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KINETICS OF CORTISOL METABOLISM AND EXCRETION. A HYPOTHETIC MODEL BASED ON THE CUMULATIVE URINARY RADIOACTIVITY IN EIGHT MULTIPLE PITUITARY DEFICIENT PATIENTS

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Summary—A new model is proposed to study the kinetics of [^{3}H]cortisol metabolism by using urinary data only. The model consists of 5 pools, in which changes of the fractions of dose are given by a system of 5 ordinary differential equations. After i.v. administration of [^{3}H]cortisol to 8 multiple pituitary deficient (MPD) patients (group I) the urines from each patient were collected in 9–15 portions during the following 3 days. From the urinary data the rate constants of cortisol metabolism were calculated. A published set of urinary data from patients with a normal cortisol metabolism (group II) was used for comparison. The *overall* half-life of the label in the circulation was 30 min for both groups; the half-life of the label excretion by both groups was 6 h and the time of maximal activity in the main metabolizing pool was 1.8 h in group I and 1.5 h in group II. The 20% of normal cortisol production rate (CPR) in the 8 MPD patients amounted to 7.2 ± 1.9 μmol/(m^2*d). Therefore, the low CPR but normal rate constants, i.e. a normal metabolic clearance rate of cortisol, in the MPD patients suggest a sensitive adjustment of the cortisol response in the target organs.

INTRODUCTION

During endocrinological investigations of growth hormone deficient (MPD) patients it was observed that the urinary excretion of cortisol metabolites was very low and the plasma cortisol levels at about 9 a.m. were <200 nM. When a low dose of [^{3}H]cortisol ([^{3}H]F) was administered for diagnostic reasons to 8 of these patients to determine their cortisol production rates (CPR), it was decided to also study the kinetics of cortisol metabolism and excretion. If a subnormal CPR were to be found it could be that these children adapted to this rate of production by a lower metabolic clearance rate of cortisol, and the rate constants should then be smaller. If the rate constants were similar to those of metabolically normal patients then the adaptation of the children to a low CPR would be at the end-organ target cells.

Nine to fifteen urine portions were collected from each MPD patient during the 3 days following the dosage of [^{3}H]F and the cumulative label excretion in the urine was calculated as a fraction of dose (FOD).

A kinetic model was designed to calculate the rate constants of cortisol metabolism using only the cumulative FOD in the urine. The model consists of 3 internal and 2 external pools and the transfer rates of the label from and to these 5 pools are given by a system of 5 ordinary differential equations. These equations were integrated and expressed in the 5 rate constants. The values of all these constants were obtained by computer-assisted fitting of the time dependent cumulative FOD in the urine of each patient separately. A pilot study to test the model was performed using the urinary data ($n = 8$) from an obese boy (patient 1).

Fortunately, in the literature a study was found with the necessary urinary data obtained from 6 experiments with 4 metabolically normal adults with which our newly developed kinetic model could be checked [1].

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Abbreviations: CPR, cortisol production rate; d, day; FOD, fraction(s) of dose; MPD, multiple pituitary deficiency; UER, urinary excretion rate of cortisol metabolites; 11-OET, 11-oxo-aetiocholanolone; THE, tetrahydrocortisone; 5αTHF, allotetrahydrocortisol; THF, tetrahydrocortisol; αHHE, 20α-cortolone; βHHE, 20β-cortolone; αHHF, 20α-cortol; βHHF, 20β-cortol; T_4, l-thyroxine; and T_3, triiodothyronine.

Table 1a. Introductory data of the 8 MPD patients and patient No. 1, somatometric data

Pat. No.	Sex	Chron age (y)	Bone age [28] (y)	Height (m)	Weight (kg)	Body surface [20] (m^2)
1	M	13.8	14.0	1.55	57.2	1.59
2	F	21.7	18.0	1.42	39.1	1.26
3	M	26.8	19.0	1.78	62.9	1.76
4	M	20.4	12.8	1.59	37.0	1.28
5	M	23.4	19.0	1.71	70.9	1.85
6	M	18.0	12.5	1.57	36.1	1.25
7	F	17.4	15.5	1.53	44.2	1.38
8	F	20.8	13.8	1.68	65.8	1.77
9	F	20.8	13.8	1.73	68.4	1.82

EXPERIMENTAL

Patients

The clinical data of the patients are presented in Table 1a. Patient No. 1 was referred because of obesity and a high urinary steroid excretion. The other 8 patients (Nos 2–9) had multiple pituitary deficiences of GH, TSH, FSH and LH and were treated with 1-thyroxine (T_4) and, except for patient No. 4, with testosterone (patients 3, 5 and 6) or ethinyloestradiol and norethisterone cyclically (patients 2, 7, 8 and 9), but not with glucocorticosteroids. Patients 4 and 6 received hGH, the other patients were no longer on GH therapy. The pubertal stages [2] for our patients were: breast development: stage 4 for patients 2 and 7, stage 3 for patients 8 and 9; pubic hair: stage 4, 1, 5, 1, 4, 1, 3, 1 and 1 for patients 1 to 9, respectively and testicular development: stage 3, 5, 1, 4 and 2 for patients 1, 3, 4, 5 and 6, respectively.

Dosage of [^{3}H]F

Radioactive cortisol, [1,2,6,7-^{3}H]F (sp. act.: 90 Ci/mmol) was obtained from Amersham (Bucks., England). The radioactive purity of the [^{3}H]F was checked and found to be = 95%. About 90 kBq (2.4 μCi) [^{3}H]F were taken into 10 ml 0.9% NaCl solution and filtered through a 0.2 μm Millex filter (Millipore Products Div, Etten-Leur, The Netherlands) into a closed 10 ml vial. At 09:00 h about 9 ml of the [^{3}H]F solution was administered within 5 min to the patient i.v. The remaining solution was used for analysis of the radioactivity [3]. The exact amounts of solution were measured by their weight. Each patient's 3 days urine collection was in 8 to 15 portions, which were stored at −18°C until use. The radioactivity in 1 ml of each of these portions was determined by liquid scintillation counting over a period of 10 or 20 min [3].

Table 1b. Introductory data of the 8 MPD patients and patient No. 1, the raw urinary data of the MPD patients

Pat. No. 2			Pat. No. 3			Pat. No. 4			Pat. No. 5			Pat. No. 6			Pat. No. 7			Pat. No. 8			Pat. No. 9		
t^a	$x_2(t)^b$	$v(t)^c$	t	$x_2(t)$	$v(t)$	t	$x_2(t)$	$v(t)$	t	$x_2(t)$	$v(t)$	t	$x_2(t)$	$v(t)$	t	$x_2(t)$	$v(t)$	t	$x_2(t)$	$v(t)$	t	$x_2(t)$	$v(t)$
0.020	0.029	531	0.037	0.058	121	0.032	0.030	146	0.039	0.051	41	0.040	0.054	26	0.083	0.126	14	0.024	0.039	303	0.024	0.046	88
0.061	0.105	335	0.079	0.061	31	0.073	0.097	67	0.080	0.119	49	0.084	0.158	30	0.167	0.300	20	0.066	0.139	133	0.066	0.153	130
0.113	0.228	176	0.120	0.275	28	0.113	0.173	62	0.119	0.184	64	0.123	0.257	28	0.208	0.375	46	0.149	0.344	125	0.149	0.359	105
0.165	0.360	104	0.162	0.385	40	0.155	0.235	132	0.161	0.254	69	0.165	0.356	27	0.288	0.476	22	0.232	0.509	96	0.232	0.498	143
0.249	0.470	24	0.204	0.473	58	0.180	0.285	277	0.242	0.375	127	0.208	0.454	54	0.344	0.542	66	0.315	0.630	80	0.315	0.586	53
0.332	0.567	45	0.245	0.517	71	0.220	0.324	177	0.327	0.485	150	0.251	0.533	82	0.375	0.574	150	0.398	0.704	178	0.398	0.682	116
0.499	0.696	95	0.287	0.576	102	0.280	0.367	87	0.411	0.657	250	0.331	0.650	58	0.406	0.621	273	0.481	0.760	99	0.481	0.725	124
0.999	0.810	50	0.370	0.680	113	0.363	0.457	68	0.494	0.718	370	0.415	0.698	67	0.458	0.645	71	0.981	0.878	75	0.981	0.828	54
1.499	0.840	65	0.454	0.795	111	0.446	0.543	96	0.994	0.803	17	0.498	0.726	55	0.500	0.666	75	1.481	0.901	65	1.481	0.853	23
1.999	0.849	65	0.533	0.839	107	0.526	0.598	96				0.998	0.801	20	1.000	0.815	86	1.981	0.910	91	1.981	0.858	30
2.499	0.853	55	0.933	0.913	77	0.915	0.678	27				1.498	0.909	38	1.500	0.847	37	2.481	0.913	77	2.481	0.860	50
2.915	0.854	54	1.537	0.937	33	1.530	0.707	35				1.998	0.928	23	2.000	0.861	78	2.908	0.913	92	2.908	0.860	60
			2.037	0.941	40	2.030	0.712	31				2.498	0.946	27	2.500	0.865	56						
			2.537	0.943	55	2.530	0.714	19				2.935	0.954	27	3.000	0.867	43						
			3.206	0.944	69	3.019	0.716	36															
dose: 126.84 kBq			dose: 74.71 kBq			dose: 77.14 kBq			dose: 58.98 kBq			dose: 51.24 kBq			dose: 67.35 kBq			dose: 113.21 kBq			dose: 124.49 kBq		

[a]Time in days; [b]the cumulative fraction of dose (^{3}H); and [c]the production rate v in ml urine per hour.

Chemicals

Standard steroids were purchased from Sigma Chemical Co (St Louis, MO, U.S.A.). Dichloromethane (DCM), methanol (MeOH), acetonitrile (CH_3CN) and other solvents and reagents were of analytical grade and obtained from Merck (Darmstadt, F.R.G.). *Helix pomatia* digestive juice was purchased from Reactifs Industrie Biologique Française (Clichy, France). Methoximation and silylation reagents were obtained from Pierce Chemical Co. (Rockford, IL, U.S.A.).

Kinetic model

For patient 1 and the 8 MPD patients a simple model for the metabolism and excretion of cortisol is proposed. As shown in Fig. 1 it consists of 3 internal and 2 external pools, in which changes of the FOD, x_1 to x_5, can be described by a system of five differential equations:

$$\begin{aligned} dx_1/dt &= -k_3x_1 && , x_1(0) = 1 \\ dx_2/dt &= k_1x_4 && , x_2(0) = 0 \\ dx_3/dt &= k_2x_5 && , x_3(0) = 0 \\ dx_4/dt &= k_3x_1 - (k_1 + k_4)x_4 + k_5x_5 && , x_4(0) = 0 \\ dx_5/dt &= k_4x_4 - (k_2 + k_5)x_5 && , x_5(0) = 0 \end{aligned} \quad (1)$$

These differential equations were integrated and expressed in the rate constants k_1 to k_5. The values of all of these constants were obtained by fitting the function x_2 to the data of the cumulative FOD in the urines of each patient separately.

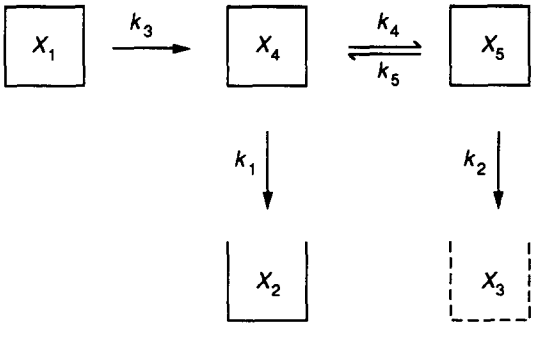

Fig. 1. Kinetic model of [^{3}H]F metabolism and excretion of its metabolites. The model consists of the 3 internal and 2 external pools X_1 to X_5, in which x_1 to x_5 represents: x_1, the FOD in the circulation (X_1); x_2, the cumulative FOD excreted in the urine (X_2); x_3, the cumulative FOD excreted in the non-urinary pool (X_3), being at least partly the faeces; x_4, the FOD in the metabolizing tissues, here defined to be the liver, intestinal tissue + kidneys (X_4); and x_5, the FOD in the gallbladder + intestinal lumen (X_5); and k_1 to k_5 are the rate constants related to mass transfer: k_1, from the body into the urine; k_2, from the body into the non-urinary pool, in part the faeces; k_3, disappearing from the circulation; k_4, from the pool X_4 to the pool X_5; and k_5, from the pool X_5 to the pool X_4. It should be noted that the dose of [^{3}H]F is taken to be 1.

CPR and urinary excretion rate (UER) of the cortisol metabolites

The CPR in μmol per day (μmol/d) was determined from the specific activity of 11-oxo-aetiocholanolone (11-OET). 11-OET was chemically derived from the cortisol metabolites THE, THF and HHE in the 3 days urine collections by side chain oxidation using $NaBiO_3$ [3, 4]. The CPR was calculated from the simple equation:

$$\text{CPR} = [^3\text{H}]\text{F}/(\text{sp. act.}*t) \quad (2)$$

where [^{3}H]F = radioactive dose of [^{3}H]cortisol (Bq), sp. act. = specific activity of 11-OET (Bq/μmol) and t = time of the urine collection (days). Using the body surface areas of the patients in m^2 (see Table 1a) the CPR is presented as μmol/(m^2*d) to normalize the values of the patients with different body heights and weights and to compare the data of children with those of adults [4–6].

The UER of cortisol metabolites was derived from the steroid profile measured in the same 3 days urine collection used for the measurements of the CPR and creatinine. The UER (μmol)/(m^2*d) was calculated from the sum of 11-OET, 11-hydroxy-aetiocholanolone and -androsterone, THE, THF, 5αTHF, α- and βHHE and α- and βHHF. The gas-chromatographic steroid profiles and the creatinine concentrations were measured as described previously [3, 4].

Statistics

The results are given as the mean ± SD unless otherwise indicated. Two-sided probability values were derived from Student unpaired or paired t-tests.

RESULTS

Kinetics of metabolism and excretion

The solution to the system of five differential equations for the FOD in the circulation (x_1) and the urine (x_2) is:

$$x_1 = e^{-k_3t} \quad (3)$$

$$\begin{aligned} x_2 = k_1[&-\alpha(1 - e^{(\lambda_1 - k_3)t})/(\lambda - k_3) \\ &- \beta(1 - e^{(\lambda_2 - k_3)t})/(\lambda_2 - k_3) \\ &+ R(1 - e^{-k_3t})/k_3Q] \end{aligned} \quad (4)$$

where

$$\alpha = (k_3 + R\lambda_2/Q)/(\lambda_1 - \lambda_2),$$
$$\beta = -\alpha - R/Q,$$
$$\lambda_1 = (-P + \sqrt{(P^2 - 4Q)})/2,$$
$$\lambda_2 = (-P - \sqrt{(P^2 - 4Q)})/2, \qquad (5)$$
$$P = -2k_3 + k_1 + k_2 + k_4 + k_5,$$
$$R = k_3(k_5 + k_2 - k_3)$$

and

$$Q = k_3^2 - k_3(k_1 + k_2 + k_4 + k_5) + k_1k_5 + k_1k_2 + k_2k_4.$$

The remaining part of the solution to equation (1), i.e. the equations for the FOD's in the other 3 pools, x_3, x_4 and x_5, as well as the mathematical conditions and criteria used for the acceptability of the model are available on request. As the results from the two possibilities:

(1) $k_1, \ldots, k_5$ are all free, though positive, parameters, and

(2) $k_1, \ldots, k_4$ are free, postive parameters, but $k_5 = k_4$,

did not differ significantly we used the second possibility. The results are shown in Table 2 and Fig. 2. The rate constants in Table 2 are expressed as reciprocal days. In only one publication [1] on the metabolic fate of radioactive cortisol [^{14}C]cortisol were the raw data of urinary radioactivities tabulated, but this enabled us to compare the kinetic parameters

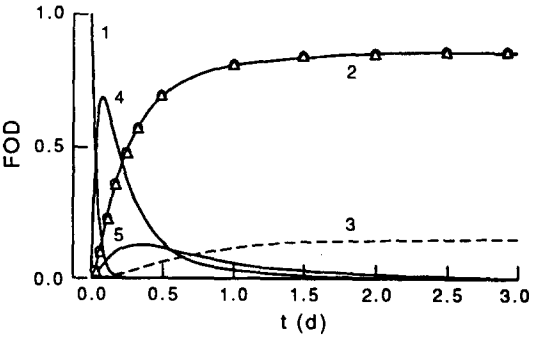

Fig. 2. Fractions of dose, x_1 to x_5, as a function of time after administration of the dose [^{3}H]F to patient No. 2 of group I. The numbers 1 to 5 indicate the corresponding FOD defined in Fig. 1. The symbols ○ and △ refer to the calculated and the corrected cumulative FOD in the urine (X_2), respectively. Using the mean rate of urine production ($\bar{v}$) during the last two days, where $x_2(v,t) = x_2(t)$, we state that $x_2(v,t) = (1 - \delta[v(t) - \bar{v}]x_2(t)$ where the mean correction factor $\delta = 6 \times 10^{-4} \pm 2 \times 10^{-4}$ (h/ml).

for the MPD patients (group I) with those of the metabolically normal, oncologic patients (group II, Nos. 1–4, $n = 6$).

For the MPD group ($n = 8$) the average k_1 (in d^{-1}) of excretion into the urine: 3.24 ± 0.64 was similar to that for the group II: $k_1 = 4.02 \pm 1.21$ ($n = 6$). The rate constant k_2 of non-urinary excretion for group I did not differ from the corresponding value of k_2 for group II, except for our patients 3, 4 and 5 (group I), whose relative high k_2 values could not be accurately determined.

The average rate constant k_3 of ^{3}H-disappearing from the circulation in the 8 MPD patients was as expected rather high: 35.6 ± 12.4 and equal of that of group II: 39.8 ± 12.0 ($n = 6$).

Table 2. Rate constants of mass transfer of cortisol and its metabolites

Pat. No.		k_1	k_2	k_3	k_4
			(a) [^{3}H]F		
Group I					
1		5.1 ± 0.00	1.1 ± 0.00	18.7 ± 0.02	1.8 ± 0.00
2		3.6 ± 0.00	1.2 ± 0.00	26.6 ± 0.07	1.2 ± 0.01
3		3.6 ± 0.07	27 ± ∞	36.4 ± 1.11	0.21 ± 0.22
4		2.2 ± 0.72	40 ± ∞	31.0 ± 11.2	0.90 ± 6.3
5		2.4 ± 0.12	31 ± ∞	42.3 ± 4.0	0.06 ± 0.69
6		3.9 ± 0.01	0.13 ± 0.00	25.9 ± 0.17	1.64 ± 0.02
7		3.0 ± 0.00	1.2 ± 0.00	26.2 ± 0.00	0.71 ± 0.01
8		3.4 ± 0.00	2.1 ± 0.02	62.8 ± 7.6	0.77 ± 0.00
9		3.8 ± 0.00	1.04 ± 0.00	33.5 ± 1.34	0.55 ± 0.00
Mean		3.4 ± 0.86		33.7 ± 12.9	0.87 ± 0.59
	Exp.		(b) [^{14}C]F		
Group II	No.				
1	1	6.1 ± 2.21	4.47 ± 1.07	21.7 ± 8.66	4.5 ± 4.28
2	2	3.3 ± 0.00	1.34 ± 0.01	36.2 ± 0.00	0.66 ± 0.00
2	3	4.2 ± 0.04	0.40 ± 0.01	53.8 ± 0.05	2.4 ± 0.04
3	4	2.7 ± 0.00	0.98 ± 0.00	46.4 ± 0.00	0.85 ± 0.00
4	5	3.3 ± 0.03	2.02 ± 0.04	31.9 ± 0.06	1.8 ± 0.04
4	6	4.5 ± 0.09	2.62 ± 0.03	48.7 ± 1.46	4.3 ± 0.22
Mean		4.01 ± 1.21		39.8 ± 12.0	2.4 ± 1.67

The individual rate constants, defined in Fig. 1, are given as reciprocal days (d^{-1}) with 90% confidence interval. The means of the rate constants are followed by the SD.

Table 3. Half-lives of cortisol (^{3}H- and ^{14}C-) in the circulation (X_1) and of its metabolites in the total body ($X_2 + X_3$)[a], times of maximal activity in pool X_4 and pool X_5, and the maximal urinary excretion rate $\dot{x}_2$ (max) at t_{max} (X_4)

	$t_{\frac{1}{2}}$		t_{max}		
	X_1	$X_2 + X_3$[a]	X_4	X_5	$\dot{x}_2$(max)[b]
Group I					
Mean	0.51	6.1	1.81	6.7	0.103
SD	0.14[c]	0.9[d]	0.33	3.0	0.017
Range	0.39–0.64	5.3–7.4	1.1–2.2	2.9–10.3	0.072–0.120
Group II [1]					
Mean	0.46	5.8	1.47	6.7	0.116
SD	0.17	1.6[d]	0.28	2.6	0.021
Range	0.31–0.77	3.9–7.9	1.1–1.8	4.1–10.1	0.091–0.141

Times are given in hours.

[a] $t_{\frac{1}{2}}(x_2 + x_3)$ was derived from $x_2 + x_3 \approx 1 - e^{-\gamma t}$ (see Fig. 3).

[b] $\dot{x}_2$(max) is dx_2/dt at $t_{max}(X_4)$, the maximal rate of label excretion in the urine, given as dose (= 1) per hour.

[c] $t_{\frac{1}{2}}(x_1)$ of patient 1 (0.89 h) was not included in the mean.

[d] $t_{\frac{1}{2}}(x_2 + x_3)$ of patient 6 (group I; 8.5 h) and exp. 3 (group II, 7.0 h) were longer than their actual first half-lives and hence not included in the mean.

The rate constant k_4 of mass transfer from the metabolizing pool (X_4) to the intestinal lumen (X_5) and back was of the same magnitude as the corresponding k_2 value for both groups of patients, except for the above mentioned three MPD patients 3, 4 and 5, where a strong, negative correlation was observed between the high k_2 and low k_4 values. Figure 2 shows the FOD in each pool for patient 2 of group I and it represents the results obtained for all patients of both groups, except for patients 3, 4, and 5 of group I, who showed hardly any radioactivity in pool X_5. For our patients it was observed that the rate of label excretion ($\dot{x}_2$) was partly dependent on the rate of urine production. Consequently the observed cumulative urinary radioactivity was corrected for this phenomenon (see Fig. 2).

The overall half-time [$t_{\frac{1}{2}}(x_1)$] of ^{3}H and ^{14}C in the circulation (X_1) and the times of maximal radioactivity in the pools X_4 and X_5: $t_{max}(X_4)$ and $t_{max}(X_5)$, respectively, are given in Table 3. The average $t_{\frac{1}{2}}(x_1)$ for the MPD patients was 0.51 ± 0.14 h (~ 30 min), similar to that for group II: $t_{\frac{1}{2}}(x_1) = 0.46 \pm 0.17$ h (~ 30 min; $n = 6$). $t_{max}(X_4$; liver, intestines + kidneys) and $t_{max}(X_5$; intestinal lumen) were 1.8 ± 0.33 and 6.7 ± 3.0 h, respectively, for our 8 MPD patients (2 to 9) and again, not different from those for group II: 1.5 ± 0.3 h and 6.7 ± 2.6 h. However, the three MPD patients Nos 3–5 showed a low $t_{max}(X_5)$: 2.9 h.

Table 3 shows that the maximal rate of label excretion in the urine $\dot{x}_2$(max) at $t = t_{max}(X_4)$ ranged from 0.072 to 0.120 h^{-1} for our group and from 0.098 to 0.142 $*h^{-1}$ for group II, with no difference between the mean values: 0.103 ± 0.017 h^{-1} (group I) and 0.116 ± 0.021 h^{-1} (group II), respectively (see footnote [b] in Table 3).

Finally Table 3 shows the approximated $t_{\frac{1}{2}}(x_2 + x_3)$ of total excretion from the body. This half-life was computed by least squares optimization from:

$$x_2 + x_3 \approx 1 - e^{-\gamma t} \qquad (6)$$

instead of using the actual sum of x_2 and x_3 in the equations consisting of three exponential terms. This approximated $t_{\frac{1}{2}}(x_2 + x_3)$ was 6.1 ± 0.9 h (group I; patient 6 excepted) and 5.8 ± 1.6 h (group II; experiment 3, patient 2 excepted). The results are graphically shown in Fig. 3 (patient 1, group I) where the solid (sigmoidal) curve represents the actual sum of $x_2 + x_3$ and the other curve the simple exponential approximation.

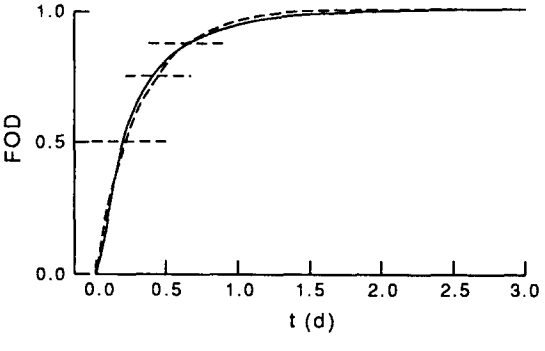

Fig. 3. Total excretion of the [^{3}H]F metabolites from the body. The total excretion is the sum of the cumulative radioactivity in the urine (x_2)and that excreted not in the urine (x_3), and it is shown as a function of time after the administration of the dose. The solid line indicates the actual sum of x_2 and x_3 according to our model. The broken line shows the sum of the two cumulative radioactivities approached using equation (6): $x_2 + x_3 \sim 1 - e^{-\gamma t}$. The results given for patient No. 1 (group I) are similar to those for all other patients with the exception of the results for our patient No. 6 and for patient 2 (exp 3) of group II (see footnote [d] in Table 3). The three horizontal dashed lines indicate the FOD values 0.50, 0.75 and 0.875.

Table 4. Cortisol production rate, and the urinary excretion rates of cortisol metabolites and creatinine of the obese boy and the eight MPD patients

	CPR[a] [μmol/(m²*d)]	UER[b] [μ/mol(m²*d)]	Creatinine [μmol/(kg*d)]
Pat. No. 1	37.4 (3.7)	38.1	222
Pat. Nos 2–9			
Mean	7.2 ± 1.9	6.1 ± 2.4	181 ± 36
Range	4.0–9.4	2.7–9.7	162–253

[a]CPR = cortisol production rate determined from the specific activity of the chemically derived 11-OET in the same 3-day urine collection as used for the UER and creatinine determination. CPR is given as the mean with % CV ($n = 3$) within parentheses.
[b]UER = urinary excretion rate of cortisol metabolites is the sum of the neutral cortisol metabolites + 11-OET, 11-hydroxy-aetiocholanolone and -androsterone, measured in the 3 days urine collection.

CPR, UER and creatinine excretion rate

The CPR values measured at 3 days from 11-OET are given in Table 4, together with the corresponding values of the UER and the creatinine excretion rate. The mean CPR in the 8 MPD patients: 7.2 ± 1.9 μmol/(m²*d) was comparable to the CPR in hypoglycemia associated with dwarfism [5]: 8.8 to 17.4 μmol/(m²*d), and was around 20% of the CPR measured in patient 1 (Table 4) as well as in normal children and adults [6]: 32.6 ± 6.9 μmol/(m²*d).

The UER was lower than the CPR (except for the patients 1 and 3) and for the MPD patients the linear relationship was: UER = −2.48 + 1.16*CPR [μmol/(m²*d); $r = 0.941$; $n = 7$]. Their mean ratio was 0.81 ± 0.14 ($n = 7$; No. 5 not included; see Table 4). The average final FOD in the urine of all patients was 0.864 ± 0.078 ($n = 9$). The creatinine excretion was quite constant during the 3 days. Its mean rate was 181 ± 36 μmol/(kg*d) ($n = 8$) and within the normal range [7, 8].

DISCUSSION

We designed and tested a new model to study the kinetics of cortisol metabolism. It is based on curve fitting of the cumulative ^{3}H-label excretion in the urine and used to calculate the values of the rate constants of cortisol metabolism and excretion.

The capacity to metabolize cortisol in our MPD patients is normal, because of the lack of a significant difference between the MPD patients and the metabolically normal patients of group II regarding: (1) the half-life of label in the circulation [$t_{\frac{1}{2}}(X_1)$]; (2) the approximate half-life of label excretion [$t_{\frac{1}{2}}(X_2 + X_3)$]; (3) the average time of maximal activity in the metabolizing pool X_4 (liver, intestines and kidneys); and (4) the maximal rate of label excretion in the urine. With this normal capacity and the lower than normal cortisol concentration in the circulation the rate of cortisol metabolism in the MPD patients must be relatively low and corresponds with the low rates of cortisol excretion (UER) and production (CPR). Therefore, it is correct to assume that the MPD patients, who did not receive glucocorticoids (periods of fever and or inflammation excepted), are more sensitive to cortisol than are the normals.

CPR

The low amounts of neutral cortisol metabolites excreted by the MPD patients (the UER in Table 4) led us to measure their CPR from 11-OET [4]. The mean ratio of UER and CPR = 0.81 ± 0.14 ($n = 7$) is in line with the final recovery of ^{3}H-radioactivity in the urine after three days: FOD = 0.86 ± 0.08 ($n = 8$). We conclude from this similarity (paired t-test: $P = 0.29$; $n = 7$), that the results of the urinary CPR are more reliable [4] than those of the recently proposed plasma secretion rate where deuterated cortisol is continuously infused [9].

Kinetics

The kinetic model considers the body to be more complex than the homogeneous entity described previously [3] but less complex than the physiological reality. The simplicity of the model was inversely related to the number of degrees of freedom and this is dependent on the difference between the number of urine collections of the patients and the number of considered parameters.

Circulation

The *overall* $t_{\frac{1}{2}}$ of ^{3}H in the circulation of the MPD patients, ~30 min, is much shorter than the reported $t_{\frac{1}{2}}$ values (slow phase) of plasma cortisol: 60 to 75 min [10], ~100 min [11], ~125 min [12] and ~73 min [13] for euthyroidal adults, but ~50 (range 47–54) min for children with idiopathic short stature [14]. Our patients were euthyroid [13, 15, 16]. The plasma free concentrations of T_4 and triicodothyronine (T_3), measured during the period of the experiments were 18 and 6 pM, respectively, in patient No. 3 with $t_{\frac{1}{2}}(X_1) = 27$ min; 18 and 8 pM in patient No. 7 with $t_{\frac{1}{2}}(X_1) = 38$ min; and 7 pM for T_4 in patient No. 5 with $t_{\frac{1}{2}}(X_1) = 23$ min, while the normal values of free T_4 range from 9 to 26 pM and that of free T_3 from 3 to 8.5 pM. Also the $t_{\frac{1}{2}}(X_1)$ in the metabolically normal patients of

group II ranged from 19 to 46 min with a mean of 28 min. It must be noted firstly that the model did not allow us to differentiate between the rapid and the slow disappearance phases of cortisol. A monoexponential process was assumed to exist, hence one $t_{\frac{1}{2}}$. However, in the quoted studies [10–14] the $t_{\frac{1}{2}}$ of cortisol was determined during the slow phase of disappearance from the circulation. Secondly that the partly but rapidly occurring oxidation of cortisol to cortisone [10] decreases the $t_{\frac{1}{2}}$ of the radioactive mixture of these two steroids, as the $t_{\frac{1}{2}}$ of label disappearing from the circulation during the slow phase is smaller for cortisone than for cortisol [11]. Finally that within 15 min after a 30 min lasting i.v. infusion of 0.25 mg labelled cortisol only 13% of the blood radioactivity was found in the unaltered hormone [1]. These facts support the correct result of the *overall* half-life of cortisol in the circulation: $t_{\frac{1}{2}} = 30$ min.

We tried to compare the value of the disappearance rate constant (k_3) with the indirectly calculated metabolic clearance rate (MCR [17]) and from the MCR also to determine the possible hepatic extraction of cortisol and the mean cortisol concentration during the period of urine collection. For this purpose we used the necessary data and equations from the literature [18–22] as explained in Table 5. By this approach the estimated MCR amounted to 132 ± 49 (range: 89 to 238) l/(m²*d), the hepatic extraction to 0.21 ± 0.06 (range 0.15–0.30) and the mean daily cortisol concentration to 61 ± 27 (range 35–106) nM. These data show that for the MPD patients the metabolic capacity is apparently normal, that the hepatic extraction (in fact the extraction by the liver, kidneys and intestinal tissue) is related to the fraction of cortisol not bound to transcortin [17] and that the lower than normal mean cortisol level (~60 nM instead of 253 ± 7 nM [13] or 220 ± 50 nM [23] corresponds to the lower than normal UER and CPR.

Table 5. Comparison of the k_3 values with the indirectly calculated MCR, the hepatic extraction (MCR/HPFR) and the estimation of the mean daily cortisol concentration (I.C.F) in the plasma of the 8 MPD patients

Pat. No. 2–9	V_p[a] (l/m²)	MCR[b] [l/(m²*d)]	MCR/ HPFR[c]	I.C.F[d] (nM)
Mean	1.28	132	0.24	61
SD	0.06	49	0.09	27
Range	1.16–1.35	89–238	0.15–0.40	35–106

[a]The plasma volumes (V_p) were calculated using data given in [18] (men) and [19] (women). The volumes were normalized to l/m² (see Table 1).

[b]The MCR was derived from the equation $MCR = k_3 \cdot V_p \cdot c$, where k_3 (see Table 2) is given in reciprocal days, V_p as shown above and the constant $c = 2.87$ was estimated as follows: using the calculated mean MCR = 0.4*MCR(05–11 h) + 0.6*MCR(20–02 h) = 0.4*149 + 0.6*91 = 114 l/(m²*d) by using the morning and evening MCRs from [21]; the mean k_3 for adults = 30/d from Table 2, group II, exp. No. 1, 2 and 5 [1]; and the mean plasma volume $V_p = 1.32$ l/m² according to Refs [17–19]: c = 114/(30*1.32) = 2.87.

[c]HPFR, the hepatic plasma flow rate was derived from [22]: 520 and 588 l/(m²*d) for men and women, respectively. The resulting ratio MCR/HPFR, a dimensionless number < 1, can be regarded as the hepatic extraction.

[d]I.C.F, the integrated concentration of cortisol (F) was estimated by calculation of the ratio of the individual CPR (see Table 4) and the individual MCR [21].

The non-invasive sampling technique for the measurements of the kinetic parameters and the urinary CPR lasts 3 days. This is far beyond the stress inducing period of blood sampling for the time of 2 to 3 h, which has been used for the measurement of the half-time of ^3H disappearance from the circulation [10–13].

Excretion

It was concluded [1] that "... the rate of excretion of the products derived from the metabolism of 80 per cent of administered hydrocortisone during the first 24 h can be characterized by an average half-life of 3.6 h in all subjects studied.". Mathematically this is a wrong conclusion. We found that the approximated $t_{\frac{1}{2}}$ of the label in the body (see Table 3) was 6 h for both groups of patients. The mass of labelled cortisol metabolites in the bile has been shown to be > 3% of the dose found in the faeces [11]. In another study about 9% of the FOD was recovered in the faeces [1], which may have been derived from a larger FOD in the bile. The influence of the enterohepatic circulation on the secretion of cortisol metabolites in the bile, the reabsorption in the intestines and on the faecal excretion can be clearly seen from the results obtained from 4 uraemic patients treated by haemodialysis [24]. Their excretion of cortisol metabolites in the faeces during the 2 weeks following the administration of [^{14}C]F was 19 to 37% of the given dose [24]. Insufficient excretion of the label by the kidneys resulted in an increased fraction of dose in the faeces. Therefore, the presumed presence of radioactivity in the intestinal lumen (X_5) and in the pool of non-urinary excretion, with the inclusion of the faeces (X_3), is likely. In our model the times of maximal activity in X_5 ranged from 4.1 to 10.1 h, mean 6.7 h, in group II of adults and from 8.2 to 10.3 h for our patients except for patients Nos 3 to 5 where 2.9 h was found. For these latter 3 patients extraordinarily high values of k_2 were observed: 27 to 40 d^{-1}, and these combined with the fairly low value of k_4:

0.06 to 0.90 d^{-1} caused the fraction of dose in X_5 to be very low. An explanation for these results in these 3 patients cannot be offered.

Radioactivity

It must be realized that the described kinetic analysis of the urinary label excretion in this study would have been almost impossible if the non-radioactive [$^{13}C_4$]cortisol [4] had been used in place of [^{3}H]F. If [$^{13}C_4$]cortisol was used, 100% of *all* cortisol metabolites would have to be recovered after extraction from the urine, after deconjugation, isolation and purification by HPLC and after side chain oxidation to the common metabolite 11-OET before measuring the isotope dilution of the [$^{13}C_4$]11-OET from the labelled cortisol metabolites in all urine samples by gas chromatography/mass spectrometry [4]. From the product of the isotope dilution and the totally recovered mass of all cortisol molecules, the apparent FOD of [$^{13}C_4$]F could so be obtained. The alternative, i.e. measuring the $^{13}CO_2$-enrichment by isotope ratio mass spectrometry in a precisely known part of *all* urinary cortisol metabolites in each urinary collection after isolation, purification and burning this mass to CO_2 could be the only other possibility next to the easy and direct measurement of radioactivity.

Radiation exposure

Another advantage of the kinetic analysis of the metabolism of [^{3}H]F is the ability to calculate the radiation exposure for the total body and the organ pools X_1 (circulation), X_4 (liver, intestinal tissue + kidneys), X_5 (gallbladder and the intestinal lumen) and even the urinary bladder (UB) using the appropriate equation in [25], the mean organ weights [26], and the integrals $\int x_1 dt$. To our knowledge this is the first time that the radiation burden of organs (pools) due to the presence of [^{3}H]F could be calculated. It was found that the highest organ dose equivalence (organ radiation burden) for the urinary bladder was $\sim 4\,\mu$Sv (Sievert). For our patients the total body impact, the so called effective dose equivalence, caused by an administered dose of 1.7 ± 0.7 kBq ^{3}H/kg body weight was $0.05 \pm 0.02\,\mu$Sv $\triangleq$ 1% of the mean daily natural effective dose equivalence in the Netherlands, and to 1 to 2% of the lowest known effective dose equivalences by X-rays, used for the examination of the knee and the hand, respectively [27].

Conclusion

To conclude we may state that our MPD patients metabolized cortisol with normal rate constants despite their state of hypocortisolaemia and low CPR ($\sim 7\ \mu$mol/(m^2*d)); their low rates of cortisol metabolism are due to the low mean cortisol levels in the circulation. An adequate biological response in the target organs of the MPD patients might have been possible due to an adaptation of the target cells.

It is remarkable that in our model kinetic aspects of the process of the disappearance of cortisol can be obtained by taking urine frequently.

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