Metabolic and Renal Clearance Rates of Purified Human Chorionic Gonadotropin

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A B S T R A C T The metabolic clearance rate (MCR) and renal clearance rate (RCR) of human chorionic gonadotropin (hCG) were measured in healthy young men and women using techniques of continuous intravenous infusion and rapid intravenous injection of unlabeled, highly purified hCG. Seven subjects received 4 d of infusion at a rate of 0.2 μg/min, followed by an additional 4 d of infusion at 0.8 μg/min. Mean serum levels of hCG established at these rates of infusion were 61.1 ± 3.3 and 237 ± 16 ng/ml, respectively (mean $\pm$SEM). The MCR determined at the low infusion rate was not significantly different from that determined at the higher infusion rate (1.83 ± 0.09 vs. 1.95 ± 0.14 ml/min per m²). The mean MCR for all subjects was 1.88 ± 0.08 ml/min per m². The MCR was not significantly different between men and women (2.04 ± 0.13 vs. 1.76 ± 0.07 ml/min per m²). The RCR also did not vary between low and high infusion rates (0.40 ± 0.03 vs. 0.40 ± 0.04 ml/min per m²). The mean RCR for all subjects was 0.40 ± 0.02 ml/min per m². There was no difference in RCR between men and women (0.42 ± 0.05 vs. 0.39 ± 0.03 ml/min per m²). Six subjects were given 1.0 mg of highly purified hCG by rapid intravenous injection. Initial serum levels of hCG were 300–400 ng/ml, and the subsequent disappearance curve was multiexponential over 8–10 d. The disappearance curve of hCG in each subject was fitted to a biexponential equation. The rapid component $t_{1/2}$ was 5.97 ± 0.63 h and the slow component $t_{1/2}$ was 35.6 ± 8.0 h. We conclude that the MCR of purified hCG in man is about 2 ml/min per m² and the RCR is 0.4 ml/min per m²; these parameters are concentration independent and do not differ significantly between healthy young men and women.

INTRODUCTION

The metabolism of human chorionic gonadotropin (hCG)[1] has been the subject of a number of reports during the past 40 yr (1–10). It has been established that the hCG molecule has a plasma metabolic clearance rate (MCR) that is quite slow relative to the MCR of the pituitary glycoprotein hormones (1–5), and that urinary excretion accounts for some, but not all, of the metabolic disposal of hCG (1, 2, 5–10). Techniques for quantitation of hCG metabolism have varied, and each has its limitations. For example, the disappearance of endogenous hCG after delivery of the placenta has been determined by radioimmunoassay (1, 4); however, a number of hCG-related molecules circulate during pregnancy and gestational trophoblastic disease (11–15), and these species may have differing rates of disappearance. Furthermore, the persistence of hCG secretion by some residual trophoblastic tissue may complicate the kinetic analysis. Some investigators have injected crude commercial preparations of hCG and have analyzed the kinetics of disappearance of hCG biological or immunological activity from blood (2, 3, 5). However, crude preparations of hCG contain immunologically active, but biologically inactive hCG-related peptides (5). Accordingly, we have used highly purified hCG to study the metabolism of hCG. Our experimental approach was to use both a single injection technique, as employed by other investigators, and a continuous intravenous infusion, the latter

[1] *Abbreviations used in this paper:* Con A-Sepharose, concanavalin A covalently bound to Sepharose 4B; hCG, human chorionic gonadotropin; hLH, human luteinizing hormone; MCR, metabolic clearance rate; PBS, phosphate-buffered saline; RCR, renal clearance rate.

technique not having been applied to the measurement of hCG metabolic parameters heretofore.

METHODS

Materials

We obtained the highly purified hCG CR121 preparation from the Center for Population Research of the National Institute of Child Health and Human Development. It was prepared by Dr. Robert Canfield and Dr. Steven Birken using the same method as used for hCG CR119 (16). We have found these two hCG preparations to be indistinguishable by a variety of criteria, including potency in bioassay, radioimmunoassay, and LH-CG radioreceptor assay systems, and gel filtration pattern on Sephadex G-100. The biological potency of hCG CR121 is 14,000 IU/mg (9,900–17,300 IU/mg, 95% confidence limits) in terms of the Second International Standard of hCG (ventral prostate weight bioassay). The hCG CR121 preparation was made suitable for human administration by millipore filtration. The antisera to hCGβ (SB6) and to hCGα (SA7) have been characterized previously (13, 17). Antiserum generated against the carboxy-terminal peptide of hCGβ was also employed (18). Concanavalin A covalently bound to Sepharose 4B (Con A-Sepharose) was purchased from Pharmacia Fine Chemicals, Piscataway, N. J. α-Methyl-D-mannoside was purchased from Sigma Chemical Co., St. Louis, Mo.

Subjects

Six male and seven female National Institutes of Health Clinical Center normal volunteers, aged 18–22 yr, participated in the study after giving informed consent. Injections or infusions of hCG were given to the women late in the luteal phase or during menstruation to avoid the ovulatory surge of luteinizing hormone.

Base-line samples

Three base-line 24-h urine specimens and three base-line blood samples (10 ml) were obtained from each subject before the administration of hCG.

Continuous infusion technique

Seven subjects (three men and four women) were given purified hCG by continuous intravenous infusion. The subjects were allowed to pursue their normal diets and activities within the constraints imposed by the intravenous infusion apparatus (IMED portable intravenous flow pump). At 0800 h of the 1st d, a loading dose of 0.2 mg of purified hCG was injected intravenously, and continuous infusion was begun of a solution of 500 ml 0.9% NaCl containing 0.15 mg hCG and 2 ml of 25% human serum albumin. The infusion was maintained at a flow rate of 40 ml/h. The intravenous solutions were prepared fresh and changed every 12 h. After 4 d (96 h), an additional 0.6 mg of purified hCG was injected intravenously, and the concentration of hCG in the infusate was increased to 0.6 mg/500 ml. The actual flow rate was determined on several occasions by measuring the weight of fluid delivered during 12 h by the pump into a tared collection flask. The mean flow rate determined in this way was 38.9±0.7 ml/h (±SD). An aliquot of the infusate given to each subject was stored at −20°C until assayed for hCG; thus, it was possible to measure the rate of infusion of hCG during each 12-h period. Blood samples (10 ml) were drawn from the contralateral arm every 12 h, just before the intravenous solution was changed. 24-h urine specimens were obtained each day during the 8-d infusion period. Because of technical difficulties with maintaining the intravenous infusion, data were not obtained in two subjects at the higher rate of infusion.

Single injection technique

At 0800 h, after overnight fasting, six subjects (three men and three women) were given a rapid intravenous injection of 1.0 mg of purified hCG in 5 ml of 0.9% NaCl into a right antecubital vein. Blood samples (10 ml) were obtained at various times during the next 16 h via an intravenous line in a left forearm vein; the line was kept open by an infusion of 0.9% NaCl at 40 ml/h. During the day of injection, the subjects were allowed a clear liquid lunch. After 8 h at bed rest, normal diet and activities were resumed. Additional blood samples (10 ml) were taken daily for the next 8–12 d, and 24 h urine specimens were collected for each of 12–15 d after injection. Creatinine concentration was measured in each urine specimen to ensure that adequate collections had been obtained.

Processing of samples

All samples of blood were allowed to clot and were centrifuged at 1500 g for 15 min to separate the serum. Urine was kept refrigerated until the end of the collection period. Aliquots of serum, urine, and infusate were stored at −20°C for subsequent analysis.

Radioimmunoassays

The hCGβ determinant radioimmunoassay employed antiserum generated against the hCGβ subunit (SB6) (17); hCG CR119 iodinated with ^{125}I by the chloramine T method (19) was used as the radioligand, and hCG CR121 was the reference preparation.

The hCGα determinant radioimmunoassay used antiserum against the hCGα subunit (SA7) (13); hCGα (CR119), iodinated with ^{125}I by the chloramine T method (19), was used as the radioligand, and hCGα (CR119) was the reference preparation.

The hCGβ carboxy-terminal peptide determinant radioimmunoassay used an antiserum to the hCGβ carboxy-terminal peptide (18) in a system which used labeled hCG CR119 as the radioligand and hCG CR121 as the reference preparation.

Serum and infusate samples

To eliminate interassay variability, serum and infusate samples from individual subjects were run in the same assay. Aliquots of infusate and sera derived from the continuous infusion technique were diluted in radioimmunoassay buffer, and potency estimates were made using the reference preparation diluted in the same buffer. Each unknown sample was run in the assay at three different dilutions, such that the resulting displacement of radioligand was between 20 and 80% in at least two of the three dilutions. Dose interpolations for the unknown samples were made using parallel line bioassay statistical methods (20).

For subjects given hCG by rapid injection, sera and standards were diluted in normal male (blank) serum to a volume of 200 μl.

Urine samples

To minimize nonspecific effects of urine on the radioimmunoassays, Con A-Sepharose was used to extract hCG from the urine specimens as described previously (21, 22). Briefly, amounts of urine up to 1.5 ml were mixed with 300 μl of a slurry of Con A-Sepharose (1:1, vol/vol in phosphate-buffered saline [PBS, 0.15 M NaCl, 0.01 M PO$_4$, pH 7.4]) and mixed by vortex three to four times during 45 min at room temperature. Recovery of the purified hCG by this procedure was 92±5% and was accounted for in calculating the results. α-Methyl-D-mannoside (0.25 M final concentration) was added to the routine radioimmunoassay buffer to inhibit adsorption of labeled hCG to the Con A-Sepharose gel.

Gel filtration

Samples of urine and infusate (1.0 ml), with 5–10,000 cpm of ^{125}I-hCGα added as a column marker, were applied to a column of Sephadex G-100 (69.5 × 1.6 cm) and eluted with PBS by upward filtration at a flow rate of 25 ml/h. Fractions of 2.3 ml were collected and an aliquot of each fraction was counted for ^{125}I.

Samples of serum (2.0 ml), with 5–10,000 cpm of ^{125}I-hCGα added as a column marker, were applied to a column of Sephadex G-100 (83.5 × 2.6 cm), and eluted with PBS by upward filtration at 25 ml/h. Fractions of 2.3 ml were collected and aliquots counted for ^{125}I. The fractions obtained from these columns were assayed in the various radioimmunoassays either directly or after Con A-Sepharose extraction. The immunoreactive moieties which eluted from the column contained sufficient carbohydrate for adsorption to Con A-Sepharose.

Analysis of data

Continuous infusion. The steady-state serum concentration of hCG during the infusion was used to calculate the MCR by the formula, MCR = IR/C, where IR is the infusion rate (ng/min) and C is the steady-state serum concentration (ng/ml). The renal clearance rate (RCR) was calculated according to the formula, RCR = U/C, where U is the quantity of hCG excreted in the urine during the period, and C is the steady-state serum concentration.

Single injection. The disappearance of hCG from serum was curvilinear on a semilog plot, and for purposes of calculating curve parameters and estimating clearance rates from the data, a curve-fitting computer program (23) was used to fit the data to an equation of the form, C = Ae$^{-\alpha t}$ + Be$^{-\beta t}$, where C is the serum concentration, t is the time, A and α are the rapid component parameters, and B and β are the slow component parameters. The MCR was calculated as the total dose administered divided by the area under the disappearance curve (integral with respect to time, from 0 to infinity). The formula used was MCR = Dose/(A/α + B/β). The apparent initial volume of distribution (V$_d$) was calculated as: V$_d$ = Dose/(A + B). The rapid and slow component half-lives were calculated as (ln 2)/α and (ln 2)/β, respectively. The RCR was calculated as the amount of hCG excreted in the urine during the first 72 h after injection divided by the integral of the disappearance curve during this same time period. The formula used was RCR = U/($\int_0^{72} C dt$), where U is the quantity of hCG excreted in the urine during the 72 h, C is the serum concentration, and t is the time.

Body surface area was estimated from height and weight by the formula of Du Bois and Du Bois (24).

Statistical analysis of the radioimmunoassays was performed as described by Rodbard (20). For comparison of means, we used Student's t test (25).

RESULTS

MCR of hCG determined by continuous infusion technique. Base-line serum levels of hCG were at or below the limits of detection of the radioimmunoassay (≤ 1.0 ng/ml) in all subjects. An initial rapid intravenous injection of hCG was given to raise rapidly the blood level to near the desired concentration. Fig. 1 shows the levels of hCG which were achieved in the serum of the subjects during the infusion of hCG at 0.2 μg/min and 0.8 μg/min. Mean serum hCG levels on the 3rd d of continuous infusion were not significantly different from those on the 4th d (paired t test, 0.05 level). For each subject, the serum hCG levels during the 3rd and 4th d were averaged, and the average value was used to calculate the MCR and RCR. For the seven subjects, the mean serum hCG levels during the final 48 h of the low and the high infusion rates were 61.1±3.3 and 237±16 ng/ml (mean±SEM), respectively. The MCR of hCG was 1.88±0.08 ml/min per m² (Table I), and there was no significant difference between the men and the women (2.04±0.13 vs. 1.76±0.07 ml/min per m²). The
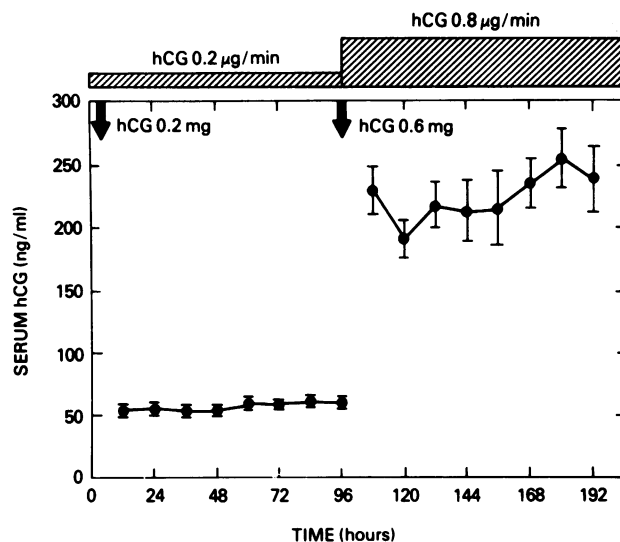

FIGURE 1 Serum levels of hCG achieved during the infusion of purified hCG at two different rates. An initial loading dose of 0.2 mg hCG was administered by rapid intravenous injection, and a continuous intravenous infusion of hCG was maintained at 0.2 μg/min for 96 h. At 96 h, an additional rapid intravenous injection of 0.6 mg hCG was given, and the infusion rate was increased to 0.8 μg/min for an additional 96 h. Blood specimens were obtained every 12 h during the 8 d, and serum hCG was determined by radioimmunoassay. The closed circles are the means of the serum hCG levels in seven subjects; the vertical lines indicate the standard deviations.

MCR of hCG at the low infusion rate did not differ significantly from that at the high infusion rate (Table I).

RCR of hCG determined by the continuous infusion technique. For the seven subjects, the mean daily hCG excretion rates during days 3 and 4 of infusion were 58.6 and 233.9 $\mu g/24$ h for the low and high infusion rates, respectively (Table II). The percent of administered hCG excreted in urine varied among the subjects from 17 to 28% (21.6 ± 1.1, mean $\pm$SEM). The RCR of hCG determined in each subject was similar at the high and low infusion rates. The mean value for the RCR was 0.40 ± 0.02 ml/min per m². There was no significant difference in RCR between men and women (0.42 ± 0.05 vs. 0.39 ± 0.03 ml/min per m²).

Characterization of serum and urine hCG immunoreactivity. Accumulation of hCG subunits or of immunoreactive metabolites in the serum or urine during the infusion could influence the accuracy of the hCG levels determined, and hence the MCR and RCR calculated from these values. Accordingly, we examined the nature of the immunoreactive hCG in serum and urine in order to ascertain the extent to which the total hCG immunoreactivity consisted of crossreactivity from hCG subunits or metabolites.

After Sephadex G-100 gel filtration of the base-line serum, no hCGβ or hCGα immunoreactivity was detected in any fraction (data not shown). Serum obtained on the final day of the 8-d continuous infusion of hCG was filtered on Sephadex G-100 and the levels of hCGα and hCGβ immunoreactivity in the fractions were determined (Fig. 2). Serum hCGβ immunoreactivity eluted in two positions: (*a*) a major peak (>90% of the total immunoreactivity), which eluted from the column with the same K_{av} as the purified hCG and (*b*) a minor peak (<10% of the total immunoreactivity), which eluted with a smaller exclusion volume. No hCGβ immunoreactivity was apparent in the position where the free hCGβ subunit would have eluted from the column (Fig. 2). The larger apparent molecular size form of hCG immunoreactivity was not apparent in chromatographs of the hCG preparation incubated with or without the subjects' sera. It may

TABLE I
MCR of hCG Determined by Continuous Infusion of Highly Purified hCG

Subject	Infusion rate	Sex	Height	Weight	Body surface area	Infusion rate	Serum hCG*	hCG MCR	
			cm	*kg*	*m²*	*ng/min*	*ng/ml*	*ml/min*	*ml/min/m²*
1	Low	M	176	65.4	1.80	$195.5 \pm 5.0\ddagger$	55.2 ± 1.5	3.56 ± 0.18	1.98 ± 0.10
	High					775.2 ± 30.0	255.8 ± 4.2	3.03 ± 0.12	1.68 ± 0.06
2	Low	M	181	69.3	1.89	189.3 ± 7.0	54.8 ± 1.4	3.46 ± 0.16	1.83 ± 0.08
	High					750.7 ± 25.7	192.0 ± 23.0	4.19 ± 0.78	2.22 ± 0.41
3	Low	M	182	80.8	2.02	193.7 ± 10.3	44.6 ± 1.4	4.35 ± 0.22	2.16 ± 0.11
	High					789.0 ± 34.2	168.2 ± 9.0	4.76 ± 0.45	2.36 ± 0.22
4	Low	F	162	61.7	1.66	185.2 ± 3.7	70.7 ± 3.2	2.63 ± 0.10	1.58 ± 0.06
	High					862.4 ± 6.5	288.8 ± 12.2	3.00 ± 0.14	1.81 ± 0.08
5	Low	F	156	53.2	1.51	201.0 ± 3.8	75.5 ± 1.6	2.66 ± 0.03	1.76 ± 0.02
	High					—	—	—	—
6	Low	F	154	54.5	1.51	161.9 ± 20.2	71.6 ± 4.0	2.31 ± 0.36	1.53 ± 0.24
	High					723.8 ± 22.8	280.2 ± 10.6	2.60 ± 0.17	1.72 ± 0.12
7	Low	F	164	57.5	1.62	175.0 ± 6.0	55.0 ± 2.2	3.19 ± 0.10	1.97 ± 0.06
	High					—	—	—	—
	Low ($n = 7$)							3.17 ± 0.26	1.83 ± 0.09
	High ($n = 5$)							3.52 ± 0.41	1.95 ± 0.14
	Combined ($n = 12$)							3.31 ± 0.23	1.88 ± 0.08

* Levels were calculated by averaging serum levels during days 3 and 4 of the hCG infusion.
‡ Mean $\pm$SEM of values obtained during four consecutive 12-h infusion periods.

TABLE II
RCR of hCG Determined by Continuous Infusion of Highly Purified hCG

Subject	Infusion rate	Sex	Body surface area	Serum hCG*	Urine hCG	hCG RCR	
			m^2	ng/ml	μg/24 h	ml/min	ml/min/m²
1	Low High	M	1.80	55.2±1.5‡ 255.8±4.2	52.3§ 242.4	0.658±0.043 0.658±0.030	0.348±0.022 0.348±0.016
2	Low High	M	1.89	54.8±1.4 192.0±23.0	55.6 184.0	0.706±0.038 0.662±0.020	0.374±0.020 0.350±0.010
3	Low High	M	2.02	44.6±1.4 168.2±9.0	52.2 245.5	0.808±0.062 1.026±0.226	0.400±0.030 0.508±0.112
4	Low High	F	1.66	70.7±3.2 288.8±12.2	65.3 208.6	0.640±0.066 0.506±0.048	0.386±0.040 0.304±0.028
5	Low High	F	1.51	75.5±1.6 —	62.0 —	0.572±0.057 —	0.378±0.038 —
6	Low High	F	1.51	71.6±4.0 280.2±10.6	52.2 288.6	0.506±0.018 0.719±0.098	0.335±0.012 0.476±0.064
7	Low High	F	1.62	55.0±2.2 —	70.8 —	0.894±0.050 —	0.552±0.031 —
	Low (n = 7) High (n = 5)					0.683±0.050 0.714±0.085	0.396±0.027 0.397±0.040
	Combined (n = 12)					0.696±0.044	0.397±0.022

* Data repeated from Table I for convenience.
‡ Mean±SEM of values obtained during four consecutive 12-h infusion periods.
§ Mean of values obtained on 24-h specimens collected on days 3 and 4.

represent some form of hCG complexed with serum proteins, as the relative proportions of hCGα and hCGβ immunoreactivity were similar to that of the intact hCG molecule. Judging from the size of this minor peak relative to the size of the hCG peak, the serum hCG levels determined by radioimmunoassay would overestimate the actual hCG concentration only slightly (<10%). With regard to other hCG-related immunoreactive substances, after 8 d of continuous infusion of purified hCG, the only detectable hCGα immunoreactivity was found in the fractions which contained hCGβ immunoreactivity; none was observed in the position where free hCGα would have eluted from the column (Fig. 2).

Gel filtration of the urine obtained on the 8th d of the hCG infusion revealed that the vast majority of the hCGβ immunoreactivity eluted in fractions corresponding to intact hCG (Fig. 3, upper panel). There was no apparent free hCGβ subunit of a size similar to that of authentic hCGβ in any of the subjects. However, the urine of the subject illustrated here did contain a small amount of hCGβ immunoreactivity

which eluted from the column with a larger exclusion volume than hCG, hCGα, or hCGβ. This substance with hCGβ immunoreactivity had a K_{av} on this column, which was essentially identical with that of the discrete degradation product of the hCGβ subunit that we have described previously (26). However, this small molecular size form of hCGβ immunoreactivity constituted a minute portion of the total hCGβ immunoreactivity eluted from the column and was not detected in the eluate obtained from gel filtration of the urine of the other six subjects. Hence, with the infusion of purified hCG, the overwhelming majority of the hCGβ immunoreactivity excreted in urine was hCG itself.

When base-line urine was gel filtered, minor amounts of hCGα immunoreactivity were observed in the position corresponding to that of free hCGα (Fig. 3, lower panel) confirming our previous experience (22). After 8 d of continuous infusion of hCG, urinary hCGα immunoreactivity eluted in the position corresponding to that of intact hCG, as expected; the peak corresponding to free hCGα did not appear to have changed
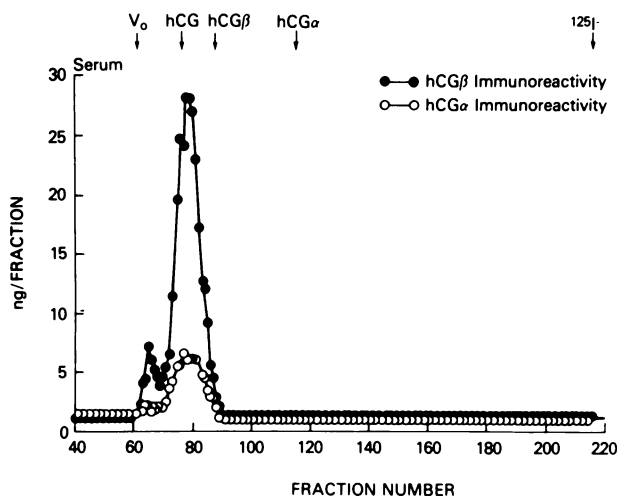
FIGURE 2 Sephadex G-100 gel filtration of serum obtained from a male subject on day 8 of continuous intravenous infusion of purified hCG. Serum (2.0 ml) was applied to a Sephadex G-100 column (83.5 × 2.6 cm) and was eluted with PBS by upward filtration at 25 ml/h. Fractions of 2.3 ml were collected and aliquots assayed for hCGβ and hCGα immunoreactivity. V_0 is the void volume and $^{125}I^-$ indicates the position of the salt peak. The positions of authentic hCG, hCGβ, and hCGα are indicated.

appreciably (Fig. 3). Thus, there is no evidence for degradation of hCG to an hCGα-like material, similar to that which has been reported to exist in pregnancy serum and urine (11, 12, 14).

The hCGβ carboxy-terminal peptide radioimmunoassay was used to obtain another estimate of the quantity and immunological character of the hCG in the serum and urine specimens obtained during the continuous infusion of purified hCG. We reasoned that if the infused hCG were unaltered, and if negligible quantities of immunoreactive metabolites had accumulated, the ratio of the carboxy-terminal peptide immunological determinant level to the conformational (SB6) determinant level should be unity, i.e., equal to that in the purified hCG. The levels of these two determinants were indeed nearly identical in serum (Fig. 4) and in urine (Fig. 5). Therefore, clearance calculations based on hCG levels measured by radioimmunoassay using carboxy-terminal peptide determinant antiserum were not significantly different from those measured employing hCGβ conformational determinant antiserum (MCR, 1.91±0.11 vs. 1.88±0.08 ml/min per m²; RCR, 0.38±0.03 vs. 0.40±0.02 ml/min per m²).

The slopes of the dose-response curves of the serum and urine specimens collected during infusion of hCG were not significantly different from that of authentic hCG in the hCG radioimmunoassay. The immunoreactive hCG material in both serum and urine
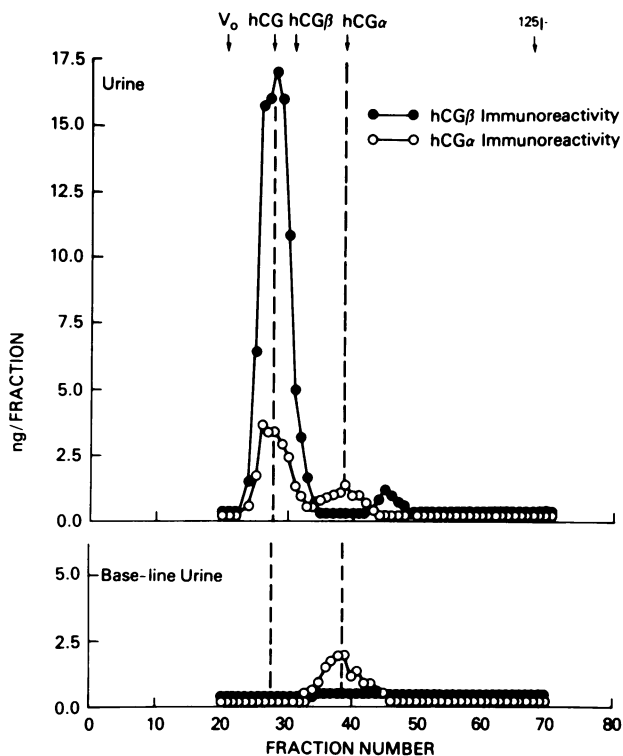

FIGURE 3 Sephadex G-100 gel filtration of urine obtained from a male subject on day 8 of continuous intravenous infusion of purified hCG (upper panel), and base-line urine from the same subject (lower panel). Urine (1.0 ml) was applied to a Sephadex G-100 column (69.5 × 1.6 cm) and was eluted with PBS by upward flow at 25 ml/h. Fractions of 2.3 ml were collected and aliquots assayed for hCGβ and hCGα immunoreactivity. V_0 is the void volume and $^{125}I^-$ indicates the salt peak. The positions of authentic hCG, hCGβ, and hCGα are indicated.
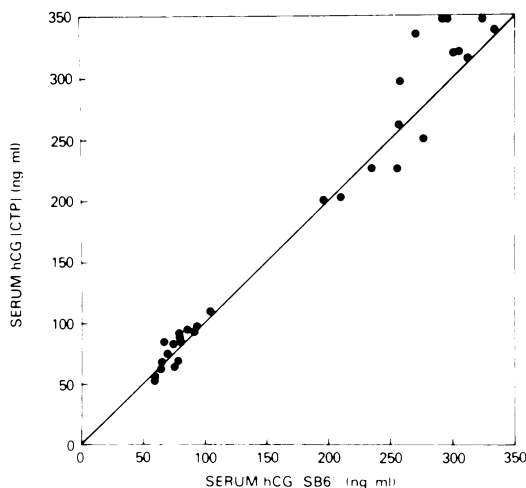

FIGURE 4 Correlation of hCG levels determined in serum by antiserum to the hCGβ carboxy-terminal peptide determinant (CTP) and by antiserum to the hCGβ subunit conformational determinant (SB6). The line of unity is drawn. $r = 0.98$; $P < 0.001$.
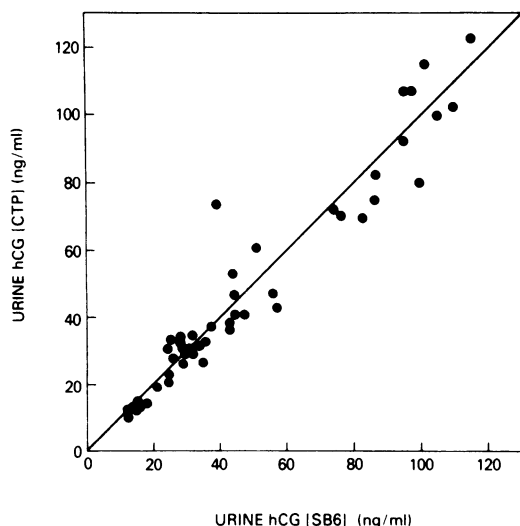
FIGURE 5 Correlation of hCG levels determined in urine by antiserum to the hCGβ carboxy-terminal peptide determinant (CTP) and by antiserum to the hCGβ subunit conformational determinant (SB6). The line of unity is drawn. r = 0.96; P < 0.001.

adsorbed quantitatively to Con A-Sepharose, indicating that no deglycosylated hCG had accumulated.

In summary, when purified hCG was administered to normal subjects by continuous infusion to achieve steady state levels, the vast majority of the accumulated immunoreactive material detected in serum and urine by our radioimmunoassays was apparently intact hCG. Parenthetically, there may be other peptide fragments present which lacked cross-reactivity in these radioimmunoassays.

Levels of hCG in serum after single injection of hCG. Base-line serum hCG levels (hCGβ determinant radioimmunoassay) were at or below the detection limit of the assay (≤1.0 ng/ml). After rapid intravenous injection of 1.0 mg of purified hCG the initial serum levels of hCG achieved were 300–400 ng/ml. Fig. 6 shows the mean hCG levels of six subjects during 7 d after injection. The serum disappearance curve is curvilinear on a semilog plot. The kinetic parameters of the computer-derived biexponential curves for each subject are given in Table III. The initial volume of distribution, when corrected for body size, was the same for men and women, and was similar to plasma volume. The rapid component $t_{1/2}$ was ~6 h, and the slow component $t_{1/2}$ was ~36 h.

MCR and RCR of hCG determined by the single injection technique. The parameters of the serum disappearance curve of hCG were used to calculate the MCR of hCG after single intravenous injection of purified hCG in each subject. The mean MCR calculated for the group was 1.61±0.09 ml/min per m² (Table IV). The urinary excretion of hCG was great-
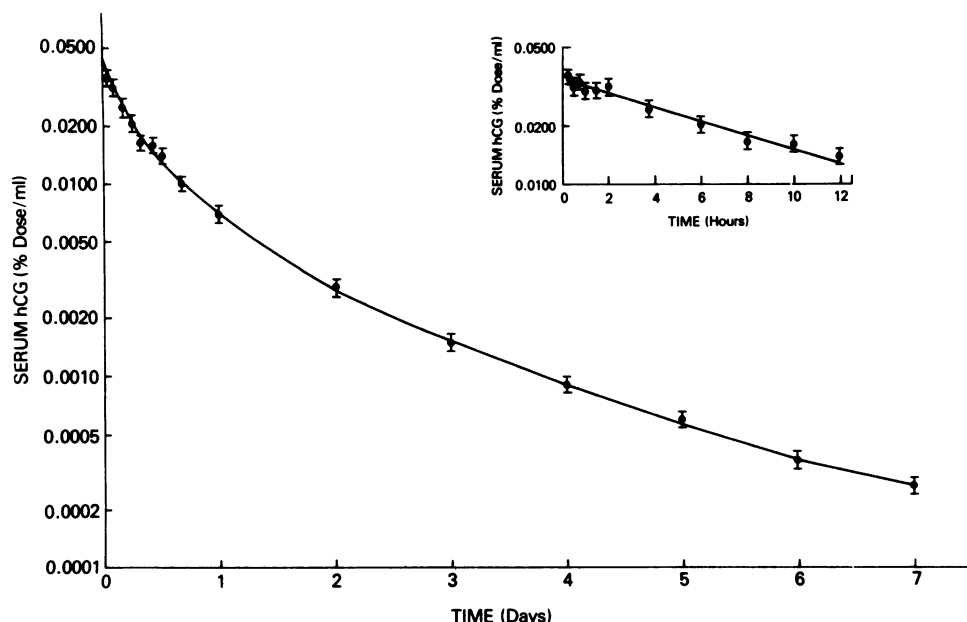

FIGURE 6 Disappearance of hCG from serum after injection of highly purified hCG (1.0 mg) in six normal subjects. Blood specimens were obtained at various times after rapid intravenous injection of hCG, and serum levels of hCG were measured by radioimmunoassay. Closed circles represent the mean serum hCG levels, expressed as a percentage of the administered dose per milliliter; the vertical lines indicate the SEM. The insert provides more detail of the disappearance during the first 12 h after injection.

TABLE III
Kinetic Parameters of hCG Determined by Single Intravenous Injections of 1 mg of Highly Purified hCG

Subject	Sex	Body surface area	A	α	B	β	t₁/₂ Fast	t₁/₂ Slow	V_d	
		m^2	% dose/ml	h^{-1}	% dose/ml	h^{-1}	h	h	ml	ml/m^2
8	M	1.77	0.0305	0.116	0.0069	0.0228	5.95	30.2	2674	1511
9	M	1.69	0.0282	0.101	0.0052	0.0198	6.83	34.9	2994	1772
10	M	1.98	0.0233	0.082	0.0017	0.0096	8.45	74.2	4000	2020
11	F	1.61	0.0193	0.120	0.0121	0.0234	5.77	29.7	3185	1978
12	F	1.95	0.0228	0.165	0.0132	0.0324	4.20	21.3	2778	1425
13	F	1.62	0.0294	0.149	0.0187	0.0294	4.63	23.4	2079	1283
Mean		1.77	0.0256	0.122	0.0096	0.0229	5.97	35.6	2952	1665
SEM		0.07	0.0018	0.012	0.0025	0.0033	0.63	8.0	260	124

est on the 1st d after injection of hCG, and declined progressively thereafter. To calculate the RCR of hCG, we used the urinary excretion of hCG measured during the first 3 d after injection and the serum concentration curve integrated during the same 3 d. As shown in Table IV, the RCR of hCG in these subjects was 0.49±0.05 ml/min per m².

DISCUSSION

Using purified hCG and a continuous infusion technique, we have determined that the MCR of hCG is ~2 ml/min per m², and that the MCR is independent of the serum hCG level over the range of 60–240 ng/ml. The MCR, expressed in terms of body surface area, does not differ significantly between healthy young men and women. Similarly, the RCR of hCG, ~0.40 ml/min per m², is independent of the serum hCG level, and is the same for men and women. Renal excretion

accounts for only 20% of the total disposal of purified hCG. The pathways for the other 80% of the disposal remain unaccounted for, but presumably involve uptake and degradation by hepatic and renal tissue (27); in addition, although it is clear that binding to gonadal receptors accounts for some of the metabolism of hCG (27), this pathway is likely to be of minor quantitative significance.

We gave purified hCG by continuous infusion, and our value for the MCR of hCG is 3.31±0.23 ml/min (or 1.88±0.08 ml/min per m²). In contrast, previous studies of hCG metabolism employed analysis of its disappearance after a single injection of crude preparations of hCG (2, 3, 5), or after removal of hCG-producing tissue, e.g., delivery of the placenta (1, 4). For example, Wide et al. (2) calculated an MCR based on bioassay measurement of hCG of 2.33 ml/min; when a radioimmunoassay was used to measure hCG, these authors found a substantially different value of the MCR,

TABLE IV
RCR and MCR of hCG Determined by Single Intravenous
Injection of Purified hCG

Subject	Sex	Body surface area	U*	$\int_0^3 C dt$‡	RCR		MCR
		m^2	ng/3 d	ng·min/ml	ml/min	ml/min/m²	ml/min/m²
8	M	1.77	284,704	210.22	0.94	0.53	1.68
9	M	1.69	292,766	198.91	1.02	0.60	1.83
10	M	1.98	283,195	155.35	1.27	0.64	1.81
11	F	1.61	215,758	242.39	0.62	0.38	1.53
12	F	1.95	240,326	210.32	0.79	0.41	1.57
13	F	1.62	294,430	313.79	0.65	0.40	1.24
Mean					0.88	0.49	1.61
SEM					0.10	0.05	0.09

* U = quantity of hCG excreted during the 3 d after injection of purified hCG.
‡ $\int_0^3 C dt$ = serum hCG concentration integrated over the 3 d after injection of purified hCG.

5.83 ml/min. Presumably, the different potency estimates by bioassay and radioimmunoassay were due to heterogeneity of the circulating hCG-related material. Further, some forms of immunologically active, but biologically inactive hCG-related molecules may have a considerably more rapid clearance than authentic hCG; examples are hCGα and hCGβ (28). Rizkallah et al. (3) used a radioimmunoassay to measure hCG after injection of a crude hCG preparation and found MCR of 2.35 ml/min (men) and 2.68 ml/min (women). Sowers et al. (5) used a crude commercial preparation of hCG, which they indicated was composed of immunoreactive impurities such as the hCGβ subunit. These contaminants may explain in part why their calculated MCR of hCG (14 ml/min) was so high.

We also administered highly purified hCG by rapid intravenous injection to permit a comparison of our curve parameters for highly purified hCG with those available in the literature. Analysis of the disappearance curve of hCG in our studies revealed it to be a multiexponential function; this is similar to observations in most previous reports (1, 3–5). Using the kinetic parameters derived for each individual, we calculated the apparent initial volume of distribution to be 2,952±260 ml (or 1,665±124 ml/m²). This value is similar to the estimated plasma volume of our subjects, and reflects the general experience with other glycoprotein hormones (29–31). Wide et al. (2) derived values of V_d of injected hCG of 1,664 ml by bioassay and 3,680 ml by radioimmunoassay. Rizkallah et al. (3) found values ranging from 1,750 to 2,830 ml. The rapid and slow component half-lives of serum hCG disappearance in our subjects were 6.0±0.6 and 36±8 h, respectively. Midgley and Jaffe (1) found similar mean values after delivery in pregnant women. These workers also injected hCG in one nonpregnant

woman and found a slow component half-life of 32 h. Rizkallah et al. (3) reported values of 5 and 24 h for the rapid and slow component half-lives; the values for men and women were the same. Yen et al. (4) measured the postpartum disappearance of hCG by radioimmunoassay, and reported a rapid half-life of 11 h and a slow half-life of 23.4 h. Wide et al. (2) detected only a single exponential disappearance after injection of hCG, with a half-life of 6–10 h; however, the latest time point at which the hCG level was determined was 24 h in two subjects and 5 h in the third.

Our value for the RCR of hCG is 0.70±0.04 ml/min (or 0.40±0.02 ml/min/m²). Wide et al. (2) reported that the RCR was 0.70 ml/min when hCG is measured by radioimmunoassay, and 0.36 ml/min when hCG is measured by bioassay in male rats. Gastineau et al. (8), using a rat ovarian hyperemia bioassay to measure serum and urinary hCG, found a value of 0.38 ml/min during normal pregnancy; using a rat prostate weight bioassay, Loraine (6) reported RCR values during pregnancy of 0.95±0.17 ml/min (SD). Midgley and Jaffe (1) found RCR values by radioimmunoassay which ranged from 0.59 to 1.75 ml/min after normal pregnancy and delivery; Johnson et al. (7) measured serum and urinary hCG by ovarian hyperemia bioassay and calculated the RCR to be 0.47 ml/min. Sowers et al. (5) also estimated the RCR of hCG by radioimmunoassay after injection of their crude hCG preparation. Their value of 4 ml/min is at great variance with the previously published data, as well as with our own.

We have recently reported studies of the metabolism of the purified hCG subunits in humans (22, 28); in Table V, the kinetic parameters of hCG metabolism are compared with those of hCGα and hCGβ. It can be appreciated that the disappearance curve parameters A and B are not substantially different for the three

TABLE V
Comparison of Kinetic Parameters and Clearance Rates of
hCG, hCGβ, and hCGα in Man

	hCG (n = 6)	hCGβ (n = 13)*	hCGα (n = 8)*
A, % dose/ml	0.0256±0.0018‡	0.0251±0.0021	0.0273±0.0025
α, h⁻¹	0.122±0.012	1.032±0.042	3.276±0.192
B, % dose/ml	0.0096±0.0025	0.0041±0.0010	0.0055±0.0013
β, h⁻¹	0.0229±0.0033	0.174±0.030	0.546±0.138
$t_{1/2}$, h			
Fast	5.97±0.63	0.68±0.03	0.22±0.02
Slow	35.6±8.0	3.93±0.68	1.27±0.32
V_d, ml/m²	1665±124	1958±131	1729±99
MCR, ml/min/m²	1.88±0.08	19.0±0.7	49.7±1.6
RCR, ml/min/m²	0.40±0.02	0.13±0.02	0.16±0.02
RCR/MCR × 100%	21.7±1.4	0.68±0.11	0.33±0.04

* Values for hCGβ and hCGα are calculated from our previously published data (22, 28).
‡ Mean±SEM.

molecules; hence, the initial volumes of distribution are not significantly different, and not unexpectedly, they approximate the plasma volume. It is readily apparent that the slopes of the fast and slow components of the disappearance curves (α and β, respectively), differ significantly among the three molecules, increasing in the order hCG $\ll$ hCGβ < hCGα; consequently, the half-lives of disappearance decrease in the order hCG $\gg$ hCGβ > hCGα. As a result of these relationships, the MCR of hCG:hCGβ:hCGα assumes the proportions 1:10:25. This relationship suggests that other factors in addition to the high proportion of carbohydrate are responsible for the slow MCR of hCG. The hCGβ subunit contains relatively more carbohydrate and sialic acid in proportion to its weight than does the intact hCG molecule (32), and yet its MCR is 10-fold greater than that of hCG. While the absolute values of the half-lives of hCG, hCGβ, and hCGα determined in man are considerably different from those determined after injection of these molecules in rats, the half-lives of disappearance in rats show the same relationships as in man, namely decreasing in the order hCG $\gg$ hCGβ > hCGα (27).

The RCR do not show the same relationships as the MCR. The RCR of hCG is approximately two to three times greater than that of either of its subunits. Therefore, the more rapid MCR of hCGα and hCGβ are not attributable to enhanced renal excretion. Our data show that 20% of the metabolic disposal of hCG is accounted for by urinary excretion but <1% of the disposal of hCGα and hCGβ is accounted for by urinary excretion. The structural features that determine these relationships are unknown.

Despite possessing structures which are basically similar to that of hCG, the pituitary glycoprotein hormones have MCR which are considerably greater than that of hCG (29–31, 33–35). The differences in MCR among these hormones are not necessarily attributable solely to their differences in carbohydrate composition. For example, the MCR of human luteinizing hormone (hLH) is much greater than that of hCG, but about the same as that of hCGβ (28–31, 33). Yet, both hCG and hCGβ are much richer in sialic acid and other carbohydrate residues than hLH. The amino acid sequence of the hLHβ subunit exhibits ~80% homology with hCGβ, but hLHβ lacks the 30 amino acid sequence at its carboxy-terminus, which is characteristic of hCGβ (32). Further study is necessary to clarify how these various differences in structure account for the much lower MCR of the hCG molecule in comparison to hLH and the other pituitary glycoprotein hormones.

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