Metabolic Clearance and Production Rates of Human Growth Hormone

ANDREW L. TAYLOR, JOSEPH L. FINSTER, and DANIEL H. MINTZ

From the Department of Medicine, University of Pittsburgh School of Medicine and the Magee-Women's Hospital, Pittsburgh, Pennsylvania 15213

ABSTRACT The metabolic clearance rate (MCR) of human growth hormone (HGH) was determined by the constant infusion to equilibrium technique utilizing HGH-^{125}I. 22 control subjects had a MCR of 229 ±52 ml/min (mean ±SD). No difference was evident between sexes, or between various age groups. Patients with acromegaly demonstrated normal MCR's. Moreover, acute elevations of plasma growth hormone concentrations in normal subjects did not alter the MCR of HGH. The MCR was relatively constant from day to day and within the day when subjects were evaluated in the supine position. In contrast, the assumption of the upright position was associated with a mean 24% decrease in the MCR.

These results were contrasted with the MCR of HGH observed in a small number of patients with altered thyroid function or diabetes mellitus. In six patients with hypothyroidism the MCR (131 ±36 ml/min) was significantly decreased ($P < 0.001$); whereas the MCR in eight patients with hyperthyroidism (240 ±57 ml/min) did not differ from control subjects. The MCR in eight patients with insulin-independent diabetes mellitus (IID) (185 ±41 ml/min) and in eight patients with insulin-dependent diabetes mellitus (IDD) (136 ±31 ml/min) were significantly different from control subjects ($P = < 0.05$ and $P = < 0.001$, respectively).

These data were interpreted to indicate that the plasma HGH-removing mechanism(s) is not saturated at physiologic plasma HGH levels, that plasma HGH levels alone may not permit distinction between variations in pituitary release of the hormone and its rate of clearance from the plasma, and that the estimation of the MCR of HGH may help clarify the mechanism of abnormal plasma HGH responses to various stimuli.

Production rates of HGH (PR) in control subjects (347 ±173 mμg/min) were contrasted with hyperthyroid patients (529 ±242 mμg/min, $P < 0.05$), hypothyroid patients (160 ±69 mμg/min, $P < 0.02$), *IID* (245 ±100 mμg/min, NS), and IDD (363 ±153 mμg/min, NS). Considerable variability in the determination of the concentrations of immunoprecipitable HGH-^{125}I and endogenous plasma HGH concentrations was encountered at apparent equilibrium conditions. Since both factors are necessary for the PR calculations, the wide 95% confidence limits of the PR's did not permit a clear interpretation of the significance of these observations.

INTRODUCTION

Investigators examining the physiologic regulation of plasma human growth hormone (HGH) have been aware that diverse stimuli (1–9) elicit changes in plasma HGH concentrations. Such changes in plasma levels alone, moreover, do not distinguish between the separate contributions of pituitary HGH release and HGH clearance from the plasma, both of which are responsible for a change in plasma concentration.

The plasma disappearance of single intravenous injections of radioisotopically labeled HGH has been analyzed to define the distribution and turnover of HGH in humans (10–12). The fractional turnover and production rates of HGH in these studies were derived from calculations which assume first order kinetics and steady-state conditions. Some of the radioisotopic plasma disappearance curves in the literature (10, 11), however, do not clearly establish whether the plasma disappearance of HGH is linear or nonlinear when plotted semilogarithmically. In the present study, the constant infu-

This work was presented at the Annual Meeting of the Midwest Section, American Federation for Clinical Research, November 1968.

Dr. Mintz' present address is University of Miami School of Medicine, P.O. Box 875, Biscayne Annex, Miami, Fla. 33152.

Received for publication 2 December 1968 and in revised form 14 July 1969.

sion to equilibrium technique of Tait (13) was employed to assess the metabolic clearance (MCR) and production rate (PR) of HGH. This technique has also been utilized in evaluating the metabolism of other polypeptide hormones (14–16) which have been shown to have a multicompartmental distribution.

METHODS

Preparation of HGH-^{125}I. Highly purified HGH[1] was isotopically labeled to specific activities of approximately 150 $\mu c/\mu g$ with ^{125}I (Iso/Serv Division, Cambridge Nuclear Corp., Cambridge, Mass.) as described by Greenwood, Hunter, and Glover (17). 50 μl of the iodination mixture with 5 μl of human plasma was placed on a 5 cm cellulose powder column (Whatman, CF11) prepared in a disposable capillary Pasteur pipette. The cellulose column had been rendered pyrogen free by exposure to dry heat (160°C) for 30 min. The column was first washed with 1.5 ml of sterile 0.1 M veronal buffer, pH 8.6, and the HGH-^{125}I subsequently eluted with three 0.5-ml washes of 0.1 M veronal buffer containing 20% acetone and 0.25% human albumin (18). Aliquots of the second and third 0.5-ml acetone elutions were utilized for the infusion studies. The sterility of the HGH-^{125}I fraction was examined before use by incubation of an aliquot on a blood-agar plate and in thioglycolate broth at 4°, 22°, and 37°C. Bacterial contamination was not encountered with the labeling and preparatory procedures. The interval between iodination and use of the eluate in human studies was 2–8 days.

Experimental procedure. The subjects of the investigation were evaluated in the supine position in the forenoon after 14–17 hr of fasting. Water intake was permitted up to the start of each study. Each patient received five drops of Lugol's solution by mouth 12 and 2 hr before study. A slow intravenous 0.85% NaCl infusion was begun through each of two indwelling plastic cannulae placed in opposite antecubital veins. 60 min were then allowed to elapse before initiating the studies. Blood samples were withdrawn through one cannula and HGH-^{125}I, in 1% human serum albumin and 0.85% NaCl was infused via a constant infusion pump through the other. After injection of approximately 5 μc as a priming dose, 15–25 μc of the diluted HGH-^{125}I was infused at a constant rate of 1 ml/min over 2.5–3 hr.

Heparinized blood specimens were obtained before and every 15 min until the termination of the infusion. The plasma was immediately separated for analysis of immunoprecipitable HGH-^{125}I and an aliquot was frozen for subsequent determination of endogenous HGH concentration.

Measurement of HGH-^{125}I and endogenous plasma HGH concentration. The plasma samples were analyzed for immunoprecipitable HGH-^{125}I content by a double-antibody precipitation method immediately after each study (19). Duplicate 1 ml aliquots of plasma in 0.1 ml of 0.1 M ethylenediaminetetraacetic acid (EDTA) were incubated with 0.2 ml of guinea pig anti-HGH serum (final concentration 1 : 8500) for 48 hr at 4°C. Sunbsequently, 0.1 ml of nonimmune guinea pig plasma and 0.1 ml of rabbit anti-guinea pig gamma globulin (Hyland Laboratories) (final concentration 1:1500) were added and the mixture was incubated for an additional 24 hr at 4°C. All dilutions were made with 0.1 M Veronal buffer, pH 8.6. After centrifugation, the supernate was decanted and the precipitate washed twice with 0.5 ml of Veronal buffer. The radioactivity counts in the precipitate were determined in a well-type scintillation counter to a statistical accuracy of greater than 98%.

Eluates from the cellulose column utilized in the infusion studies were subjected to hydrodynamic flow chromatoelectrophoresis on Whatman 3MC chromatography paper (20). The eluates contained no free ^{125}I and less than 15% ^{125}I-labeled material that migrated beyond the chromatogram origin. In a series of control studies, fractions of column eluates were incubated with guinea pig anti-HGH sera at final concentrations of 1:8500 for 48 hr. Chromatoelectrophoresis of these mixtures revealed that in excess of 95% of the undamaged HGH-^{125}I migrated beyond the chromatogram origin, predominantly as antibody-bound HGH-^{125}I. Aliquots of the eluates in 0.5 ml of human plasma were also examined by the double-antibody precipitation technique. 90% or more of the undamaged HGH-^{125}I, as assessed by paper chromatography, was precipitated in the double-antibody procedure utilized in the studies. The slight discrepancy between the per cent of undamaged HGH-^{125}I and immunoprecipitable HGH-^{125}I as assessed by paper chromatography and double-antibody precipitation techniques, respectively, may be due to "incubation damage" to the isotopically labeled HGH (20), incomplete precipitation by the rabbit anti-guinea pig antisera, or the inability of paper chromatography to totally distinguish "radiation damaged" (21) immunononreactive from unmodified immunoreactive HGH. A fivefold increase in the concentration of guinea pig anti-HGH and or rabbit anti-guinea pig sera did not alter this discrepancy. Further, the addition of the rabbit anti-guinea pig antibody to the incubation mixture at 24 rather than at 48 hr incubation did not enhance the immunoprecipitability of the HGH tracer. When unlabeled HGH in final concentrations up to 200 $m\mu g$/ml were added to the HGH-^{125}I tracer in human plasma in vitro, the immunoprecipitability of the tracer was not significantly altered.

The immunoprecipitability of the HGH-^{125}I in each infusion mixture was determined in 0.5 ml of the infusate added to 0.5 ml of the patient's plasma obtained before the experiment. 75–85% of the radioactivity counts in the infusate were immunoprecipitable. Immunoprecipitability in aliquots of the infusion mixture obtained at various times throughout the infusion period did not vary. By radioautography of chromatograms of the column eluates or infusates, no aggregation of HGH-^{125}I was observed (22).

The endogenous plasma HGH concentration was measured in duplicate by a modification (23) of the dextran-coated charcoal immunoassay method of Herbert (24), using HGH-^{131}I as the tracer. Neither the ^{125}I content or the trace amounts of HGH-^{125}I in the plasma interfered with the ^{131}I-counting or immunoassay procedure.

Calculations. The plasma immunoprecipitable HGH-^{125}I concentrations reached equilibrium by 60 min after the start of the constant infusion. The plasma metabolic clearance rate (MCR) was determined from four or more consecutive samples in which the immunoprecipitable HGH-^{125}I and the endogenous plasma HGH concentrations were constant.

The plasma metabolic clearance rate (MCR) and the production rate (PR) were calculated according to the general formula of Tait (13):

$$MCR = \frac{r}{x'c}$$

where r is the rate of the infusion of HGH-^{125}I in cpm/min and x'c is the plasma HGH-^{125}I level in cpm/ml after equilibrium has been reached. Only the immunoprecipitable HGH-^{125}I in the infusion solution was used to calculate the

[1] Gift from the National Pituitary Agency through Dr. A. E. Wilhelmi, Lot No. HS 503A.

TABLE I

Determination of MCR of HGH by Use of Unlabeled HGH

Subject No.	Immunoassayable HGH infused	Estimated pituitary* HGH production	Total "infused"‡	Plasma HGH level	MCR unlabeled HGH	MCR§ I^{125}-labeled HGH
	mμg/min	*mμg/min*	*mμg/min*	*mμg/ml*	*ml/min*	*ml/min*
8	2880	160	3040	18	169	166
17	7680	520	8200	25	328	347

* Estimated on basis of a prior determination of the MCR utilizing HGH-^{125}I × basal plasma HGH concentration.

‡ Sum of unlabeled HGH infused and the estimated pituitary production rate.

§ See Table III.

rate (r) of HGH-^{125}I infusion. The production rate (PR) of HGH was calculated as:

$$PR = MCR \times i$$

where i is the plasma concentration of endogenous unlabeled HGH determined by radioimmunoassay.

The coefficient of variation of the plasma immunoprecipitable HGH-^{125}I was calculated by standard statistical methods (25). The significance of the differences in the means of the MCR, PR, and plasma endogenous HGH concentrations in each of the study groups were analyzed by Student's *t* test.

Subjects and patients. Adults were evaluated after informed consent. The results of five studies are not included because plasma equilibrium of either immunoprecipitable HGH-^{125}I or endogenous plasma HGH, or both, was not achieved. The control group consisted of 13 males and 9 females, aged 17–75 yr. Four of the patients who served as controls had recently recovered from bacterial pneumonia, one had recovered from saphenous vein thrombophlebitis, and 16 were normal nonhospitalized volunteers. 16 patients had diabetes mellitus, eight required insulin, and eight were managed by diet and oral hypoglycemic agents. Two insulin-requiring and two insulin-independent patients with diabetes mellitus were hospitalized for diabetic control at the time of study. The insulin-independent patients with diabetes mellitus consisted of five women and three men, aged 52–63 yr. The insulin-dependent patients with diabetes mellitus consisted of eight men, aged 29–61 yr. Except in one instance, exogenous insulin or oral hypoglycemic agents were withheld for 24 hr before study in the patients with diabetes mellitus. Diabetic patients with clinically detected liver, kidney, or muscle diseases were not included in the study. All patients with diabetes mellitus were clinically well hydrated. There were five female and one male patients with acromegaly aged 24–52 yr. Each of the patients with acromegaly had abnormal glucose tolerance to orally administered glucose and elevated plasma HGH concentrations. The five women had been treated for acromegaly within the previous 9 months with heavy-particle irradiation or low voltage X-ray therapy. There were 14 patients with untreated thyroid disease; eight had hyperthyroidism and six had hypothyroidism. In each instance, clinical impressions were confirmed by determination of protein-bound iodine and plasma thyroxine concentrations. The patients with hypothyroidism consisted of six women, aged 42–67 yr, and the patients with hyperthyroidism consisted of six women and two men, aged 22–51 yr.

Control studies. Two normal subjects (Nos. 8 and 17) who underwent studies after the usual protocol were also evaluated during the constant infusion of unlabeled HGH. After the 60 min rest period, a 50 μg priming dose of HGH was injected intravenously followed by a constant infusion of HGH at 2.9 and 7.7 μg/min. Heparinized blood samples were withdrawn at the usual times over 3 hr. The plasma obtained and an aliquot of the infusion was frozen until analyzed for the concentration of HGH by radioimmunoassay.

Three normal subjects (Nos. 17, 21, and 22) underwent studies in the supine position after the usual protocol except that unlabeled HGH was infused at a constant rate (12, 10, and 10 μg/min, respectively) beginning 120 min after the initiation of the HGH-^{125}I infusion and continuing for an additional 90 min.

Four subjects (Nos. 12–15) underwent studies in the supine and erect positions starting at 8:00 a.m. After 135 min of the constant HGH-^{125}I infusion and plasma sampling in the supine position, the subjects stood erect and plasma samples were collected every 15 min for 60 min while the infusion was maintained. The supine study was repeated 36 hr later (8:00 p.m.) in the same subjects.

RESULTS

The MCR of HGH after the infusion of unlabeled HGH in patients 8 and 17 were calculated to be 169 and 328

TABLE II

Effect of Acute Elevations of Plasma HGH Concentration on MCR

Subject	Study period	MCR	Plasma HGH	MCR % change during infusion
		ml/min	*mμg/ml*	%
17	Control	347	1.5	−5
	Infusion	330	63	
21	Control	158	1.1	0
	Infusion	157	52	
22	Control	272	2.1	−8
	Infusion	251	44	

TABLE III

Results of Constant Infusion of HGH-^{125}I in Control, Hypothyroid, Hyperthyroid, and Acromegalic Patients

Patient No.	Age	Sex	Weight	Height	Immunoprecipitable HGH-^{125}I infused	Plasma level at equilibrium	MCR		Endogenous plasma HGH	PR
	yr		*kg*	*cm*	*cpm/min*	*cpm/ml* ±SD	*ml/min*	*ml/min per m²*	*mμg/ml* ±*SD*	*mμg/min*
Control subjects										
1	52	M	79	187	12,257	67 ±2	183	91	1.2 ±0.3	220
2	45	M	65	173	16,782	72 ±2	231	131	1.8 ±0.5	416
3	25	M	64	170	15,189	67 ±2	227	131	1.3 ±0.5	295
4	43	M	71	163	23,846	136 ±3	176	101	2.0 ±0.7	352
5	42	F	59	163	12,535	63 ±3	198	123	3.0 ±0.6	594
6	21	F	68	170	16,847	77 ±3	218	123	2.5 ±0.6	545
7	27	F	65	160	14,019	63 ±3	224	135	0.9 ±0.3	202
8	30	M	91	191	11,261	68 ±0.6	166	76	0.7 ±0.3	115
9	18	M	82	170	20,706	110 ±6	187	97	1.1 ±0.6	206
10	21	F	70	171	15,079	82 ±5	183	101	0.7 ±0.2	128
11	17	F	64	168	13,419	69 ±5	195	114	0.7 ±0.3	137
12	25	M	95	183	17,624	64 ±4	275	128	1.3 ±0.5	358
13	26	M	75	185	16,620	69 ±5	240	122	0.7 ±0.4	168
14	22	M	84	180	13,167	47 ±4	280	139	1.4 ±0.6	392
15	26	M	84	183	12,173	41 +3	294	144	0.7 ±0.4	206
16	19	M	68	175	12,527	38 ±4	329	182	1.5 ±0.7	494
17	38	M	77	176	23,962	69 ±3	347	181	1.5 ±0.6	521
18	54	F	84	170	14,637	60 ±8	244	126	2.6 ±0.5	634
19	74	F	69	165	15,901	69 ±9	230	134	2.5 ±0.8	575
20	75	F	47	155	44,571	239 ±18	187	132	1.8 ±0.4	337
21	26	F	56	168	10,350	66 ±3	158	97	1.1 ±0.6	174
22	25	M	73	178	20,248	74 ±1	272	143	2.1 ±0.7	490
Mean ±SD							229 ±52	125 ±26	1.5 ±.7	347 ±173
Hypothyroid patients										
23	62	F	48	155	5,039	67 ±0.4	75	52	1.9 ±0.7	143
24	52	F	60	168	14,117	93 ±2	151	91	1.3 ±0.5	196
25	55	F	84	168	14,092	106 ±1	132	68	2.0 ±0	264
26	42	F	57	160	13,100	74 ±0.6	177	113	1.0 ±0	177
27	54	F	77	163	5,808	56 ±0.1	104	57	0.6 ±0.2	62
28	67	F	70	157	8,515	59 ±0.2	146	86	0.8 ±0.1	117
Mean ±SD							131 ±36*	78 ±23*	1.3 ±0.6	160 ±69‡
Hyperthyroid patients										
29	22	M	74	183	3,700	18 ±0.9	208	107	0.8 ±0	166
30	42	F	95	156	21,410	87 ±0.7	245	127	1.5 ±0.6	368
31	51	F	56	160	4,912	14 ±0.4	350	224	2.0 ±0	700
32	27	F	56	157	28,786	110 ±1	262	170	2.3 ±0.5	603
33	45	F	54	151	28,170	99 ±0.1	285	197	1.8 ±0.5	513
34	48	F	50	163	28,724	160 ±6	180	119	2.2 ±0.3	396
35	49	F	54	166	12,500	64 ±4	196	124	2.6 ±0.6	510
36	41	M	61	173	22,641	116 ±4	195	114	5.0 ±1.6	975
Mean ±SD							240 ±57	147 ±43	2.3 ±1.2§	529 ±242§

* Significantly different from controls $P < 0.001$.
‡ Significantly different from controls $P < 0.02$.
§ Significantly different from controls $P < 0.005$.

TABLE III—*Continued*

Patient No.	Age	Sex	Weight	Height	Immunoprecipitable HGH-^{125}I infused	Plasma level at equilibrium	MCR		Endogenous plasma HGH	PR
	yr		*kg*	*cm*	*cpm/min*	*cpm/ml* ±SD	*ml/min*	*ml/min per m*	*mμg/ml* ±SD	*mμg/min*
Acromegalic patients										
37 a	32	F	91	169	16,132	50 ±0.2	323	162	140 ±10	45,220
b					16,662	50 ±0.6	333	167	133 ±13	44,290
38	42	F	66	160	33,890	149 ±8	228	137	24 ±3.6	5,472
39	24	F	77	163	13,700	68 ±2	202	111	58 ±6	11,716
40	43	F	82	168	16,254	97 ±1	168	88	20 ±1.5	3,360
41	29	M	84	173	11,273	56 ±4	203	104	192 ±12	38,976
42	52	F	68	165	16,449	55 ±5	297	170	13 ±0.8	3,861
Mean ±SD							238 ±62	129 ±34		

ml/min (Table I).[3] The plasma HGH levels measured at equilibrium were 18 mμg/ml and 25 mμg/ml, respectively. The MCR's of the unlabeled HGH were similar to the MCR's obtained in the same subjects using HGH-^{125}I for the infusion. The results demonstrating the effect of acute elevations of plasma HGH concentration on the MCR of HGH are presented in Table II. The MCR of HGH was unaffected in one subject (No. 21) and was reduced by 5 and 8% in two others (Nos. 17 and 22) when the plasma HGH concentrations were acutely increased to 52, 63, and 44 mμg/ml, respectively.

In four subjects, no diurnal variation in MCR was observed when studies were performed at 8:00 a.m. and repeated at 8:00 p.m. (Fig. 1). These four subjects were also evaluated in prone and erect positions beginning at 8:00 a.m. Standing erect was associated with a mean 24% decrease in the MCR.

The MCR's and PR's for the constant infusion studies were derived from the data listed in Tables III and IV. The mean coefficient of variation of the plasma immunoprecipitable HGH-^{125}I was 3.2% (range 0.1–9%). The mean coefficient of variation of the values used to determine the mean endogenous plasma HGH concentration was not different from that previously reported from this laboratory for single samples at the same plasma HGH concentration (26). The 22 control patients had a mean MCR of 229 ±52 (mean ±SD) ml/min and a mean PR of 347 ±173 mμg/min. The mean plasma HGH concentration was 1.5 ±0.7 mμg/ml. No difference in MCR, PR, or endogenous plasma HGH was observed between the sexes. The blood glucose concentration was normal in all patients except in the diabetic patient groups. The blood glucose did not vary more than 5% during the study in the control or patient study groups.

The six hypothyroid patients had a mean MCR and PR of 131 ±36 ml/min and 160 ±69 mμg/min, respectively (Table III and Fig. 2). The mean MCR and PR observed in the hypothyroid patients were significantly decreased when compared to the control subjects ($P <$ 0.001 and < 0.02, respectively). The mean plasma HGH concentration of 1.3 ±0.6 mμg/ml was not significantly different from the control subjects. The eight hyperthyroid patients had a mean MCR of 240 ±57 ml/min which was not different from the control value (Table III and Fig. 2). The mean plasma HGH concentration of

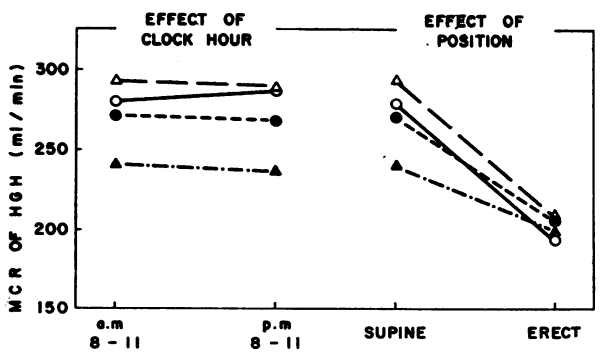

FIGURE 1 Effect of clock hour and patient position on the MCR of HGH in four control subjects.

[3] Since the plasma HGH concentration observed may represent a function of both the HGH infused and the endogenous HGH released into the blood, the calculation of r, the "infusion" rate, includes an estimate of endogenous production based on the basal plasma HGH concentration and the MCR determined by a prior HGH-^{125}I infusion. Although inclusion of endogenous HGH production may introduce an error in the calculation of MCR, the relative contribution of this fraction represented only 5 and 7% of the amount of HGH infused. Accordingly, any error introduced should be minimal. Patients with monotrophic growth hormone deficiencies were not available during these studies to aid in further validating that the MCR of labeled and unlabeled HGH are similar.

TABLE IV

Results of Constant Infusion of HGH-^{125}I in Insulin-Independent and Insulin-Dependent Diabetic Patients

Patient	Age	Sex	Wt	Height	Immunoprecipitable HGH-^{125}I infused	Plasma level at equilibrium	MCR		Endogenous plasma HGH	PR	Blood sugar
	yr		*kg*	*cm*	*cpm/ml* ±SD	*cpm/ml* ±SD	*ml/min*	*ml/min per m²*	*mμg/ml* ±SD	*mμg/min*	*mg/100 ml*
Insulin-independent diabetic patients											
43	52	F	68	156	7,720	56 ±2	139	83	0.5 ±0.1	70	113
44	61	F	75	157	16,137	114 ±6	141	81	1.8 ±0.4	254	94
45	55	F	102	163	20,160	106 ±5	190	93	1.7 ±0.2	323	164
46	52	F	68	155	11,624	77 ±2	151	91	1.5 ±0.1	227	126
47	63	M	73	173	11,881	56 ±3	213	115	1.3 ±0.5	277	85
48	59	M	65	160	20,890	111 ±3	189	114	1.5 ±0.5	284	146
49	59	M	91	180	21,570	83 ±2	258	123	1.5 ±0	387	145
50	60	F	69	145	25,501	127 ±0.5	201	126	0.7 ±0	141	113
Mean ±SD							185 ±41*	103 ±18*	1.3 ±0.5	245 ±100	
Insulin-dependent diabetic patients											
51	34	M	61	173	13,912	88 ±2	158	92	4.3 ±0.4	679	274
52	44	M	65	170	15,071	102 ±3	147	85	2.7 ±0.3	397	70
53	53	M	68	178	19,067	166 ±2	115	65	2.3 ±0.4	265	248
54	42	M	54	173	16,856	102 ±2	165	101	2.4 ±0.1	396	237
55	34	M	76	175	8,045	66 ±1	122	64	3.3 ±0.3	403	248
56	29	M	76	183	18,168	103 ±3	176	90	1.8 ±0.5	317	338
57	61	M	92	178	5,233	44 ±0.6	118	57	2.5 ±0.6	295	116
58	30	M	60	175	5,430	64 ±1	85	49	1.8 ±0.5	153	330
Mean ±SD							136 ±31‡	75 ±19‡	2.6 ±0.8§	363 ±153	
Control subjects											
Mean ±SD							229 ±52	125 ±26	1.5 ±0.7	347 ±173	

* Significantly different from controls, $P < 0.05$.
‡ Significantly different from controls, $P < 0.01$.
§ Significantly different from controls, $P < 0.001$.

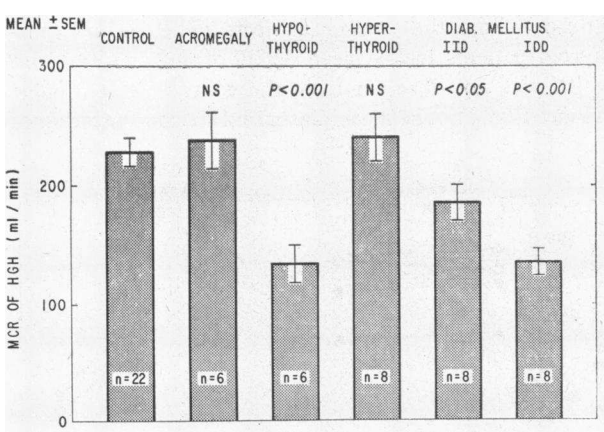

FIGURE 2 A comparison of the mean metabolic clearance rates of human growth hormone (MCR of HGH, ml/min) between control subjects and patients with acromegaly, hypothyroidism, hyperthyroidism, and insulin-independent (IID) and insulin-dependent (IDD) diabetes mellitus. NS, not significant; n, No. of subjects in each group.

2.3 ±1.2 mμg/ml was significantly greater than the control value ($P < 0.05$). The mean PR of 529 ±242 mμg/min in patients with hyperthyroidism was significantly higher ($P < 0.05$) than in control subjects. If the patient who demonstrated the highest plasma HGH concentration (No. 36) is excluded from the calculations, the mean MCR, mean plasma HGH concentration, and mean PR for the remaining patients with hyperthyroidism did not differ from normal subjects.

The six patients with acromegaly (Table III and Fig. 2) demonstrated MCR's which ranged from 168 to 333 ml/min (mean 238 ±62 ml/min). The mean MCR was not different from control subjects. The corresponding PR's ranged from 3360 to 45,220 mμg/min. The plasma HGH concentrations ranged from 13 to 192 mμg/ml. One patient (No. 37) was evaluated on two occasions separated by 2 months. The results were in close agreement.

The eight patients with insulin-independent diabetes

mellitus (Table IV and Fig. 2) had a mean MCR and PR of 185 ±41 ml/min and 245 ±100 mμg/min, respectively. The mean basal plasma HGH concentration was 1.3 ±0.5 mμg/ml. The mean MCR was significantly lower than in control subjects ($P < 0.05$). The PR and endogenous plasma HGH concentrations were not significantly different from the control subjects. The mean MCR of 136 ±31 ml/min in the eight patients with insulin-dependent diabetes mellitus (Table IV and Fig. 2) was significantly decreased when compared to the control subjects ($P < 0.001$), and patients with insulin-independent diabetes mellitus ($P < 0.02$). The mean basal endogenous plasma HGH concentration of 2.6 ±0.8 mμg/ml was significantly increased when compared to the control value ($P < 0.01$) and patients with insulin-independent diabetes mellitus ($P < 0.01$). The mean PR of 363 ±153 mμg/ml was not significantly different from the control value. There was no correlation between the fasting blood glucose concentration and the endogenous plasma HGH concentration or the MCR of HGH-^{125}I.

Correction of the MCR and PR of HGH for differences in body surface area (Tables III and IV) did not alter the relative differences determined between various patient groups and control subjects.

DISCUSSION

The constant infusion technique was employed for all studies after it was established that under the conditions of the experimental protocol, relatively steady-state concentrations of both infused radioisotopically labeled hormone and endogenous unlabeled hormone could be attained. The similarity of the MCR's of HGH (Table I) derived from data obtained during the constant infusion of unlabeled HGH (when allowances were made for endogenous production) to that obtained during the infusion of radioisotopically labeled HGH supports the validity of the tracer in estimating the MCR of HGH.

In the control group as a whole, there were no significant differences in the MCR of HGH between sexes, nor between subjects less than or greater than 45 yr of age. Constancy of the MCR both within the day (Fig. 1) and from day to day was observed (patients 8 and 17, Table I, and patient 37, Table III). The only maneuver studied which resulted in a change in MCR was the assumption of an upright position. Although the MCR of HGH is considerably less than hepatic blood flow (27), the reduction in the latter, upon assumption of the upright position (28), is comparable to the reduction in MCR which was observed. Whether these observations are related, remains to be elucidated.

The measurements of the MCR before and after acute elevation of plasma HGH to a new steady-state level, revealed no alterations in the MCR. Further, the MCR of HGH in patients with acromegaly and chronic elevations of plasma HGH concentrations did not differ from control subjects. This dissociation of MCR and plasma HGH concentration implies that the plasma HGH-removing mechanisms are not saturated at physiologic plasma HGH levels. As suggested by Stern, Farquhar, Silvers, and Reaven (15), this is a desirable feature of a control system in which rapid adjustments of plasma concentrations are necessary. Similar to the current experiments, elevated plasma concentrations of other polypeptide hormones, insulin (15), luteinizing hormone (LH) (14), follicle-stimulating hormone (FSH) (16), and human placental prolactin (HPL) (29) do not appear to alter their respective MCR's from the plasma.

The mean daily PR of HGH calculated from the equilibrium PR in the supine control subjects was 0.5 mg. This is in close agreement with the prediction of Glick, Roth, Yalow, and Berson (3). Although this amount approximates the mean daily replacement requirements for linear growth in hypopituitary dwarfs (30), the actual integrated HGH secretion may well differ from this because of several factors. Firstly, although the MCR appears relatively constant and independent of changes in plasma HGH concentration, alterations in posture during the 24 hr day would affect the MCR and thus the PR of HGH. Secondly, several factors (3, 31–34) encountered throughout the 24 hr day could directly modify the plasma concentration of HGH through enhancement or inhibition of the rate of pituitary secretion of HGH.

Even the precision of the calculation of the basal PR of HGH is open to question. From its mathematical expression, the variability of the PR must reflect both the variability of the MCR and the plasma HGH concentration. It is clear from the data in Tables III and IV, that either the steady state attained or the reproducibility of the assay was more satisfactory for the determination of the concentration of the labeled tracer than it was for the measurement of endogenous HGH plasma concentration. In our experiments, these alternatives were not distinguished. Using logarithmic transformations of the two variables used to calculate each PR (25) yields 95% confidence limits of the PR which are as large as the PR's determined for most of the control subjects. Accordingly, interpretation of the significance of the differences in the PR's observed within and between various groups of patients must remain uncertain.

The determination of the MCR of HGH as a component of the physiologic regulation of plasma HGH contration may clarify the mechanism of alterations in plasma HGH responses to provocative stimuli. Impoverished plasma HGH responses to insulin-induced hypoglycemia have been reported in patients with hypothyroidism (35, 36) and hyperthyroidism (37). The

results of the present studies suggest that if the blunted plasma HGH responses in patients with hyperthyroidism are encountered, they cannot be explained on the basis of increased peripheral clearance of the hormone. Recent observations, moreover, indicate that plasma HGH responses to hypoglycemia are normal in patients with hyperthyroidism (38). In patients with hypothyroidism, the reduced MCR of HGH indicates that abnormalities in the growth hormone–releasing mechanism account for the diminished plasma HGH responses to insulin-induced hypoglycemia. In accord with an abnormality of the growth hormone–releasing mechanism, Lewis, Cheever, and VanderLaan (39) with a bioassay, and Daughaday, Peake, Birge, and Mariz (40) with an immunoassay, determined that hypothyroidism diminishes the content of HGH in the rat hypophysis.

The explanation for the reduction in MCR in both diabetic groups is not apparent. Clinical indications of dehydration, alterations in circulation, or hepatic, renal, or muscle disease were sought but not found. In contrast to the findings reported, Boucher (12) found that the MCR in patients with insulin-dependent diabetes mellitus was normal. However, because the plasma decay curve of injected radioisotopically labeled HGH was treated as a single exponential function, the results cannot be compared.

Despite the markedly low mean MCR in the insulin-dependent diabetic group, the mean PR was normal because of the higher mean plasma HGH concentration. This elevation is at variance with the normal fasting HGH levels in patients with diabetes mellitus recorded by several investigators (3, 41–43). In most of the reports, however, the heterogeneity of the diabetic and contrast groups and the circumstances of the study which might affect the plasma HGH concentration are not defined (8, 9, 32, 33, 44–46). In the present study, both control subjects and patients were exposed to similar stimuli under carefully controlled conditions. Secondly, relatively steady-state concentrations of plasma HGH were a requisite in this study so that subjects who demonstrated spantaneous wide fluctuations in plasma HGH levels were excluded. Thirdly, the timing of blood sampling may yield results which are not comparable to those from samples obtained shortly after awakening in the morning. Finally, insulin was withheld during the morning of the study and the resultant decreased glucose utilization may have affected the basal pituitary release of HGH (3) in the patients with insulin-dependent diabetes mellitus.

Abnormalities in growth hormone metabolism in diabetes mellitus have been postulated and sought by several investigators. Apart from the increased plasma HGH responses to tolbutamide in patients with prediabetes (47), and decreased responses to arginine in maturity onset diabetes (48) normal plasma HGH responses to various provocative stimuli have been generally observed (3, 41–43, 49–51). The results of our studies emphasize, however, that plasma HGH levels alone, do not permit distinction between abnormalities in pituitary release of the hormone and its rate of clearance from plasma. Further studies may help clarify the mechanism and significance of the reduced MCR of HGH observed in patients with diabetes mellitus.

ACKNOWLEDGMENTS

We are indebted to Mrs Katherine McDade and Mr. John Bobik for their excellent technical assistance.

This work was supported by a research grant from the U. S. Public Health Service (AM 1090) and the facilities of the Clinical Research Center (5 MO-1FR 00056-07) from the National Institutes of Health.

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