Metabolic Clearance Rate of Radioiodinated Human Calcitonin in Man

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ABSTRACT The characteristics of the disappearance of radioiodinated synthetic human calcitonin from plasma have been studied in man. After single injection the disappearance curve was multiexponential. The number of exponentials of the theoretical curve fitting the best with the experimental data varied individually. Metabolic clearance rate was determined both from single injection and constant infusion studies, and fast initial distribution volume from the former. Metabolic clearance rate values in normal man calculated from constant infusion studies were 82.3 ±3.4 ml/min per m^2. Values derived from single injection studies were similar, 77.0 ±4.7 ml/min per m^2. These results were compared to those obtained in end stage, renal failure patients. Metabolic clearance rate was considerably lower and volume of fast initial distribution slightly larger in that group. This fact emphasizes the important role of kidneys in the utilization and/or the degradation of human calcitonin.

INTRODUCTION

Studies of the metabolism of iodine-labeled human calcitonin (HCT) have been made possible by the use of synthetic human calcitonin (1). In this report we discuss the results of studies of calcitonin metabolism in two groups of patients, normal subjects and uremic patients with nonfunctioning kidneys, the latter group being considered of interest in view of the role of the kidneys in the utilization and degradation of polypeptide hormones (2, 3). Single injection and constant infusion techniques were used to calculate metabolic clearance rate (MCR) (4). The results indicate a good agreement between the two methods. Considerably lower values were found in uremic subjects.

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METHODS

Subjects

An informed consent was obtained from all the subjects prior to studies. 13 subjects with normal renal function and without any evidence of disorders of calcium metabolism were studied. They were compared with 13 uremic patients in end stage renal failure treated by chronic hemodialysis. In the latter patients, the tests were always performed immediately prior to hemodialysis. One of them (LOY . . .) had had bilateral nephrectomy. The mean values of urea, calcium, and phosphorus concentration for each group are indicated on Table I.

Iodination

Synthetic HCT was kindly supplied by Ciba Ltd., Basel, Switzerland. Iodination was performed with 125-iodine (Amersham Radiochemical Centre) according to the Hunter and Greenwood method (5) with minor modifications. Separation of iodide and damaged components from pure ^{125}I-HCT was done by QU SO G 32[1] microfine granules of precipitated silicates (6). Efficiency of iodination and specific activity of ^{125}I-HCT were calculated from chromatoelectrophoresis strips according to the method of Berson and Yalow (7). Specific radioactivities were from 70 to 228 μCi/μg (mean = 123 μCi/μg). The same chromatoelectrophoretic method was used to estimate damage of the purified ^{125}I-HCT which never exceeded 10%.

Experimental protocol

The tracer was stored at −20°C and used within 1 wk of iodination. Each batch was tested for sterility by standard bacteriological techniques before its use. 20–100 μCi (0.16–0.82 μg) of ^{125}I-HCT was administered into an antecubital vein in normal subjects and into the venous side of the brachial arteriovenous fistula in hemodialyzed subjects. In the single injection studies, the cannula was flushed with

[1] Philadelphia Quartz Co., Philadelphia, Pa.

TABLE I

*Biological Characteristics**

Subjects	Plasma		
	Calcium	Phosphorus	Urea
		mmole/liter	
Normal	2.51 ±0.03	0.80 ±0.04	3.85 ±0.25
Renal failure	2.30 ±0.07	1.61 ±0.19	20.78 ±1.61

* Mean ±SEM.

saline after administration of tracer. Blood was sampled periodically from the opposite antecubital vein at 2, 4, 6, 8, 10, 12, 15, 20, 30, 45, 60, 90, 120, 150, and 180 min after the injection. In constant infusion studies ^{125}I-HCT was diluted in 500 ml of 5% dextrose and administered by means of a constant infusion pump at 90 ml/hr for 8 hr without any prior loading dose. Preliminary studies had revealed that constant plasma levels of iodinated hormone were obtained only after 6 hr of infusion. Blood samples were taken into disposable syringes containing 100 U of sodium heparin and kept in ice. Samples were centrifuged within 1 hr at +4°C, and the plasma was separated.

Analysis of plasma hormone

The total radioactivity of a 1 ml sample of plasma was determined in an automatic gamma counter (Nuclear-Chicago Corp., Des Plaines, Ill. ^{125}I-HCT was measured by the following methods: (*a*) precipitation from 1 ml of plasma immediately after the centrifugation with 2 ml of cold 10% trichloracetic acid (TCA) with two further washings, and (*b*) chromatoelectrophoresis. Separation of pure ^{125}I-HCT remaining at the origin from damaged products and iodine was performed on Whatman 3 M C strip (46 cm × 2.5 cm) in 0.05 M veronal buffer, pH 8.6. 100-µl duplicates of each sample were run at constant voltage (500 v) for 1½ hr. After the separation conditions had been established, the strips were divided into two parts from 0 to 8 cm and from 8 to 25 cm. This enabled the recovery of 100% of the applied radioactivity and the separation of

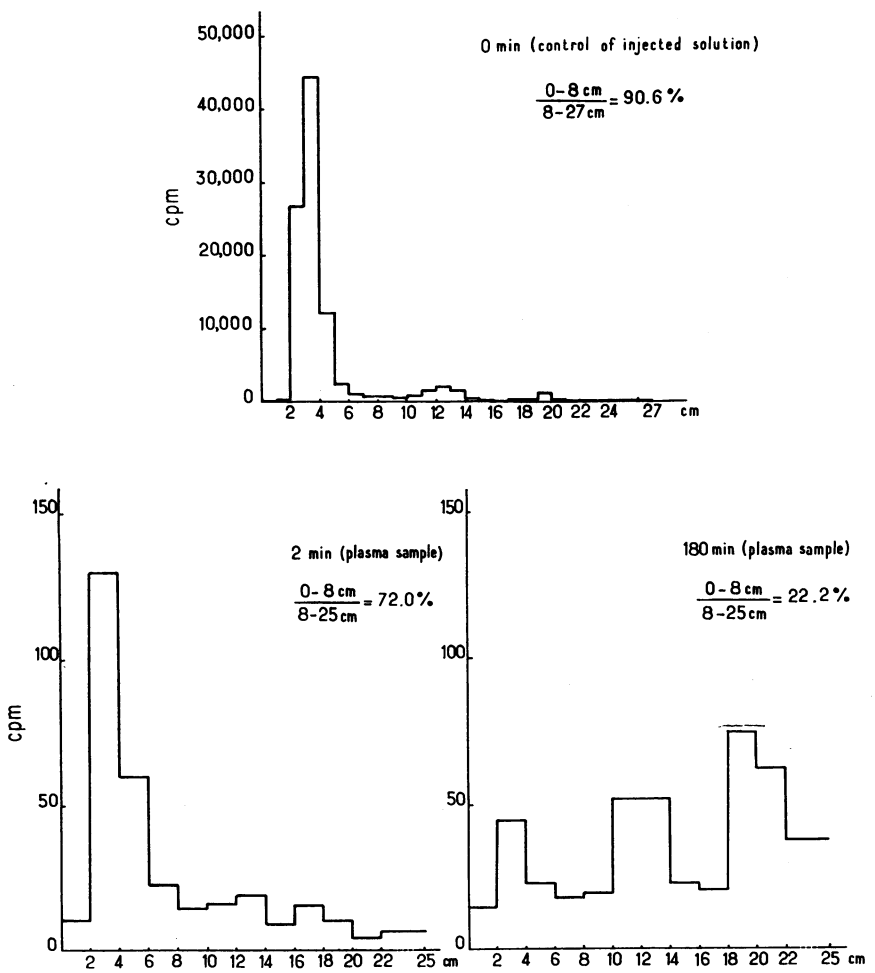

FIGURE 1 Plot of radioactivity against the length of the chromatoelectrophoretic strips of samples taken at times 0 (control of the injected solution), and 2 min and 180 min after injection.

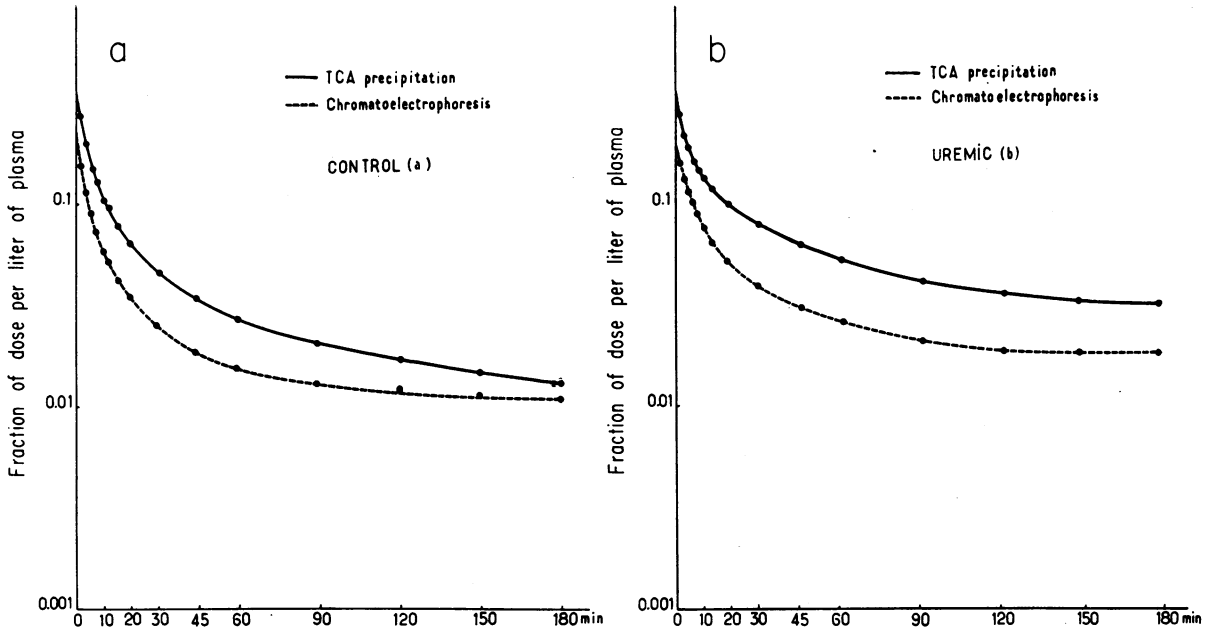

FIGURE 2 *a* and *b* Comparison of the two techniques (TCA precipitation and chromatoelectrophoresis) for assessment of radioiodinated HCT in plasma of control subject (a) and uremic patients (b).

^{125}I-HCT which remains at the origin from damaged components which move behind the albumin and from iodide which moves in advance of the serum albumin. In each test, the strips obtained from the initial and final samples were cut every 2 cm in order to obtain a more complete plot of the radioactivity curve against the length of the strips. A good separation of radioactive calcitonin by using the 0–8 cm portion of the strip was confirmed on all occasions (Fig. 1).

Calculation and computational methods

For all calculations of MCR, the administered radioactivity and the values for iodinated calcitonin in plasma are those corrected for damage after chromatoelectrophoretic analysis. Concentration of radioactive HCT in plasma was calculated as the product of radioactivity in 1 ml of plasma, expressed in cpm, by the percentage of radioactivity remaining in the first 8 cm in the chromatoelectrophoretic system. The chromatoelectrophoresis of the injected or infused solution was performed immediately prior to injection and at the end of the infusion. 50 μl of solution was added to 2 ml of normal human plasma, and 100 μl of the mixture was submitted to chromatoelectrophoresis as a control for the amount of protein. MCR, defined as the volume of plasma which contains the amount of HCT irreversibly metabolized per min, was derived from both single injection and constant infusion studies.

a) *Single injection studies.* The data of the disappearance curves expressed in fractions of the administered dose per liter of plasma described a nonlinear curve on semilog paper when plotted against time, indicating a multiexponential function. Theoretical curves of best fit were derived from the experimental data utilizing a computational program (8) adapted on IBM 360–75. This program gave for each experiment the number of exponentials, the parameters of each, and the difference between the experimental data and the corresponding points of the theoretical curve. When the program supplied several solutions, the solution containing the minimal number of exponentials necessary for a good fit was chosen. When the best theoretical curve was found to be the sum of three exponentials, $Ae^{-\alpha t} + Be^{-\beta t} + Ce^{-\gamma t}$, the metabolic clearance rate was calculated according to MCR (ml) $= \dfrac{1000}{\dfrac{A}{\alpha} + \dfrac{B}{\beta} + \dfrac{C}{\gamma}}$ and the fast initial volume of distribution (V) according to V (ml) $= \dfrac{1000}{A + B + C}$ (4). When the optimal theoretical curve was obtained by the sum of two exponentials ($Ae^{-\alpha t} + Be^{-\beta t}$), MCR and V were calculated respectively according to $\dfrac{1000}{\dfrac{A}{\alpha} + \dfrac{B}{\beta}}$ and $\dfrac{1000}{A + B}$. A, B, C, α, β, and γ were the parameters of the exponential functions. A, B, C were expressed in fractions of the administered dose per liter of plasma and α, β, γ in min^{-1}. The validity of the fit was estimated by the mean fractional deviation which is the mean difference between experimental and theoretical points expressed as a fraction of the theoretical value. This parameter for all subjects was 0.0502 $\pm$0.0057 (mean $\pm$SEM).

b) *Constant infusion studies.* The calculation of MCR using this technique is simplified in the presence of a constant plasma level of radioactive hormone. In this situation, MCR (ml) $= \dfrac{1000}{C^*}$, where C* was the stable plasma level of labeled hormone expressed in fraction of the administered dose per min per liter of plasma (4). C* was taken as the mean of the last points of the constant infusion curve during the latter hours, chosen so that they did not deviate from the mean by more than 5%.

In vitro studies

These experiments were undertaken in order to quantitate the amount of HCT degradation by the plasma itself and to estimate the fraction of MCR which may be related to this phenomenon. The degradation of ^{125}I-HCT was studied in vitro at 37°C in a water bath after mixing with plasma from uremic and normal subjects. Eight experiments (four uremic and four control plasma) were performed. Samplings were taken after incubation for 1, 2, 3, 4, 5, 6, and 24 hr. Control samples were removed prior to commencement of incubation so as to estimate the damage of the ^{125}I-HCT.

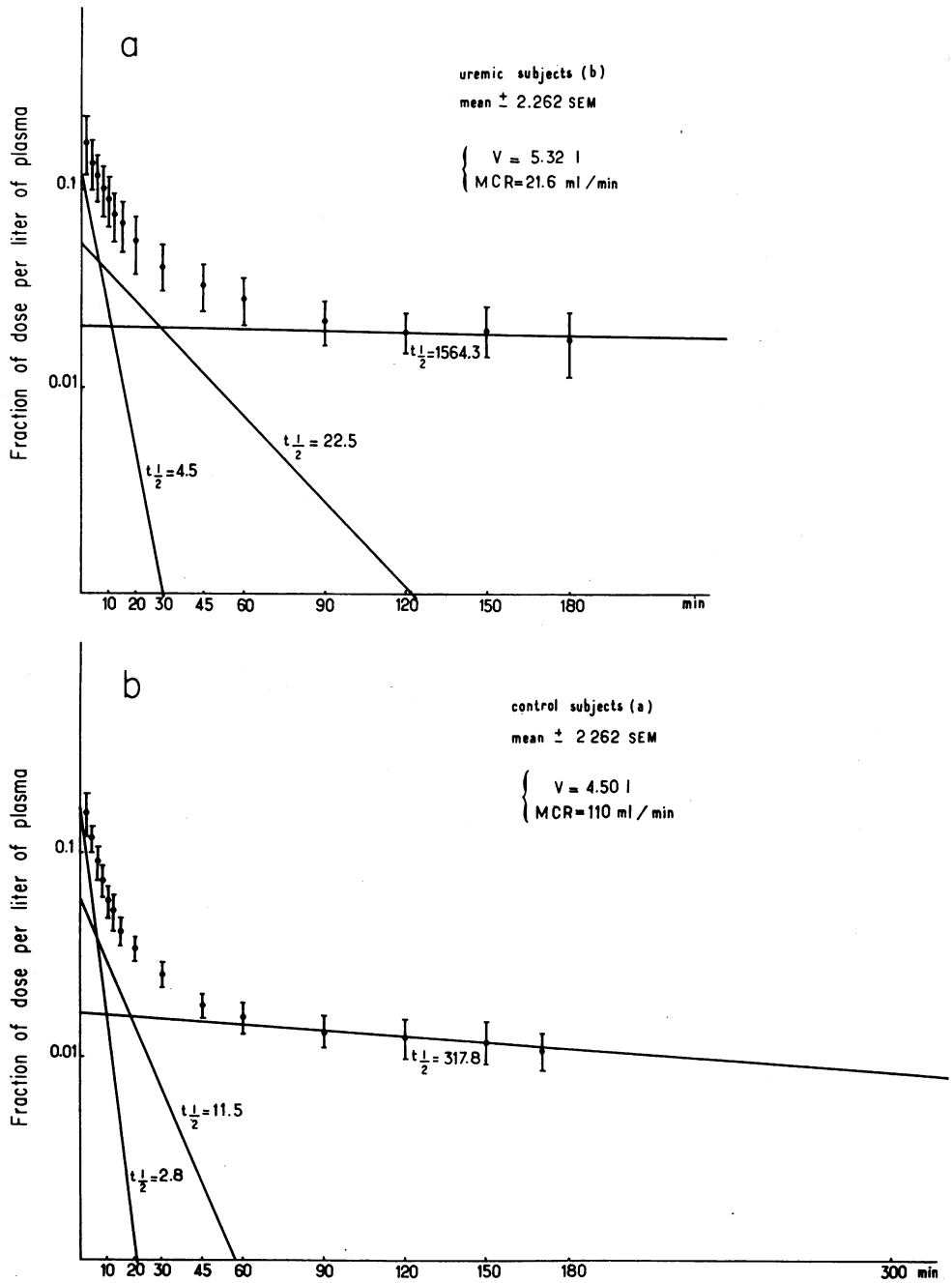

FIGURE 3 *a* and *b* Disappearance of radioiodinated HCT from plasma plotted semilogarithmically in control subjects (a) and uremic patients (b). Each point represents the mean values obtained by chromatoelectrophoresis in 10 studies and the vertical bars, the product of SEM by 2.262 (t corresponding to a probability equal to 0.05 for 9 degrees of liberty).

TABLE II

Metabolic Clearance Rate in Normal Subjects

Subject	Sex	Age	Weight	Body area	MCR	V
		yr	*kg*	m^2	*ml/min per* m^2	*ml/kg*
Single injection						
DJE...	M	38	47.2	1.44	88.5	55.9
BOG...	M	38	58.9	1.79	94.2	68.6
AUG...	M	32	74.9	1.85	48.6	64.2
ARC...	M	49	54.0	1.58	64.2	86.1
BRAN...	M	35	78.5	1.88	79.5	83.0
*CHAN...	M	24	61.2	1.72	80.6	83.1
*MOU...	M	33	64.5	1.75	70.0	70.9
TES...	M	28	55.0	1.55	76.7	67.1
BEN...	M	28	65.0	1.81	98.7	78.1
MOUH...	M	29	63.2	1.74	69.2	50.0
Mean ±SEM					77.0 ±4.7	70.7 ±3.8
Constant infusion						
BEN...	M	21	64.0	1.79	88.4	
DIA...	M	21	66.8	1.85	76.8	
BER...	M	50	67.5	1.76	81.6	
Mean ±SEM					82.3 ±3.4	

* Indicates that the disappearance curve is fitted by the sum of two exponentials.

Chromatoelectrophoresis was performed in duplicate on every sample, and the percentage of intact hormone was calculated. These values were plotted against time on semilog paper. Manipulations were performed under sterile conditions in order to avoid hormonal degradation by bacterial enzymes.

RESULTS

Single injection studies. The damage of the labeled HCT was never above 15% of the hormonal radioactivity before the injection or the infusion. TCA precipitation was above 90% in all instances. The mean dis-

TABLE III

Metabolic Clearance Rate in Uremic Subjects

Subject	Sex	Age	Weight	Body area	MCR	V
		yr	*kg*	m^2	*ml/min per* m^2	*ml/kg*
Single injection						
MI...	M	47	63.7	1.76	39.9	78.3
*LOY...	M	34	52.0	1.59	30.9	69.2
GES...	M	25	57.7	1.65	16.0	112.7
‡RAS...	M	22	73.0	1.80	40.8	116.4
PEN...	F	21	37.4	1.29	11.2	82.9
‡LHO...	M	29	54.8	1.62	38.5	77.6
DUH...	F	29	52.3	1.62	15.8	122.6
PER...	F	21	39.8	1.33	17.4	69.3
GOT...	M	44	56.0	1.62	20.7	102.1
GUS...	M	44	58.8	1.65	22.6	86.3
Mean ±SEM					25.4 ±3.5§	91.7 ±6.3§
Constant infusion						
LOY...	M	34	53.5	1.61	36.7	
DIG...	M	33	47.0	1.43	35.0	
REN...	F	34	60.0	1.60	27.8	
Mean ±SEM					33.2 ±2.7	

* This subject is binephrectomized. He was studied successively by the two methods.

‡ Indicates that the disappearance curve is fitted by the sum of two exponentials.

§ $P < 0.01$ when compared to the same values in normal subjects.

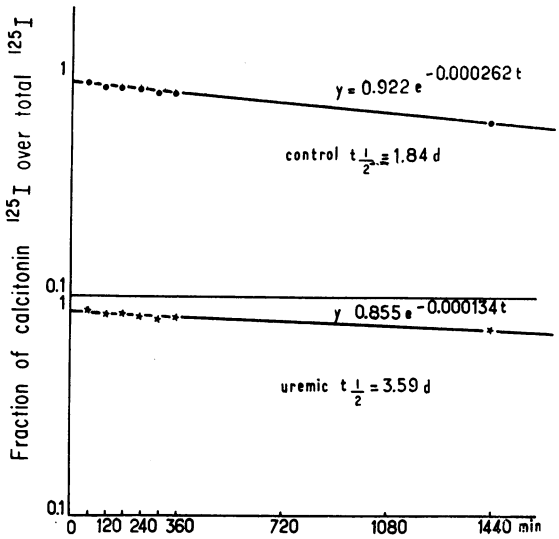

FIGURE 4 Time degradation of radioiodinated HCT in vitro plotted on semilog paper in presence of normal plasma (control) and patient plasma (uremic) Equations of the monoexponentials and half-time values (days) have been indicated.

appearance curves of ^{125}I-HCT as determined by TCA precipitation and chromatoelectrophoresis after single intravenous injection in normal and uremic subjects are shown in Figs. *2a* and *2b*, respectively. These curves have been drawn from the means of all the experimental data. As illustrated in Fig. 2, the absolute value of TCA precipitable radioactivity in plasma is consistently greater than the amount of labeled hormone as determined by chromatoelectrophoresis. It is likely that these results are due to the fact that TCA precipitation does not adequately separate intact hormone from its labeled degradation products. Figs. *3a* and *3b* show clearly that the mean disappearance curves in both groups are curvilinear and are described by the sum of three exponential functions. In 16 experiments, the individual curves were best described by the sum of three exponential functions. However in four cases the experimental data could be optimally fitted by the sum of two exponentials. The levels of ^{125}I-HCT were significantly higher in the uremic group at each time studied except during the first 10 min. MCR and V calculated from single injection studies are shown in Tables II and III. There is a highly significant difference between the values found in the two groups of subjects. V is larger and MCR clearly lower in uremic than in normal subjects. The multiexponential nature of the disappearance curve and the individual variations in the number of exponentials did not permit meaningful calculation of half-times of disappearances.

Constant infusion studies. When isotopically labeled calcitonin was administered by continuous infusion, a constant plasma level was achieved at the end of 6 hr, and further values remained within ±5% of the mean. The MCR calculated from these results are shown in Tables II and III, the values in both groups being slightly higher than those obtained from single injection studies. MCR of uremic subjects were again shown to be clearly lower than those of normal controls.

In vitro studies. The two degradation curves are both represented by monoexponentials as shown by the linear disappearance on semilog paper (Fig. 4). The slope of the curve obtained with uremic plasma is lower than that obtained with normal plasma.

Recovery of radioactivity from urine. The time course of appearance of total radioactivity in urine after single intravenous injection of labeled hormone in normal subjects is shown in Fig. 5. At the end of 48 hr, 95% of the total administered dose had been recovered. 2.4% of the urinary activity could be precipitated with TCA. In uremic hemodialysed subjects, most of the radioactivity was found in the dialysate.

DISCUSSION

The amount of human calcitonin injected can be considered as a tracer. About 0.16 μg was administered in the single injection studies. The fast initial volume of distribution of HCT represents as a mean 7.07% of the body weight in normal subjects (Table II), i.e., 4.9 liters for a subject weighing 70 kg. If 2 μg/liter is taken as the normal plasma concentration of human calcitonin (9), the amount of injected HCT would be equal to 1.6% of the amount in the initial volume of distribution. How-

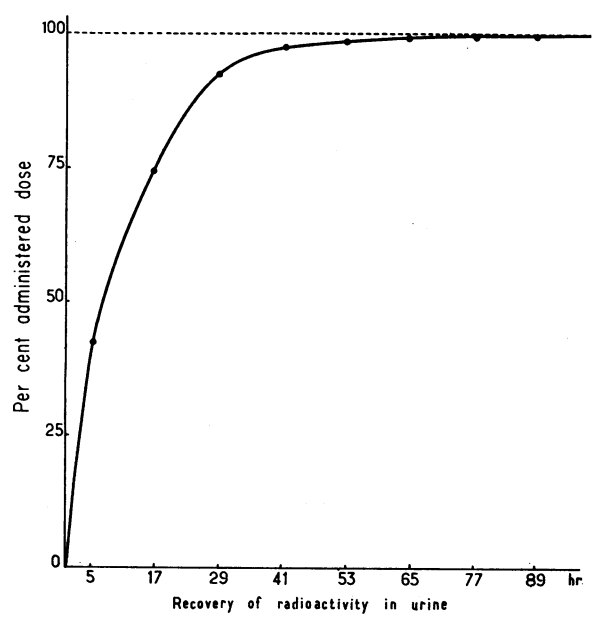

FIGURE 5 Recovery of radioactivity in urine. Cumulative urinary radioactivity after single intravenous injection of radioiodinated HCT in normal man is shown.

ever, if 2 μg/liter is considered a maximum value and if the actual concentration is on the order of three times less, the amount of injected HCT would still remain within a reasonable range.

Chromatoelectrophoresis is held as an adequate method for estimating the pure radioactive polypeptide hormones (7), although it is possible that the last and slowest part of the disappearance curve may be modified in part by interference with iodinated degradation products. In these studies the percentage of activity remaining at the origin of the strip over the total activity decreased rapidly from more than 90% immediately prior to the injection to about 25% 3 hr later. The limit between the first radioactive peak corresponding to the active hormone and the second peak corresponding to damaged products was less clear cut in the later blood samples in each test. Thus, overestimates of ^{125}I-HCT concentration could lead to underevaluation of the MCR.

Computational program supplied the theoretical curves of best fit to the experimental data. In 16 of 20 tests, a three exponential model provided the curve of best fit, and in the four remaining cases a two exponential model appeared to suffice. As it is difficult to describe the metabolism of HCT according to a three compartmental system in some subjects and a two compartmental one in others, we have preferred not to calculate the value of the different volumes and fluxes which are dependent on the exponential equation chosen. We calculated only MCR and volumes of fast initial distribution which could be estimated independently of these equations. The volume of distribution calculated in normal subjects is larger than the plasma volume. The same result has been found by West, O'Riordan, and Care (10) from single injection studies of pig calcitonin in the pig. We cannot provide a clear explanation of these high values which are at variance with the volume rather closer to plasma volume obtained in studies with other polypeptide hormones (3). As the mean figure of MCR calculated from disappearance curves is valuable since it is very close to that obtained from constant infusion studies, it is possible that the distribution volumes calculated from the same curves are correct. If not, error might come from the binding of HCT to an intravascular protein. Leggate, Care, and Frazer (11) demonstrated that pig calcitonin was partly free and partly bound to a plasma protein. An hypothetic initial reversible binding of HCT to a protein which would migrate with albumin would explain an underevaluation of HCT concentration in plasma. On the other hand, if calcitonin diffuses very rapidly out of the intravascular space, it could also lead to underestimation of the actual value of the time zero intercept.

The determination of the MCR of a hormone by the use of an iodinated tracer administered by single injection is always controversial. It has been confirmed by many studies that injected unlabeled hormone or endogenous hormone have disappearance curves similar to those obtained using labeled hormones (12–14) although some discrepancies might have been found by other authors with different methods (15, 16).

The mean MCR of HCT in normal subjects was equal to 77 ml/min per m^2. If we assume a maximum HCT plasma concentration of 2 μg/liter (9) and a body area of 1.73 m^2, the secretion rate of HCT would be close to 0.250 μg/min, about 25–40 MRC milliU/min.[2] This is approximately equal to the dose administered per min during a constant infusion which will produce a physiological effect on plasma concentrations of calcium and phosphorus (17) and on renal excretion of phosphorus, calcium, and sodium (18, 19). MCR of HCT, 111 liters/day per m^2, can be compared to the values found with other polypeptide hormones in man. It is lower than that of growth hormone, 200 liters/day per m^2 (15, 20), and larger than those of thyroid stimulating hormone, 61 liters/day per m^2 (21), of luteinizing hormone, 35 liters/day (22), and of follicle stimulating hormone, 20 liters/day (14).

MCR of ^{125}I-HCT can be divided into two parts, the first, possible degradation of active hormone by human plasma at body temperature as previously shown by Tashjian and Voelkel (23), Milhaud and Hankiss (24), and Maier, Nehar, Rittle, and Staehelin (25), and the second, irreversible disappearance from the fast initial distribution compartment due to the utilization and degradation of HCT by different tissues. The degradation in plasma can be calculated by multiplying the slope of the degradation curve in vitro by the value of the plasma volume estimated to 4.5% of the body weight. Thus, the first component of MCR can be estimated to be 0.8 ml/min for a normal subject weighing 70 kg. It represents a very low percentage of total MCR. As the percentage of ^{125}I active hormone over total plasma radioactivity is much less in vivo than in vitro over any given period, it is likely that active hormone and damaged products have very different metabolic degradation rates in vivo, the disappearance from plasma of active hormone being much faster.

MCR is much lower in uremic than in normal subjects. The only binephrectomized patient had a MCR similar to those whose kidneys were present although nonfunctional. It may be concluded that the presence of kidneys in itself is not sufficient and that normal HCT degradation needs functioning kidneys. The decrease of MCR in these patients is likely to be the consequence of the nonutilization and/or the nondegradation of HCT by kidneys. Milhaud and Hankiss (24) have shown that HCT is rapidly destroyed in vitro in the presence of kidney slices. The role of the kidney in the metabolism of

[2] Research standard B unit for thyroid calcitonin, Medical Research Council, London.

most of the polypeptide hormones is now well established. MCR of human growth hormone is decreased in subjects with renal failure (20). Fractional irreversible loss rate of insulin from plasma is also much lower in patients with end stage renal failure than in normal subjects (3). Half-life of parathormone is longer in a nephrectomy renal failure group than in control subjects (2). In those studies (2, 3), no difference was found between anephric patients and those with chronic renal failure. The aspect of renal function which is necessary for normal polypeptide hormone degradation is open to question. The decrease of the MCR of HCT in patients with renal failure could have functional consequences. HCT has the same effect as parathormone on phosphate and sodium renal excretion (18, 19). Their reduced destruction would result in an increase in the phosphate and sodium loss per nephron, a major feature of the uremic kidney. HCT has an opposite effect to parathormone on bone resorption. The bone lesions of chronic renal failure are usually attributed to hyperparathyroidism. It is possible that the important individual discrepancies observed in patients with chronic renal disease might be due to variations of the balance between parathormone and calcitonin production and destruction.

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