\(^\circ\)C and subsequently adding methanol and evaporating to dryness under nitrogen.

Each extract was pre-chromatographed in the Bush B\(\text{B}_3\) system with, in the case of saliva and plasma, 10 \(\mu\)g of non-radioactive betamethasone added as a carrier. The betamethasone area was eluted, acetylated and re-run as a spot in the Smith VI system. The area running with betamethasone acetate standard was eluted for radioactivity counting. In the case of the 2nd. saliva collection, the 2nd. plasma collection and the corresponding urine collection the rest of the acetate chromatograms were cut into 1\(\frac{1}{2}\) cm strips and eluted for radioactivity counting. The whole of each prechromatogram above betamethasone, i.e. more polar, was eluted and re-run in the Smith II system. Two steroid bands located in previous studies (see results below) were cut out and eluted for radioactivity counting. The rest of the chromatogram, in the case of the 2nd saliva and plasma collections and the corresponding urine collection, was cut into 1\(\frac{1}{2}\) cm strips and eluted for counting. When it was found that the polar and acetate chromatograms of the saliva extracts did not contain all the radioactivity of the extracts, the non-polar regions of all the saliva and plasma pre-chromatograms and those of the corresponding urine collections were cut into 1\(\frac{1}{2}\) cm strips and eluted for counting.

11-dehydrobetamethasone was prepared by oxidizing betamethasone acetate with chromic acid (Lieberman et al. 1953). The product was deacetylated by solution in 1 ml of methanol adding 0.2 ml of 1% aqueous KHCO\(_3\) and refluxing for 5 minutes. 2 ml of water was then added and the steroid extracted twice with 10 ml of ethyl acetate. The extract was washed once with 1 ml of water and evaporated to dryness. The resulting product had an \(R_F\) value of 0.70 in the Bush B\(\text{B}_3\) system.

**Radioactivity measurement**

The radioactivity of some of the native specimens (plasma and saliva) and of the initial urine, plasma and saliva extracts was assessed on a Technical Measurements Corporation LP2A liquid scintillation counter, all other samples being assessed on a Packard Tri-Carb 314EX automatic liquid scintillation counter.

0.5 ml of each native urine specimen was mixed with 4.5 ml absolute ethanol and made up to 10 ml double strength toluene scintillator (10 g/l. P. P. O., 0.6 g/l. dimethyl-P. O. P. O. P.) and counted on the Tricarb with an efficiency of approximately 6% (H. V. tap 5-100, window 10–50 volts, gain 100).

0.5 ml aliquots of each plasma and saliva specimen were mixed with 2 ml 1.5 M hyamine chloride (Meade & Stiglitz 1962) and made up to 10 ml with single strength toluene scintillator (5 g/l. P. P. O., 0.3 g/l. dimethyl P. O. P. O. P.). Extracts and paper eluates were dissolved in 1.0 ml ethanol and made up to 20 ml with single strength scintillator, efficiency on the Tricarb counter being approximately 17%.

The accuracies of the activity assessments were estimated to be ±3% for native urines; ±7% for native salivas and plasmas and for extracts and the betamethasone

500
acetate eluates; ± 3% for most eluates, rising to ± 5% for samples containing only 0.01 m\(\mu\)c.

**RESULTS**

Fig. 1 shows the urinary excretion in \(\mu\)c/h of (1) the total radioactivity, (2) the radioactivity extractable with ethyl acetate, (3) the radioactivity associated with betamethasone. During the hours 4, 7 and 11 meals were taken by the subject of the study and the rate of urine flow fell. We have found that the excretion of the polar steroid triamcinolone is little affected by the rate of urine flow but that the less polar steroids, cortisol and cortisone, are so affected. By raising the rate of urine flow from 2–3 ml a minute to 8–10 ml a minute their excretion can be increased by 50%\(\%\). Non-polar 17-oxosteroids (unconjugated) we find to be extensively reabsorbed. The irregularities in the curves of Fig. 1 may well be explained by this factor. The delay in reaching the peak rate of excretion was likely to have been due to the betamethasone having been given in microcrystalline form rather than in the finely milled form present in tablets. It is of interest to note that only 2–3%\(\%\) of the betamethasone given was recovered in the urine of the first day and from data to be considered below this was not due to extensive tubular reabsorption. In other studies we have recovered 10–15%\(\%\) of orally administered prednisolone and 30–35%\(\%\) of orally administered triamcinolone over shorter periods.

Fig. 2 shows the cumulative urinary excretion of radioactivity as a percentage of the dose given. 37.7%\(\%\) of the radioactivity was excreted in 24 hours.

![Fig. 1](image-url)

*Fig. 1.* The radioactivity, in \(\mu\)c/h, excreted in urine in the first 72 hours after the ingestion of tritiated betamethasone. Complete columns = radioactivity in native urine; columns to the top of the shaded area = radioactivity extractable with ethyl acetate; black area = radioactivity of extract attributed to betamethasone.
Plot of the cumulative urinary excretion of radioactivity expressed as % of dose given.

hours and 60% in 72 hours. In a parallel experiment in the same individual using tritiated cortisol 85.6% of the radioactivity was excreted in 24 hours and 92% in 72 hours.

Figs. 3 and 4 show the concentration of radioactivity in the plasma and saliva, the concentration of the ethyl acetate extractable radioactivity and the concentration that was due to betamethasone. The figures suggest that a small fraction was not extractable with ethyl acetate. No glucuronic acid conjugated metabolites would be expected to diffuse into the salivary cells owing

---

**Fig. 3.**
The amount of radioactivity in μC/100 ml of plasma, the amount extracted with ethyl acetate and the amount attributed to betamethasone, for the 3 plasma collections.
The amount of radioactivity in $\mu$Ci/100 ml of saliva, the amount extracted with ethyl acetate and the amount attributed to betamethasone, for the 4 saliva collections.

to their highly polar nature. (Christy & Fishman (1961) found no tetrahydrocortisone glucuronide in dog cerebrospinal fluid when the blood concentration was high.) Little can be said of the volume of distribution of the radioactivity or of the half-life of the betamethasone and its metabolites because 40% was not accounted for and because the time at which alimentary absorption was complete was not known. If 100% was absorbed by the 8th hour and the radioactivity unaccounted for disappeared at the same rate as that excreted in the urine, then the volume of distribution at the 8th hour, relative to the plasma concentration, was 100 l. This was more than twice the subjects total body water and would show that the concentration of radioactivity bound outside the vascular compartment was greater than that in the plasma. If half-life estimates are based on measurements from the 8th hour onwards figures are available for two saliva collection periods – the 8th and 9th hours and the 11th and 12th. These give a half-life for betamethasone in saliva of approximately 10 hours. The half-life of cortisol in saliva in a parallel study was only 1 hour.

Fig. 5 shows the distribution of the radioactivity in the paper chromatograms of the 2nd plasma and saliva collections and the corresponding urine collection. In the case of the plasma and saliva chromatograms the columns show $\mu$Ci present per 1 cm strip per 100 ml extracted. The columns for the urine show $\mu$Ci present per 1 cm strip per minute-volume of urine excreted and extracted. The first section is of the polar region of the $B_3$ chromatogram re-chromatographed in the Smith II system; the second section is of the betamethasone region of the $B_3$ chromatogram acetylated and re-run in the Smith VI system; the third section is of the region of the $B_3$ chromatogram that was less polar than betamethasone. If the subject's glomerular filtration rate
The distribution of radioactivity on the chromatograms ('polar', 'acetate' and 'non-polar') from the extracts of the 2nd. plasma and saliva collections and the corresponding urine collection. The radioactivity of the plasma and saliva expressed as mc per 1 cm strip per 100 ml extracted and chromatographed. Urinary radioactivity expressed as mc per 1 cm strip per minute – volume of urine excreted, extracted and chromatographed.

(G. F. R.) was 100 ml a minute and no steroid was excreted by the kidney other than by filtration and none reabsorbed, then the amount excreted per minute should be the same as the concentration of diffusible per 100 ml of plasma. The subject's G. F. R. was not measured at the time of the urine collections but on other occasions the average rate was 85 ml a minute. When the concentration of the highly polar metabolite 1 in plasma is compared with the rate of excretion in the urine it is apparent that either a great deal was reabsorbed in the kidney or that the steroid was protein-bound up to a maximum of about 70%. From what is known of the binding of polar steroids (Westphal 1961) and of their reabsorption, mentioned above, the latter is the likely explanation. The absence of metabolite 1 from the saliva is in keeping with its high polarity (Burgen 1956). Metabolites 1 and 2 were not identified but were likely to be 6β,20ξ-dihydroxybetamethasone and 6β-hydroxybetamethasone for the following reasons: Using larger quantities of non-radioactive betamethasone metabolite 1 was found to have an Rf of 0.09 in the Smith II system and to be INH positive and BT negative. Metabolite 2 had an Rf of 0.35 and was both INH and BT positive. These findings, that are consistent with the suggested structures, are in keeping with that is known of polar steroid metabolites, e.g. 6β-hydroxytriamcinolone (Florini et al. 1961); 6β-hydroxycortisol (Frantz et al. 1961); 6α-methyl-6β,17α,21-trihydroxy-pregn-4ene-3,20-dione-17-acetate (Helmreich & Huseby 1962); 6β,17α,21-trihydroxy-
6α-methyl-pregnen-3,20-dione (Castegnaro & Sala 1962); and 6β,17α,20ξ:21-tetrahydroxy-2α-methyl-pregn-4-en-3-one (Bush & Mahesh 1959b). Metabolites 2 and 3 were excreted as if 2/3rds. protein-bound and their concentration in the saliva was consistent with their decreasing polarity.

In the middle ‘acetate’ strip of Fig. 4 2/3rds of the radioactivity ran with betamethasone acetate (Metabolite 5). When standard betamethasone was acetylated by our procedure 10% of the product ran in the position of metabolite 6. It is likely therefore that this artefact accounted for much of metabolite 6 in Fig. 4.

The metabolite of major interest is No. 9 in the non-polar chromatogram. The metabolites 8 and 10 on either side of it show the expected correspondence between plasma, saliva and urine. (The peak for 10 in urine looks insignificant but it represents only a small fraction of the amount of radioactivity actually measured which showed a highly significant peak). By contrast the concentration of metabolite 9 in saliva is far too high for either the plasma concentration or the rate of excretion in the urine. The most reasonable explanation of this finding is that it is a metabolite that is formed in the salivary glands. In Fig. 6 the concentration of betamethasone and of metabolite 9 in plasma and saliva and their rate of excretion in the urine are set out for each of the comparable periods. The structure of metabolite 9 has not yet been firmly established but the following observations suggest that it is 11-dehydrobetamethasone. (1) It runs in the Bush B3 system with 11-dehydrobeta-methasone. (2) On acetylation it runs in the Smith VI system with acetylated 11-dehydrobetamethasone. (3) On oxidation with sodium bismuthate it runs in the Smith VI and Bush 3b systems with the product of 11-dehydrobetamethasone similarly oxidized.

![Graph showing radioactivity in plasma, saliva, and urine](https://example.com/graph6.png)

Fig. 6.

The amount of radioactivity in μC per 100 ml of plasma and saliva attributed to betamethasone and metabolite 9 and the amount attributed to these two excreted per minute in the urine — for each corresponding collection.
DISCUSSION

From the amount of the presumed 11-dehydrobetamethasone excreted in the urine it is apparent that the salivary glands constitute only a fraction of the extra-hepatic tissue that is able to metabolize betamethasone. The amount of the metabolite is small compared with the total excretion of metabolites but when the liver's contribution is excluded it may well be quite large in relation to the hormones economy in extra-hepatic cells. The same applies to the metabolism of cortisol to cortisone in the salivary gland (Greaves & West 1963). This finding is of considerable clinical interest. It is generally assumed that if a patient's excretion of 17-hydroxycorticosteroids is in the normal range and if the excretion increases when ACTH is given then the effect that corticosteroid therapy has upon a disease he suffers from cannot be attributed to replacement therapy. This presupposes that if the adrenal is secreting cortisol in normal quantities, the effective concentration of cortisol at all its sites of action will be normal. The finding of considerable intra-cellular metabolism of cortisol and betamethasone means that the effective concentration within certain tissue cells could be abnormal in the presence of a normal plasma concentration. A deficient concentration of cortisol in the cells of a particular tissue or area of skin might make then vulnerable to their environment and lead to cell breakdown and secondary inflammation. In this circumstance a raised plasma level of cortisol would be necessary to produce a normal level in the defective tissue. The intra-cellular metabolism of corticosteroid hormones also raises the possibility of enzymatic adaptation to elevated concentrations leading to loss of therapeautical effect. These considerations are prompted by the unexpected efficacy in rheumatoid arthritis of prednisolone in a dose of comparable magnitude to the patient's daily cortisol secretion and by the observation that small doses of prednisolone remain effective in many patients for years whereas comparable doses of cortisone rarely do. A cortisol analogue that tissue cells were unable to metabolize at all might remain effective indefinitely.

The findings of this study in respect of unaltered betamethasone in saliva, blood and urine have a bearing on the factors that determine a cortisol analogue's potency. From the quantity of unaltered betamethasone excreted in the urine (which accounts for most of that filtered) it is apparent that it is almost entirely metabolized - as is cortisol - so that its potency cannot be ascribed to the body's inability to metabolize it. (In other studies we have measured the amount of several analogues excreted and have found the relative amounts excreted for clinically equivalent doses of the following order: cortisol (100 mg given and a high rate of urine flow maintained) 1; prednisolone (pregna-1,4-diene-11β, 17α, 21-trihydroxy-3,20-dione) 0.75; triamcinolone (9α-fluoro-16α-hydroxyprednisolone) 4, - reflecting its reduced
The concentration available within the salivary gland was of the same order as that found for cortisol at physiological levels, i.e. 15 μg/100 ml (Greaves & West 1963) yet the dose of betamethasone given is considered to be clinically equivalent to more than 200 mg of cortisol. The half-life of the betamethasone in saliva, approximately 10 hours, is ten times that which we have found for tritiated cortisol in saliva (unpubl.) and suggests that the site of action may be within the cell. (That the site of action is in the cell is strongly suggested by the observation that the concentration of triamcinolone, a very polar steroid, is much lower in the saliva than the plasma (Bailey et al. 1961). This is the likely explanation for its relatively low potency when compared with betamethasone). If this lengthened half-life determined the potency relative to cortisol one might expect to find that an equivalent dose of cortisol produced a concentration 10 times that of betamethasone. We have found that a dose equivalent to only 2 mg of betamethasone produces a concentration more than 100 times greater (Bailey et al. 1961).

The picture that emerges is of an ingested corticosteroid being distributed throughout the body and the potency depending mainly upon the binding at its site of action within the tissue cells. When the binding is minimal, as with cortisol, the plasma and intra-cellular half-lives may be similar but when the intra-cellular binding is high the plasma half-life may be much shorter and may bear no relation to the steroid's potency (cf. prednisolone and dexamethasone which have similar half-lives in plasma).

Assuming, as we do, that the salivary metabolite found was 11-oxobetamethasone the original hypothesis of Bush (1956), that the action of corticosteroids might depend upon reversible oxidation and reduction of the 11-hydroxyl group, may need to be reconsidered in the case of extra-hepatic tissues. The hypothesis was considered untenable when it was discovered that 2-methylcortisol was more potent than cortisol and 2-methylcortisone very much less potent than cortisone. Liddle et al. (1956) showed this to be the case in man in respect of electrolyte metabolism and Dulin et al. (1957) showed this to be the case in rats in the liver glycogen deposition assay and in an anti-inflammatory assay. Bush & Mahesh (1958) produced further evidence against the hypothesis by failing to find 11-oxo derivatives after administering 9α-fluorocortisol. Our studies suggest that there may have been too little 11-oxo-9α-fluorocortisol for them to detect by the methods they used and yet enough within tissue cells to be of significance. Further studies of the effectiveness within tissue cells of 11-oxo derivatives of the more potent cortisol analogues are desirable.

ERRATUM

* In the article by Murphy et al. (Some aspects of the metabolism and disposition of betamethasone) in Acta endocrinologica 45 (1964) 498, page 507 line 4 should read 0.15 μg/100 ml and not 15 μg/100 ml.
ACKNOWLEDGEMENTS

Thanks are due to Glaxo Laboratories Ltd., for the tritiated betamethasone and to the Medical Research Council and The Sheffield Regional Hospital Board for research grants.

REFERENCES


Received on August 1st, 1963.