CORTICOSTEROID METABOLISM AND RHEUMATOID ARTHRITIS

BY

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It is generally held that cortisol (hydrocortisone) is metabolized in the liver (Peterson, Wyngaarden, Guerra, Brodie and Bunim, 1955; Bush, 1962). If this is the case the effective concentration of cortisol in all other tissues will be determined by its concentration in non-protein-bound (n-p-b) form in plasma. If, on the other hand, cortisol is metabolized by enzymes in extra-hepatic tissue cells, it is these enzymes that will be the final determinant of the effective concentration within such cells. It will be necessary therefore to study the activity of these enzymes in any diseased tissue (*e.g.* connective tissue) that is sensitive to small changes in the concentration of plasma cortisol. In this paper evidence will be presented for the existence of extensive extra-hepatic metabolism of cortisol and detail will be given of attempts made to detect abnormal extra-hepatic metabolism of cortisol and prednisolone in rheumatoid arthritic patients.

Materials and Methods

The tritiated cortisol used (obtained from the Radiochemical Centre, Amersham) had specific activities of 106 mc./mg. (1963) and 85 mc./mg. (1965). If not used within one month of receipt it was re-purified by paper chromatography. It was administered by mouth unless otherwise stated. The extraction, paper chromatography, and radioactivity counting procedures were those used in a study of tritiated betamethasone metabolism (Murphy, West, and Bethel, 1963), with the following exceptions:

The initial paper chromatogram was divided into four sections (polar, cortisol, cortisone, and non-polar) and before the second chromatographic runs both the cortisol and cortisone eluates were acetylated; in the studies of specimens from patients and their controls the four chromatograms were cut into a total of 160 1-cm. strips for radioactivity counting.

The tritiated prednisolone was generally labelled at the Radiochemical Centre, Amersham, at our request in

March, 1964. It had a specific activity of 1.8 mc./mg. and the pattern of its metabolites in urine was the same as that found following the administration of un-labelled prednisolone.

All specimens were processed as described for the patients above. The total number of paper chromatograph strips counted was approximately 14,000 and each was counted three times for 10 minutes or for 10,000 counts.

In the first study of the excretion of cortisol and cortisone (Table I, p. 520), separation and measurement was by Bush techniques as described in detail by Greaves and West (1963). The methods used for the separation and estimation by gas chromatography of cortisol and its metabolites (Tables II, III, and upper part of Table IV) have been published by Bailey (1964, 1965), and those for the estimation of prednisolone and its metabolites are in the press (Bailey, 1967). 17-hydroxycorticosteroids [17(OH)CS] were measured by the method of Appleby, Gibson, Norymberski, and Stubbs (1955).

Extra-Hepatic Metabolism of Corticosteroids

(a) 1 mc. tritiated cortisol was taken by one of us and after 30 min. saliva was collected for four 1-hr periods. The volumes were 104, 114, 104, and 112 ml. At the mid-point of each saliva collection blood was taken which yielded 64, 48, 54, and 54 ml. of plasma. The specimens were ultrafiltered undiluted at 37° C., using a LKB ultrafilter 6300A. Fig. 1 (opposite) shows the quantities of radioactivity found in the polar, cortisol acetate, and cortisone acetate chromatograms of the second plasma and saliva specimens.

Fig. 2 (opposite) shows the concentrations of n-p-b cortisol and cortisone in the four hourly collections of saliva and the corresponding plasma samples. It will be seen that in each case the concentration of diffusible cortisol was lower in the saliva than in the plasma and that the concentration of diffusible cortisone was higher in the saliva than the plasma.

Fig. 1.—Disposition of radioactive cortisol, cortisone, and their unconjugated polar metabolites on the chromatograms from the second collection of plasma and saliva (see text). Each chromatogram is 40 cm. long.

Fig. 2.—Concentration (in m μ c./100 ml.) of non-protein-bound tritiated cortisol and cortisone in four consecutive hourly collections of saliva and in plasma taken at the mid-point of each saliva collection.

Fig. 3.—Disposition of tritiated cortisol and its metabolites in human milk taken 3 hrs after an oral dose of tritiated cortisol.
2. 20-dihydrocortisol.
3. 20-dihydrocortisone and tetrahydrocortisol.
5. tetrahydrocortisone.
8. 11-oxo-androstenedione.
* Unknown. Each chromatogram is 20 cm. long.

(b) In similar but less extensive studies, one patient with ankylosing spondylitis and one with rheumatoid arthritis were given tritiated cortisol and two rheumatoid arthritic patients were given tritiated prednisolone. The rheumatoid patients each showed the same pattern of salivary metabolites as in Fig. 1 (prednisolone falling and prednisone rising). The spondylitic patient showed the pattern seen at the bottom of Fig. 7; in his case the rate of saliva flow was very low (0.24 ml./min.) and the cortisol was almost entirely replaced by metabolites. The ratio of the 11-hydroxy to 11-oxo-17-oxosteroids (non-polar region) is the reverse of the normal plasma ratio which is seen in Fig. 6 (overleaf).

(c) To a patient who was about to stop breast-feeding 100 μ c. tritiated cortisol were given, and 3 hours later 50 ml. of milk was expressed. Fig. 3 shows the chromatograms. It will be seen that the relative concentration of cortisol and cortisone

are the reverse of those found in plasma. Peak 2 has the R_f of 20-dihydrocortisol, Peak 3 of 20-dihydrocortisone, Peak 5 of tetrahydrocortisone, and Peak 8 of 11-oxo-androstenedione. There is no peak corresponding to 11-hydroxyandrostenedione which in plasma normally exceeds Peak 8. These findings and those given above provide evidence of extensive 11 β -hydroxysteroid dehydrogenase activity in the salivary and mammary glands.

(d) In the same study as (a) above, urine was collected for 3 days. From hourly specimens corresponding to the plasma collections, the renal excretion of n-p-b cortisol and its n-p-b unconjugated metabolites were estimated (Fig. 4).

Fig. 4.—Renal clearance of unconjugated non-protein-bound tritiated cortisol, cortisone, and three groups of metabolites at different rates of urine flow. For detail see text.

The curves of Fig. 4 made it possible to make an approximate calculation of the mean plasma concentrations of the steroids during varying periods of the following 72 hours, from their rate of excretion in the urine. Fig. 5 shows a semi-log plot of their rate of excretion over the 72 hrs. From knowledge of the mean plasma concentration of n-p-b cortisol and its n-p-b unconjugated metabolites in the four hourly periods, together with the concentrations deduced for periods covering the rest of the 72 hrs, it was possible to make an approximate calculation of the amounts of n-p-b cortisol and of n-p-b unconjugated cortisol metabolites available to the liver for metabolism and/or conjugate formation. It was found that, if one excluded the cortisone and the most polar metabolites, the liver received more than three times as much n-p-b unconjugated metabolite as n-p-b cortisol. Had the liver extracted all these metabolites from 1 litre of plasma a minute the conjugates formed would have accounted for

Fig. 5.—Rate of urinary excretion of tritiated cortisol and its unconjugated metabolites following an oral dose of 1 mc. (106 μ g.) tritiated cortisol.

1. 6-hydroxycortisol and metabolites of similar polarity.
2. 20-dihydrocortisol and metabolites of similar polarity.
3. 20-dihydrocortisone and metabolites of similar polarity.
4. 17-ketosteroids and metabolites of similar polarity.

approximately two-thirds of the tritiated cortisol administered.

(e) In (d) above, the concentration of radioactively steroids available to the liver was calculated from ante-cubital vein blood and not from portal vein blood. To reveal the pattern in portal vein blood 1 mc. tritiated cortisol was given by intravenous injection to a patient about to have an operation for carcinoma of the stomach. Portal vein blood was taken exactly 30 min. later. The plasma was diluted five times with saline before ultrafiltration. Fig. 6 (opposite) shows ultrafiltrate chromatograms after only 30 min. the quantity of unconjugated metabolites already equalled that of cortisol itself. A comparison of the concentration of radioactive cortisol and its unconjugated metabolites in protein bound and free form in the brachial artery and portal vein blood, taken simultaneously, showed a marked fall in cortisol concentration and a change in the pattern of metabolites. A valid interpretation of these findings cannot be given, as it could not be assumed that a steady state existed between the steroids in the blood and in the intestinal cells. An important finding from this study was that the pattern of cortisol and its unconjugated metabolites present in plasma ultrafiltrate plus residue was different from that of the same plasma before ultrafiltration at 37° C. This phenomenon is being further studied.

Fig. 6.—Chromatograms of tritiated cortisol and its unconjugated metabolites from the ultrafiltrate of 7 ml. portal vein plasma taken 30 minutes after 1 mc. tritiated cortisol had been given intravenously. Each column is 1 cm. wide.

Renal Clearance of Cortisol and Prednisolone and Their Unconjugated Metabolites

As a result of the evidence given and discussed, it is concluded that at physiological concentrations unconjugated cortisol metabolites are largely of extra-hepatic origin. In a search for evidence of abnormal extra-hepatic metabolism of corticosteroids in rheumatoid arthritic patients, we have therefore examined such metabolites rather than their conjugates and have extracted them from urine rather than plasma as the concentrations present in plasma are very low indeed. In order to interpret the urinary findings prior knowledge of the renal clearance of cortisol and its unconjugated metabolites was necessary. Fig. 3 shows the renal excretion of cortisol, cortisone, and three groups of metabolites. If the curves for cortisol and cortisone could be transposed, the finding would be that the excretion varied directly with the rate of urine flow and the polarity of the steroid. To test the hypothesis that the apparently anomalous positions of the cortisol and cortisone curves were due to the active tubular reabsorption of cortisol and the diffusion back into the lumen of some of the cortisol after oxidation to cortisone, a large dose of corticosterone (100 mg.) was given to the same subject together with approximately 15 μ c. tritiated cortisol, and the renal clearance of cortisol and cortisone was measured again. Urine was collected for two 30-min. periods and 200 ml. blood were taken at the mid-point of each collection. In the first period 51 per cent. of the n-p-b plasma cortisol was excreted at a rate of urine flow of 1 ml./min. and G.F.R. of 100 ml./min. and in the second period 61 per cent. was excreted at a rate of urine flow of 4 ml./min. and G.F.R. of 110 ml./min. The percentage of n-p-b plasma cortisone excreted was 16 and 36 per cent. respectively. Thus the clearance of cortisol rose and that of cortisone fell. (Low counts in the plasma ultrafiltrates gave an accuracy of ± 5 per cent.). For the effect of corticosterone

on cortisol metabolism and the interpretation of these findings see the Discussion (p. 522).

The excessive dose of radioactivity that was needed to provide the data of Fig. 3 precluded a repetition of the study but a single renal clearance of tritiated cortisol and cortisone performed on a rheumatoid patient with exactly the same techniques was made and the effect of rate of urine flow on the excretion of non-radioactive cortisol and cortisone of four normal individuals was measured. All the findings were consistent with the evidence provided above of the renal handling of cortisol and its unconjugated metabolites. Similar but less extensive studies were made of the renal clearance of tritiated prednisolone and its unconjugated metabolites and of the effect of rate of urine flow on the excretion of non-radioactive prednisolone and prednisone in four normal individuals given unlabelled prednisolone at four dosage levels. The findings duplicated those for cortisol and its metabolites, except that prednisolone was found to be actively excreted.

Urinary Excretion of Cortisol and Some of its Unconjugated Metabolites

Table I (overleaf) shows the 24-hr urinary excretion of cortisol and cortisone and of 17(OH)CS for patients and controls under various circumstances. The data show that there is no marked difference between the patients and controls in the quantities of cortisol and cortisone excreted or in the ratio of cortisol to cortisone excreted.

Table II (overleaf) shows the 24-hr urinary excretion of 17(OH)CS in mg. and of cortisol and some of its unconjugated metabolites in μ g. by six rheumatoid arthritic patients (Cases 36-41) receiving long-term ACTH therapy of the type described by West (1962). 20(OH)F is 20-dihydrocortisol; 20(OH)E, 20-dihydrocortisone; THF, tetrahydrocortisol; THE, tetrahydrocortisone; 6(OH)F, 6-hydroxycortisol; 6(OH)E, 6-hydroxycortisone together with a small amount of an unknown contaminant. At the time

TABLE I
24-HR URINARY EXCRETION OF CORTISOL AND
CORTISONE BY CONTROLS AND PATIENTS IN
VARIOUS CIRCUMSTANCES

Subject	State	Therapy	17(OH)CS (mg.)	Cortisol (μ g.)	Cortisone (μ g.)
1	N	—	13.4	26	30
2	N	—	18.5	31	55
3	N	—	13.8	20	40
4	N	—	12.6	43	73
24	RA	—	20.6	32	76
25	RA	—	7.6	45	55
26	RA	—	16.4	34	50
27	RA	—	10.6	35	82
28	RA	—	8.8	40	51
29	RA	—	13.5	23	57
			10.6	34	34
3	N	Cortisol			
		20 mg.*	16.4	30	26
			16.4	25	22
		40 mg.*	22.0	80	120
			23.4	91	171
		60 mg.*	29.0	186	138
			30.4	217	167
		80 mg.*	38.8	372	332
			43.0	332	360
		100 mg.*	54.0	676	400
			52.4	600	400
		120 mg.*	59.6	1130	704
			64.2	1281	664
5	N	180 mg.*	50.8	2697	530
30	RA	50 mg.†	17.0	57	85
			21.6	80	194
3	N	ACTH	20.2	131	193
			37.8	383	258
			34.0	456	172
			43.8	1256	716
			37.0	1500	300
64	AS	ACTH†	39.8	1053	738
			45.9	1752	840
			35.0	623	454
			26.0	340	240
			34.4	300	100
31	RA	ACTH†	30.4	500	200
			27.8	387	222
			30.0	348	268
			32.6	850	330
32	RA	ACTH†	15.6	149	91
			12.0	34	20
			23.0	200	134
33	RA	ACTH†	22.4	228	146
			21.8	100	50
34	RA	ACTH†	34.6	1006	572
35	RA	ACTH†	55.0	1394	1030

N—Normal subjects (Nos. 1-23).

RA—Rheumatoid arthritis (Cases 24-63).

AS—Ankylosing spondylitis (Cases 64-66).

*divided doses.

†long-term therapy.

17(OH)CS = total 17-hydroxycorticosteroids.

of the assays patients 38, 40, and 41 had the most persistently active joint inflammation. Patient 39, in whom disease activity was mild, was not markedly hypercortisol. For the interpretation of the variable ratio between 17(OH)CS excretion and that of cortisol and cortisone, it must be noted that the rate of urine flow at the time of maximum steroid excretion was not known. The clinical effects following the ACTH injections suggested that some patients had a high concentration of circulating

cortisol for a short period and that others had a much lower concentration for a prolonged period.

Table III (opposite) shows the excretion of cortisol and some of its unconjugated metabolites over short periods of time with known rates of urine flow. It will be seen that:

(1) The excretion of cortisol and its metabolites by three rheumatoid patients (Cases 42, 46, 47) was similar to that of the controls;

(2) The excretion of Case 43 with acute rheumatoid arthritis and fever was markedly different from that of the controls, especially in the case of 6-hydroxycortisol;

(3) A normal control Subject 3 given the same dose of BTZ (phenylbutazone) as Case 43, for the same length of time, did not have an abnormal excretion pattern;

(4) The addition of aspirin to the therapy of Case 43 did not increase the excretion of cortisol or change the pattern of metabolites.

Urinary Excretion of Prednisolone and Some of Its Unconjugated Metabolites

Table IV (overleaf) shows the results of a study of the excretion of prednisolone and some of its unconjugated metabolites by seven normal individuals and by fifteen rheumatoid arthritic patients—the latter having received prednisolone for prolonged periods. The upper part of the Table records the excretion of prednisolone, 20-dihydroprednisolone [20(OH)P^{one}], 20-dihydroprednisone [20(OH)P^{one}], and 6-hydroxyprednisolone in seven normal subjects and fifteen patients. The lower part records the percentage of the total urinary radioactivity that was found in peaks corresponding to the above-named steroids on paper chromatograms of urine extracts from one normal subject (3) and seven rheumatoid patients (51, 61, 62, 54, 55, 63, 59) given 30-50 μ c. tritiated prednisolone. A 12-hr period was chosen because it had been found that 90 per cent. of the unconjugated metabolites were excreted in less than this time and because this period enabled collections to be made in the day time when supervision could be maintained and the rate of urine flow not allowed to fall to a low level. Prednisolone phosphate dissolved in water was given in each case. The results show that, with two exceptions, it was only the patients with complications who excreted a markedly abnormal pattern of metabolites. The two exceptions were:

(1) Case 54 who, when given 5 mg. prednisolone phosphate with tritiated prednisolone, excreted only 1.5 per cent. as 6-hydroxyprednisolone;

(2) Case 55, who excreted 20.8 per cent. of the dose as 6-hydroxyprednisolone. This patient had taken three tablets of sodium amytal nightly for 5 years (see Discussion).

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TABLE II
URINARY EXCRETION ($\mu\text{g.}/24 \text{ hrs}$) OF CORTISOL AND SOME OF ITS METABOLITES BY SIX RHEUMATOID PATIENTS (NOS. 36-41) ON LONG-TERM ACTH THERAPY

Case No.	Specimen Volume (ml.)	17(OH)CS (mg.)	Cortisol ($\mu\text{g.}$)	Cortisone ($\mu\text{g.}$)	20(OH)F ($\mu\text{g.}$)	20(OH)E and THF ($\mu\text{g.}$)	THE ($\mu\text{g.}$)	6(OH)F ($\mu\text{g.}$)	6(OH)E* ($\mu\text{g.}$)
36	1625	16.4	44	92	86	141	22	—	—
37	1320 1560	23.6 23.2	46 122	77 171	101 197	167 225	70 103	56 96	25 20
38	840 1080 2155	26 35.8 55.5	155 159 1827	97 104 513	545 471 1602	205 284 740	87 114 90	295 227 630	80 105 121
39	1750 1740	34 44.8	87 225	111 192	107 198	174 141	13 42	260 325	43 24
40	1710	19.0	231	286	616	357	39	197	47
41	1920	20.8	996	525	1310	825	131	343	66

20(OH)F=20-dihydrocortisol; 20(OH)E=20-dihydrocortisone; THF=tetrahydrocortisol; 6(OH)F=6-hydroxycortisol; THE=tetrahydrocortisone; *6(OH)E=6-hydroxycortisone with a relatively small amount of an unknown contaminant.

TABLE III
EXCRETION OF CORTISOL AND SOME OF ITS METABOLITES IN 4-HR AND 2-HR URINE SPECIMENS BY ELEVEN CONTROLS AND SIX RHEUMATOID PATIENTS

Urine Collection	Subjects	State	Therapy	Urine Flow (ml./min.)	Excretion ($\mu\text{g.}$)				
					Cortisol	Cortisone	20(OH)F	20(OH)E	6(OH)F
8-12 a.m.	Controls 6-9	N	None	1.5-4	5-20	22-35	32-55	12-27	28-79
	42	RA	Aspirin	7	5.5	26.5	30	23	22
	43	RA ¹	BTZ	3	31	34	237	33	413
	Control 3	N ²	BTZ	1	4.5	9	20	8	34
	44	RA ³	F-20 mg. F-20 mg.†	3.5 4	110 115	145 144	205 285	38 37	124 246
	45	RA ⁴	F-20 mg.	3.5	166	107	353	43	—
8-10 a.m.	Controls 10-16	N	None	6.5*	2-14	18-27	8-29	5-15	8-19
	46	RA	Aspirin	4.2 3.1	7 4	30 21	19 11	8 —	20 22
	47	RA	Aspirin	5.6	16	19	19	8	19

(1) Early acute febrile rheumatoid arthritis.

(2) Control receiving same dose and duration of phenylbutazone therapy as 43.

(3) After 50 mg. cortisol daily for 1 year.

(4) No previous corticosteroid therapy.

†plus 0.7 g. aspirin at 8 and 10 a.m.

*mean. Other abbreviations as in Table II.

The pattern for Case 66 (AS:) shows clearly the effect of a greatly reduced renal function; the excretion of prednisolone and of those of its metabolites that can be further metabolized falls and the excretion of the most polar metabolite, 6,20-dihydroxyprednisolone, which is not normally measurable, increases. Patient 60 (RA:) shows the increase in the excretion of highly polar metabolites known to occur in late pregnancy.

Corticosteroid Metabolites in Synovial Fluid

Before the aspiration of knee effusions, tritiated cortisol or tritiated prednisolone was given to two

patients with rheumatoid arthritis and to one with ankylosing spondylitis. Plasma was taken at the time of the knee aspiration in the two who received tritiated cortisol and saliva was collected from 30 minutes before this time until 30 minutes afterwards. Fig. 7 shows the chromatograms from the synovial fluid and saliva of one of the patients. In the synovial fluid of each patient, the ratio of cortisol to cortisone, or of prednisolone to prednisone, and the ratio of their two unconjugated 11-oxygenated 17-oxosteroids was that found in plasma and very different from that found in saliva (*cf.* Fig. 1). As mentioned above, the unusually low concentration of cortisol and cortisone in the saliva chromatograms of

TABLE IV

Upper Part: URINARY EXCRETION OF PREDNISOLONE, PREDNISON, 20-DIHYDRO-PREDNISOLONE (20 OH P^{ol}) 20-DIHYDRO-PREDNISON (20 OH P^{one}), AND 6-HYDROXY-PREDNISOLONE (6 OH P^{ol}) BY 7 CONTROLS AND 15 PATIENTS

Lower Half: EXCRETION OF TRITIATED PREDNISOLONE AND METABOLITES EXPRESSED AS PERCENTAGE OF TOTAL URINARY RADIOACTIVITY IN ONE NORMAL SUBJECT AND 7 PATIENTS

Subject No.	Sex	Age (yrs)	Diagnosis	Prior Treatment	Dose (mg.)	Specimen		Creatinine (g.)	Prednisolone (μg.)	Prednisone (μg.)	20(OH) P ^{ol} (μg.)	20(OH) P ^{one} (μg.)	6(OH) P ^{ol} (μg.)	Percentage rec. of added Cortisone
						Time (hrs)	Vol. (ml.)							
17	F	20-25	Normal	Nil	5	12	510	0.63	200	92	659	117	482	73
18	F	20-25	Normal	Nil	5	12	780	0.69	198	174	662	236	572	—
19	F	20-25	Normal	Nil	5	12	870	0.91	316	166	508	96	364	—
20	F	20-25	Normal	Nil	5	12	1020	0.81	474	218	879	217	72	—
21	M	19	Normal	Nil	5	12	460	0.63	366	200	770	233	276	76
22	M	32	Normal	Nil	5	12	640	—	762	231	819	117	553	—
23	M	30	Normal	Nil	3 × 10	24	1190	—	2170	750	2737	762	3835	—
48	M	57	Sciatica + RA	BTZ	5	12	790	0.82	256	88	460	55	318	72
49	M	46	RA	Anal.	5	12	570	0.65	199	62	455	62	504	78
65	M	24	AS	BTZ	5	12	910	0.73	532	203	712	89	321	77
50	M	57	RA	PP	5	12	1000	0.74	206	76	484	42	755	72
51	M	45	RA	PP	5	12	530	0.74	246	70	979	193	219	69
52	F	55	RA	PP	5	12	740	0.71	156	138	805	186	188	—
53	M	46	RA	ECP	12.5	24	1690	1.01	760	166	2070	317	330	—
54	F	43	RA	PP	2 × 10	24	850	—	1060	703	2965	870	850	—
55	F	75	RA	PP	5	12	770	0.35	302	89	341	48	1160	71
56	M	56	RA ⁽¹⁾	PP	5	12	1000	0.44	134	196	731	100	73	—
57	F	58	RA ⁽²⁾	PP	5	12	295	0.28	131	60	581	62	391	65
66	M	42	AS ⁽³⁾	PP	5	12	1190	0.47	60	43	69	6	205 _a	77
58	M	52	RA ⁽⁴⁾	PP	10	12	970	—	955	173	1120	135	1350	—
59	M	27	RA ⁽⁵⁾	PP	2 × 5	24	2054	1.16	277	198	980	98	771	64
60	F	38	RA ⁽⁶⁾	PP	2 × 5	24	1290	1.02	439	510	2530	738	1210 _β	49
Percentage of Total Radioactivity Excreted in 12 hrs														
3	M	55	Normal	Nil	5	12	1840	—	5.6	2.3	8.6	—	5.0	—
51	M	45	RA	PP	5	12	1900	—	3.1	0.6	6.1	3.0	1.4*	—
61	F	46	RA	PP	5	12	1670	—	3.4	—	5.6	—	2.3*	—
62	F	30	RA	PP	5	12	1530	—	3.6	1.4	—	—	—	—
54	F	43	RA	PP	5	12	2020	—	4.0	3.5	5.5	—	1.5	—
55	F	75	RA	PP	5	12	2180	—	3.4	0.9	3.3	—	20.8	—
63	F	53	RA	PP	5	12	2310	—	9.1	—	5.6	—	8.8*	—
59	M	28	RA ⁽⁶⁾	PP	5	12	1220	—	1.4	0.9	3.9	—	8.0*	—

Therapy: BTZ—phenylbutazone. Anal.—analgesics. ECP—enteric coated prednisolone tablets. PP—prednisolone phosphate tablets. Diagnoses: RA, rheumatoid arthritis; AS, ankylosing spondylitis; (1) incipient congestive cardiac failure; (2) hepatomegaly, splenomegaly, diabetes, and agranulocytosis; (3) blood urea 180 mg./100 ml.; (4) febrile rheumatoid arthritis; (5) amyloidosis; (6) 1st stage of labour. α and β, excretion of 6-20-dihydroxy-prednisolone 246 μg. and 627 μg. respectively. *Extract made without Na₂SO₄.

Fig. 7 (opposite) was associated with a very low rate of saliva flow. The saliva may have been largely sucked from the buccal mucus membrane.

Discussion

In this paper the results are given in rather abbreviated form of several years of laboratory work directed to the metabolism of corticosteroids in rheumatoid arthritic patients. Much time has been spent on the development of techniques which have been, or are about to be, published elsewhere. In this type of sponsored research one tends to pass on from one study to another before the first is finished if it has yielded enough information for one's purpose or if it has appeared to be unprofitable, since time and resources are limited and must be conserved for what appears to be the most worthwhile approach.

The ability of extra-hepatic tissues, including connective tissues, to transform steroids *in vitro*

has been reported in more than fifty publications. The saliva studies reported above are an extension of those of Greaves and West (1963) and of Murphy and others (1963), in which evidence of extra-hepatic metabolism of corticosteroids *in vivo*, under physiological conditions, were first reported. The conclusion that, under physiological conditions, cortisol is mainly metabolized in extra-hepatic tissues is based on the findings given above in conjunction with the following observations:

(1) That when unconjugated cortisol metabolites are given intravenously they disappear four times as fast as cortisol so injected (Peterson, Pierce, Wynaegarden, Bunim and Brodie, 1957).

(2) That when cortisol and its metabolites 20-dihydrocortisol and tetrahydrocortisol are given intravenously the recovery in the urine of conjugated metabolites is higher from the metabolites than from cortisol (Bradlow, Fukushima, Zumoff, Hellman,

Fig. 7.—Chromatograms of tritiated cortisol and its unconjugated metabolites in synovial fluid taken 1 hr after the administration of 0.5 mc. tritiated cortisol intravenously. The saliva was collected for 30 min. before and after the aspiration (flow 0.25 ml./min.). Each column is 1 cm. wide.

and Gallagher, 1962, for 20-dihydrocortisol. Rapaport and Migeon, 1962, for tetrahydrocortisol). It would appear likely that most of the body tissues are able to metabolize cortisol and that, although their capacity to do so is limited, they make a major contribution to the turnover of cortisol at physiological concentrations. It may be that, through their ability to destroy cortisol, cells control the effective concentration of this hormone at its site, or sites, of action, since it would be surprising if all tissue cells needed the same concentration at all times.

We have found that when 100 mg. corticosterone is administered the urinary excretion of cortisone and 20-dihydrocortisone falls markedly, whereas the excretion of cortisol goes up markedly (Greaves and West, 1965). The fall in cortisone excretion is reasonably explained by corticosterone decreasing the oxidation of cortisol to cortisone through competition for 11 β -hydroxysteroid dehydrogenase. Hence the deduction, made in the text, that the fall in

renal clearance of cortisone was due to corticosterone inhibiting the conversion of cortisol to cortisone in the renal tubular epithelium. The rise in the clearance of cortisol, when corticosterone was given, is interpreted as due to a competitive inhibition of the active tubular reabsorption of cortisol (Schedl, Chen, Greene, and Redd, 1959). This would mean not only that the renal tubular epithelium was able to convert less cortisol to cortisone but that there was less to be converted and so less cortisone available to diffuse into the lumen and cause the apparent active renal secretion of cortisone that the position of the cortisone curve of Fig. 3 denoted.

The finding of an abnormal excretion of the highly polar metabolites of cortisol and prednisolone in certain patients cannot be interpreted as yet, since it is known that steroid hydroxylations may be enhanced by certain drugs (Conney, Schneidman, Jacobson, and Kuntzman, 1965; Burns, Cucinell,

Koster, and Conney, 1965) and in various disease states (Werk, MacGee, and Sholiton, 1964). The failure to find an abnormal pattern of cortisol or prednisolone metabolites in the urine of patients with uncomplicated rheumatoid arthritis represents only a first step towards the elucidation of the metabolism of these hormones in extra-hepatic tissues. Even if the sites of origin of all the known metabolites of cortisol are discovered there will still remain the 40 per cent. that have not yet been identified. It is therefore fitting to conclude by quoting the late Philip Hench, who said in his Nobel lecture 14 years ago: "These hormones still belong to the physiologist and clinical investigator as much as, if not more than, to the practising physician", and to comment that this is still true.

Summary and Conclusions

The existence of extensive metabolism of cortisol in extra-hepatic tissues has been deduced from studies of the disposition of non-protein-bound tritiated cortisol and its non-protein-bound unconjugated metabolites in body fluids. Evidence of abnormal extra-hepatic metabolism of cortisol and prednisolone in rheumatoid arthritic patients has been sought:

(1) By measuring the urinary excretion of cortisol and its unconjugated metabolites and of prednisolone and its unconjugated metabolites;

(2) By studying the pattern of tritiated cortisol and tritiated prednisolone metabolites in synovial fluid. No evidence of an abnormality common to rheumatoid arthritic patients has been found, but it is pointed out that the study of the metabolism of corticosteroids at the cell level is as yet in its infancy.

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Métabolisme des corticostéroïdes et arthrite rhumatoïdale

L'existence d'un métabolisme très actif du cortisol dans les tissus extra-hépatiques a été déduite d'études portant sur la distribution du cortisol non-lié aux protéines traitées par l'hydrogène radio-actif et de ses métabolites non-conjugués et non-liés aux protéines dans les liquides de l'organisme.

On a recherché des signes évoquant un métabolisme extra-hépatique anormal du cortisol et de la prednisolone chez des malades atteints d'arthrite rhumatoïdale par: 1. mesure de l'élimination urinaire du cortisol et de ses métabolites non-conjugués et de la prednisolone et de ses métabolites non-conjugués; 2. étude de la distribution des métabolites du cortisol et de la prednisolone traités par l'hydrogène radioactif dans le liquide synovial. On ne mit en évidence aucune anomalie commune à tous les sujets porteurs d'arthrite rhumatoïdale, mais il faut insister sur le fait que l'étude du métabolisme des corticostéroïdes à l'échelon cellulaire n'en est encore à l'heure actuelle qu'à ses premiers pas.

Metabolismo corticosteroide y artritis reumatoide

La existencia de un metabolismo muy activo del cortisol en los tejidos extrahepáticos ha sido deducida de los estudios de la disposición del cortisol no proteico tratado con hidrógeno radioactivo y de sus metabolitos no conjugados y no proteicos en los líquidos del organismo.

Evidencia de metabolismo extrahepático anormal de cortisol y prednisolona en enfermos con artritis reumatoide ha sido buscada por: 1. determinación de la excreción urinaria de cortisol y de sus metabolitos inconjugados y de la prednisolona y sus metabolitos inconjugados; 2. estudio de la disposición de los metabolitos de cortisol y prednisolona tratados por hidrógeno radioactivo en el líquido sinovial. No hubo evidencia de una anomalía en enfermos con artritis reumatoide, pero hay que tener en cuenta que el estudio del metabolismo corticosteroide a nivel celular todavía está en su infancia.