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Research Article

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Metabolic Clearance and Production Rates of Prolactin in Man

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A B S T R A C T Metabolic clearance rates (MCR) and production rates (PR) of prolactin (PRL) have been determined by the constant infusion to equilibrium technique in 11 normal subjects, 6 patients with hyperthyroidism, 4 patients with hypothyroidism, and 9 patients with hyperprolactinemia. PRL MCR was also determined in four patients during dopamine infusion. Mean PRL MCR was 46 ± 1 ml/min per m^2 in women and 44 ± 3 ml/min per m^2 in men, and was significantly correlated with body mass ($r = 0.84$, $P < 0.001$). In contrast with controls, PRL MCR was higher in hyperthyroidism (MCR $= 52\pm8$ ml/min per m^2, $P < 0.05$), was slightly lower in hypothyroidism (MCR $= 38\pm10$ ml/min per m^2, $P =$ NS), and was significantly correlated with serum thyroxine ($r = 0.46$, $P < 0.02$). PRL MCR was lower than controls in hyperprolactinemia (MCR $= 40\pm5$ ml/min per m^2, $P < 0.01$) and was inversely correlated with serum PRL ($r = -0.72$, $P < 0.001$). PRL MCR was not significantly changed by dopamine infusion.

Mean PRL PR for women and men was 211 ± 74 and 187 ± 44 $\mu g/d$ per m^2, respectively ($P =$ NS). In hyperthyroidism the PRL PR was elevated (PR $= 335\pm68$ $\mu g/d$ per m^2, $P < 0.02$), but in hypothyroidism the increase (PR $= 233\pm159$ $\mu g/d$ per m^2) was not significant. In hyperprolactinemia the PRL PR was extremely high (PR $= 31,000\pm29,000$ $\mu g/d$ per m^2). Dopamine infusion decreased PRL PR from 270 to 66 $\mu g/d$ per m^2 indicating that its effect was on pituitary PRL secretion and not PRL metabolism.

To evaluate possible circulating PRL heterogeneity that might arise during infusion, gel filtration of infusate and serum obtained during the MCR procedure was performed. Labeled monomeric PRL (peak III, Kav (partition coefficient) $= 0.4$) was partially converted to two larger forms (peaks I and II) in vivo. Peak I (Kav $= 0$) was 30–40% immunoprecipitable, although peak II (Kav $= 0.2$) was not immunoprecipitable. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of peak I resulted in $\geq90\%$ conversion to peak III and restoration of full immunoactivity. Thus, peak I is a noncovalently linked aggregate that is partially immunoactive, and therefore able to alter MCR determinations. These studies demonstrate the impact of hormone heterogeneity on MCR estimations and suggest that gel filtration of immunoprecipitable material be an integral part of future MCR measurements.

INTRODUCTION

The circulating concentration of a hormone is determined not only by the production rate of that hormone (PR),[1] but also by its metabolic clearance rate (MCR). Knowledge of hormonal MCR has become necessary for a complete understanding of physiologic and pathologic changes in peripheral hormone concentrations. Since 1963, the constant infusion to equilibrium technique of Tait (1) has been used to measure the MCR of both steroids and polypeptide hormones. The advantage of this method over previous single-injection techniques is that knowledge of the complex disappearance curve of an infused hormone is not necessary for MCR calculations. The only prerequisites are that equilibrium be reached during the infusion and that labeled and unlabeled hormones be metabolized identically (1).

The MCR of growth hormone (GH), luteinizing hormone, follicle-stimulating hormone, thyrotropin-stimulating hormone (TSH), and the subunits of TSH have been determined by this method (2–9). Although

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[1] *Abbreviations used in this paper*: GH, growth hormone; Kav, partition coefficient; MCR, metabolic clearance rate; PAGE, polyacrylamide gel electrophoresis; PR, production rate; PRL, prolactin; SDS, sodium dodecyl sulfate; T_3, triiodothyronine; T_4, thyroxine; TRH, thyrotropin-releasing hormone; TSH, thyrotropin-stimulating hormone.

the MCR of prolactin (PRL) has been measured in both the rat (10) and sheep (11) in this manner, comparable data in humans have not been reported. The following study was designed to determine the MCR of PRL in humans, and, using ambient hormone levels, to calculate the daily production rate of PRL in normal subjects, and in various pathologic states (hyperthyroidism, hypothyroidism, and hyperprolactinemia). In addition, the effect of dopamine on the MCR and PR of PRL was investigated because dopamine causes a substantial reduction in circulating PRL levels (12). To validate these methods, gel filtration and immunologic characterization of radioiodinated PRL were performed to assess the impact that hormone heterogeneity and metabolism might have on MCR calculations.

METHODS

Iodination of human PRL. Highly purified human PRL was supplied by the National Pituitary Agency (VLS-3, VLS-4) and by Dr. Albert Parlow (AFP-1582-C). The hormone was reconstituted in 0.01 M NH_4HCO_3 buffer, pH 8.2, and stored at $-70°C$. Iodination with ^{125}I to a specific activity of 50–100 $\mu Ci/\mu g$ was carried out using a modified chloramine T method or the lactoperoxidase method (13). The labeled PRL was chromatographed on a 1.5 × 90-cm Sephadex G-100 column (Pharmacia Fine Chemicals, Div. of Pharmacia Inc., Piscataway, N. J.) using phosphate-buffered normal saline, 0.02% sodium azide, pH 7.4. Three characteristic protein peaks were obtained (peaks I, II and III). For these MCR studies only peak III, or "native," PRL was used.

Comparison of labeled PRL disappearance rates. To justify the use of ^{125}I-PRL for MCR studies, it was necessary

to document that the disappearance of unlabeled and labeled PRL from sera was similar and not influenced by iodination. 20 μg of PRL (VLS-3) were injected into the jugular vein of a dog, and blood samples were taken at 0, 2, 5, 15, 20, 40, and 60 min. Subsequently, 10 μCi ^{125}I-PRL (VLS-3) were injected, and blood was sampled every 10 min for 90 min. Serum was analyzed for labeled and unlabeled PRL by immunoprecipitation and radioimmunoassay.

Determination of the MCR and PR of PRL

MCR procedure and immunoprecipitation. 80 ml phosphate-buffered saline, pH 7.4, containing 10–20 μCi ^{125}I-PRL and 1% human serum albumin were sterilized by Millipore filtration (Millipore Corp., Bedford, Mass.). An intravenous bolus of 20–30 ml was given initially so that equilibrium could be achieved more rapidly. After 5 min, the remaining 50–60 ml were infused over 3 h at a constant rate (0.23–0.25 ml/min). Samples were drawn at 0, 5, 30, 60, 90, 120, 135, 150, 165, and 180 min. Apparent equilibrium was considered to have occurred when the number of immunoprecipitable counts per milliliter in sera did not vary by $> \pm 10\%$.

At equilibrium, labeled PRL was immunoprecipitated from 0.8 ml of sera or infusate by the double antibody technique using a final rabbit anti-PRL antibody titer of 1:100. The immunoprecipitability of infusate ranged from 45 to 83% depending on the hormone used for iodination, the specific activity, and the age of the iodinated PRL.

Determination of PRL MCR. The mean counts per minute per milliliter from at least three points during the final hour of infusion were averaged. The infusion rate in counts per minute was calculated as the product of the infusion rate of the pump in milliliters per minute and the immunoprecipitable counts per minute per milliliter of ^{125}I-PRL in the infusate.

$$MCR \ (ml/min) = \frac{Immunoprecipitable \ ^{125}I\text{-PRL in infusate (cpm/ml)} \times infusion \ rate \ (ml/min)}{Serum \ concentration \ of \ immunoprecipitable \ ^{125}I\text{-PRL at equilibrium (cpm/ml)}}.$$

Serum PRL concentrations. Serum PRL was determined by a homologous radioimmunoassay using amniotic fluid PRL standards calibrated against MRC Research Standard A 71/222 (Medical Research Council of Canada). 1 ng of our PRL standard is equivalent to 16 μU of the MRC standard. A double antibody system was used to separate bound from free hormone. 50-μl serum samples were incubated at 4°C with 100 μl rabbit anti-PRL antibody (1:10,000 final concentration) and 750 μl 0.05 M phosphate buffer containing 0.1% bovine serum albumin. After 48 h, 100 pg ^{125}I-PRL tracer were added and, after 24 h at 4°C, samples were precipitated with goat anti-rabbit gamma globulin. The sensitivity of the assay is 1 ng/ml, with interassay variation of 15% and intraassay variation of 8%. In this assay, the mean normal serum PRL concentration for females is 4.8 ± 2.2 ng/ml (range, 0 to 12 ng/ml), and is 3.7 ± 2.7 ng/ml (range, 0 to 10 ng/ml) for males. To eliminate interassay variation, all basal PRL determinations were performed in the same assay.

Determination of PRL PR. The PRL PR was calculated as the product of the endogenous serum PRL concentration and the MCR. Serum PRL levels were drawn between 8:00 and 10:00 a.m. The PRL PR (micrograms/day) = MCR (milliliters/minute) × [PRL] (nanograms/milliliter) × 1,440 min/d.

Patients. Control volunteer subjects and patients with various diseases were studied in the Clinical Research Center of the Massachusetts General Hospital, Boston, Mass., after informed consent was obtained. All subjects except those with thyroid disease were pretreated with 270 mg KI before the ^{125}I-PRL infusion, to block uptake of free ^{125}I by the thyroid. For testing with thyrotropin-releasing hormone (TRH), 200 μg TRH (Protirelin, Abbott Laboratories, North Chicago, Ill.) was injected as a bolus, and blood was sampled at 0, 10, 20, 30, 45, 60, and 120 min (14).

Controls. 11 healthy subjects (5 men aged 23–65 yr and 6 premenopausal women aged 24–53 yr, in the follicular phase of the menstrual cycle) were studied. All had normal pituitary, thyroid, renal, and liver function. One subject was studied twice to test the precision of the procedure, as well as to test the possibility that PRL labeled by the chloramine T method might be cleared differently from that labeled by the lactoperoxidase technique. One man and two women had blood sampled hourly through an indwelling catheter for 24 h to characterize a daily secretory profile for PRL.

Thyroid disease. Six patients with Graves' disease (five women and one man, mean age 33 yr) were studied before therapy. The patients were hyperthyroid by clinical criteria, and all had elevations of serum thyroxine (T_4) and

triiodothyronine (T₃), and undetectable serum TSH levels. Four patients with primary hypothyroidism were studied (three women and one man, mean age 52 yr). One patient had Hashimoto's thyroiditis, two patients had had thyroid ablation 5 and 10 yr earlier with ¹³¹I, and one patient had propylthiouracil-induced hypothyroidism of at least 3-mo duration. All had markedly decreased serum T₄ and T₃ with concomitant elevations of serum TSH levels. Serum T₄, T₃, and TSH were measured by radioimmunoassay (14, 15).

Hyperprolactinemia. Eight patients (six women and two men, mean age 30 yr) with PRL-secreting pituitary adenomas, and one woman with idiopathic hyperprolactinemia were studied. Seven patients were euthyroid, and two patients (patients 27 and 29) had secondary hypothyroidism. Two patients with tumors were studied both before and after transsphenoidal hypophysectomy, and the remaining six were studied before proton beam pituitary irradiation.

Dopamine infusion. Four subjects (three normal and one with idiopathic hyperprolactinemia) underwent a standard MCR determination. 1 wk later, they had the same procedure performed together with a simultaneous dopamine infusion. Dopamine, 200 mg (Intropin, Amar-Stone Laboratories, Mount Prospect, Ill.), was mixed in 500 ml 5% dextrose in water and infused at the rate of 4 μg/kg per min. Blood pressure and pulse were monitored every 30 min and did not change significantly over 2 h. After 2 h of infusion, the PRL MCR was determined by the previously described methods, during which time the dopamine infusion was continued.

Gel chromatography of ¹²⁵I-PRL. To investigate the heterogeneity of labeled PRL, gel chromatography of infusate and sera was performed. Infusate (20,000 cpm) or 4 ml of serum obtained at 5 and 180 min of the constant infusion were chromatographed on a 1.5 × 90-cm Sephadex G-100 column at 4°C. All samples were chromatographed within 24 h of the infusion. 1-ml fractions were collected and counted, and radioactive peaks were immunoprecipitated with excess anti-PRL antisera (1:100).

Polyacrylamide gel electrophoresis of PRL. To investigate the molecular nature of the heterogeneous species of PRL, each iodination peak (Fig. 5A) was subjected to denaturing polyacrylamide gel electrophoresis (PAGE). Approximately 100,000 cpm of peaks I, II, and III were placed in 2% sodium dodecyl sulfate (SDS), 0.05 M Tris buffer, pH 6.8, with or without 1% β-mercaptoethanol. Samples were applied to 0.6 × 9 cm SDS containing 7.5% polyacrylamide–0.2% bis-acrylamide (Eastman Organic Chemicals Div., Eastman Kodak Co., Rochester, N. Y.) gels, and electrophoresed with a current of 2 mA/gel in a continuous 0.05 M Tris–0.4 M glycine–0.1% SDS buffer system, pH 8.4. Gels containing ¹²⁵I-PRL were sliced and counted. Slices were incubated in 0.05 M PO₄, 0.1% SDS solution for 2 h at 37°C, and the eluted counts were immunoprecipitated as described above. The concentrations of SDS or β-mercaptoethanol used in these studies did not affect immunoactivity of labeled PRL.

Statistics. The significance of differences was analyzed by Student's *t* test. Linear regression analysis was performed by the method of least squares.

RESULTS

Labeled and unlabeled PRL disappearance curves. The initial rate of disappearance (distribution phase) of ¹²⁵I-PRL and unlabeled PRL was similar in the dog (*P* for slopes = 0.4, NS). These data validated the use of labeled PRL in subsequent studies.

PRL MCR and PR in normal subjects. A typical example of the MCR procedure is shown in Fig. 1. Equilibrium was reached by 90–120 min, after which the variation of immunoprecipitable counts was no >±10%. Women had a mean MCR of 79±10 ml/min (46±1 ml/min per m²) and men had a mean MCR of 78±11 ml/min (44±3 ml/min per m²) (Table I). Because there was a significant positive correlation (*r* = 0.84, *P* < 0.001, Fig. 2) between the MCR and body surface area, all results have been corrected for surface area. One subject (subject 6) had a MCR measured twice, with the repeat value (45 ml/min per m²) being very similar to the original value (48 ml/min per m²). It was also demonstrated in this subject that there was no difference in the MCR of PRL iodinated with chloramine T vs. that iodinated with lactoperoxidase (13).

PRL PR was calculated on the basis of basal morning serum PRL levels and expressed as micrograms per day per square meter of body surface. Mean PRL PR for women was 211±74 μg/d per m² (range, 187 to 570 μg/d per m²). Mean PRL PR for men was 187±44 μg/d per m² (range, 192 to 443 μg/d per m²). There was no significant difference between these two groups (*P* ≅ 0.7).

PRL MCR and PR in thyroid disease. The mean PRL MCR in hyperthyroid patients was 52±8 ml/min per m², which was significantly higher than the control value of 46±4 ml/min per m² (*P* < 0.05) (Table II). The mean PRL MCR in the hypothyroid patients was decreased (38±10 ml/min per m²) but was not significantly lower than control or hyperthyroid subjects (Table II). There was a small but significant direct correlation between PRL MCR and serum T₄ levels (*r* = 0.46, 0.01 < *P* < 0.02, Fig. 3), but no such relationship was observed for serum T₃ levels (data not shown).
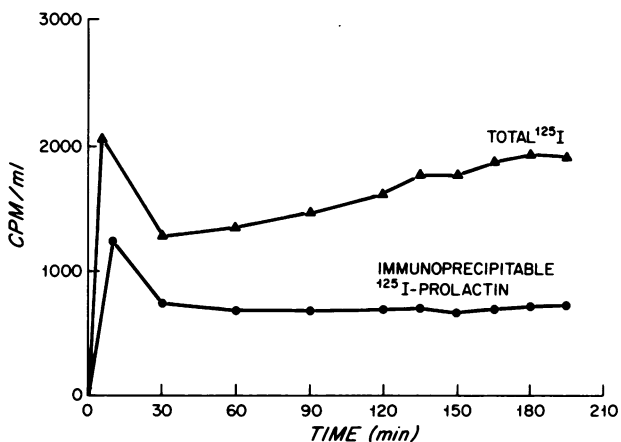

FIGURE 1 Total ¹²⁵I and immunoprecipitable ¹²⁵I during the course of a MCR determination. Apparent equilibrium is reached by 90–120 min.

TABLE I
Normal Controls

Subject	Age	Sex	Surface area	T_4	T_3	TSH	Basal PRL	Peak PRL after TRH	PRL MCR	PRL MCR/m²	PRL PR	PRL PR/m²
	yr		m²	μg/dl	ng/dl	μU/ml	ng/ml	ng/ml	ml/min	ml/min/m²	μg/d	μg/d/m²
1	45	F	1.64	7.1	141	1.9	4.6	24	79	48	523	318
2	53	F	1.7	7.5	146	3.8	1.8	33	72	42	187	110
3	29	F	1.54	5.8	152	1.4	2.2	7.0	66	43	209	136
4	25	F	1.62	9.9	168	1.3	3.1	17	74	45	330	201
5	24	F	1.8	4.3	86	0.6	2.8	16	95	53	383	214
6	29	F	1.96	6.3	158	1.8	4.4	45	90	45	570	285
Mean (women)	34						3.2	24	79	46	367	211
±SD	±10						±1	±12	±10	±1	±144	±74
7	28	M	1.86	5.6	153	2.1	3.2	12	74	40	341	184
8	25	M	2.04	5.5	128	3.3	3.0	12	101	49	436	212
9	23	M	1.76	6.9	133	3.4	4.1	18	75	43	443	254
10	65	M	1.54	9.2	86	3.0	1.9	12	70	45	192	123
11	24	M	1.64	7.8	150	0.7	2.2	22	73	44	231	162
Mean (men)	33						2.9	15	78	44	329	187
±SD	±15						±0.8	±4	±11	±3	±102	±44
Mean (combined)	33			6.4	136.5	2.1	3.0	20	79	46	349	200
±SD	±13			±1.3	±26	±1.1	±1.1	±10	±11	±4	±128	±63

Mean basal PRL for the hyperthyroid patients was 4.5±1.1 ng/ml, which was significantly higher than the normal control value of 3.0±1.1 ng/ml ($0.01 < P < 0.02$). In addition the mean PRL PR for this group was 335±68 μg/d per m², which was significantly greater than the normal value of 200±63 μg/d per m² ($0.01 < P < 0.02$). In the hypothyroid subjects, mean basal PRL was 4.1±2.1 ng/ml, and the mean PRL PR was 233±159 μg/d per m². Both values were slightly higher than normal, but the differences were not significant.

The mean peak PRL after TRH in hyperthyroid

FIGURE 2 Positive correlation of PRL MCR with body surface area in normal subjects ($r = 0.84$, $P < 0.001$).

subjects was 6.9±2.8 ng/ml, which was significantly lower than the control value of 19.8±10 ng/ml ($P < 0.01$). Hypothyroid subjects had a mean peak PRL after TRH of 43.2±16 ng/ml, which was significantly higher than controls ($0.01 < P < 0.02$).

PRL MCR and PR in hyperprolactinemic patients. Eight patients with a PRL-secreting pituitary adenoma and one patient with idiopathic hyperprolactinemia were studied (Table II). The mean PRL MCR of 40±5 ml/min per m² was significantly lower than the mean value obtained for normal controls ($0.02 < P < 0.05$). There was a highly significant inverse correlation between the logarithm of the serum PRL and the PRL MCR ($r = -0.72$, $P < 0.001$, Fig. 4). Two patients (patients 22 and 23) were studied pre- and postoperatively. In patient 22, PRL MCR preoperatively was 33 ml/min per m² and was identical 5 d postoperatively. In patient 23, pre- and postoperative values were 38 and 35 ml/min per m², respectively.

PRL PR in the eight patients with pituitary tumors were elevated, and ranged from 6,400 to 81,500 μg/d per m² (mean 34,770±28,400 μg/d per m²). Two macroadenoma patients had PRL PR determinations before and after transsphenoidal hypophysectomy. In patient 22, PRL PR fell from 18,900 to 1,370 μg/d per m² and in patient 23, PRL PR fell from 8,800 to 957 μg/d per m². Thus, although surgery lowered the PRL PR, a normal PRL PR was not achieved immediately in either of these patients.

Dopamine infusion. Four subjects who had undergone a prior PRL MCR measurement (three normal and one with idiopathic hyperprolactinemia) were restudied during a 5-h infusion of dopamine (Table
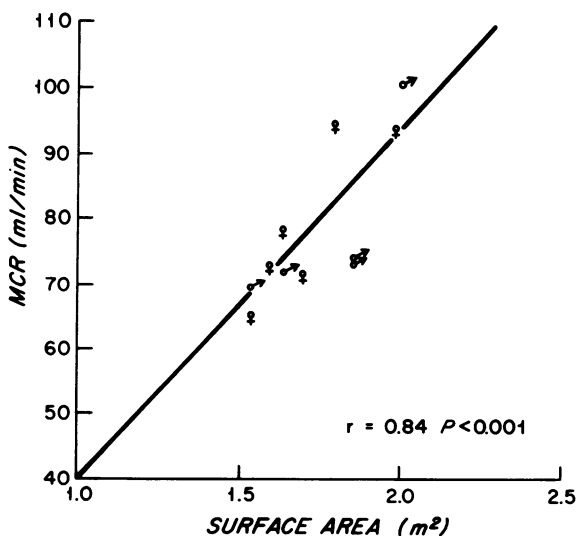
III). Although serum PRL declined markedly during the infusion in all cases, there was no substantial change in the PRL MCR as compared with base-line values (mean 47±6 vs. 45±5 ml/min per m²). In the normal subjects, serum PRL fell from a mean of 4.2 to a mean of 0.8 ng/ml with a concomitant fall in PRL PR from 279 to 66 µg/d per m² ($P < 0.001$). In the hyperprolactinemic patient, serum PRL declined from 25 to 4 ng/ml, reflecting a decrease in PRL PR from 1,500 to 240 µg/d per m².

Gel chromatography of labeled PRL. Gel filtration studies were performed because of the possibility that PRL was altered after infusion, forming iodinated species with molecular weights different from native or peak III PRL, yet that were immunoprecipitable with anti-PRL antisera. These species would then be included in the MCR calculations as "immunoprecipitable ^{125}I," and yet would not represent native PRL. These additional counts would, therefore, falsely lower the true PRL MCR for the reason that these counts appear in the denominator of the MCR equation (MCR = infusion rate ÷ circulating concentration).

Fig. 5A shows the elution profile of ^{125}I-PRL after iodination with lactoperoxidase (13). Three protein peaks (I, II, and III) were evident. Using excess anti-PRL antisera (1:100), 20–30% of peak I, 0% of peak II, and 65–90% of peak III counts were immunoprecipitable. Peak III (native PRL), when rechromatographed after incubation in buffer at 37°C for 4 h, was not transformed into either peak I or peak II material (Fig. 5B). However, when this material was incubated in fresh serum at 37°C for 4 h, a small amount of peak I material was formed (Fig. 5C).

Fig. 6A–C show typical gel filtration patterns of infusate and serum at 5 and 180 min during the infusion in one normal subject. The patterns were similar

TABLE II
Patients

Patient	Age	Sex	Surface area	T₄	T₃	TSH	Basal PRL	Peak PRL after TRH	PRL MCR	PRL MCR/m²	PRL PR	PRL PR/m²
			m²	µg/dl	ng/dl	µU/ml	ng/ml	ng/ml	ml/min	ml/min/m²	µg/d	µg/d/m²
Hyperthyroidism												
12	37	F	1.4	16.3	342	<0.4	6.0	13	65	46	562	401
13	20	F	1.55	17.4	445	<0.4	5.7	6.0	63	41	517	334
14	31	F	1.54	19.9	343	<0.4	4.5	5.8	103	63	667	445
15	54	F	1.57	14.7	594	<0.9	3.7	6.2	74	47	402	256
16	25	M	1.6	35	1500	<0.3	3.7	3.2	83	52	442	276
17	33	F	1.45	16	388	<0.3	3.4	7.4	88	61	431	297
Mean	33			19.9	602		4.5*	6.9	79	52‡	504	335§
±SD	±11			±7.1	±410		±1.1	±2.8	±14	±8	±91	±68
Hypothyroidism												
18	54	F	1.69	1.1	71	48	2.2	29	67	39	212	124
19	50	F	1.55	2.8	50	88	3.3	54	33	21	157	99
20	65	M	1.56	1.1	27	109	3.1	26	73	47	326	210
21	39	F	2.0	1.2	42	101	7.7	64	89	45	987	499
Mean	52			1.6	48	87	4.1	43	65	38	420	233
±SD	±9			±0.7	±16	±23	±2.1	±16	±20	±10	±332	±159
Hyperprolactinemia												
22 Pre-"	31	F	1.9	5.8	100	1.0	392	63	33	35,900	18,900	
Post-							28	63	33	2,600	1,370	
23 Pre-"	21	F	1.8	8.5	143	1.0	165	69	38	15,800	8,800	
Post-							19	62	35	1,720	957	
24	30	F	1.8	10.4	188	1.2	600	90	49	76,500	42,500	
25	20	F	1.6	6.4	122	2.7	165	58	36	15,300	8,530	
26	26¶	F	1.47	6.7	100	1.0	28	61	42	2,480	1,690	
27	25**	F	1.8	3.5	51	3.1	114	70	39	11,500	6,400	
28	29	F	1.55	7.9	123	1.0	1100	78	49	119,300	76,900	
29	55**	M	2.08	0.5	19	<0.3	1320	89	43	169,500	81,310	
30	30‡‡	M	2.35	14	184	<0.5	680	83	35	81,310	34,600	
Mean				7.1	114	1.4	509	70	40‡	58,620	31,100	
±SD				±3.6	±52	±0.8	±432	±11	±5	±54,170	±29,100	

* $P < 0.02$ compared with control.
‡ $P < 0.05$ compared with control.
§ $P < 0.01$ compared with control.
" Values before and after transsphenoidal hypophysectomy.
¶ Idiopathic hyperprolactinemia.
** Secondary hypothyroidism.
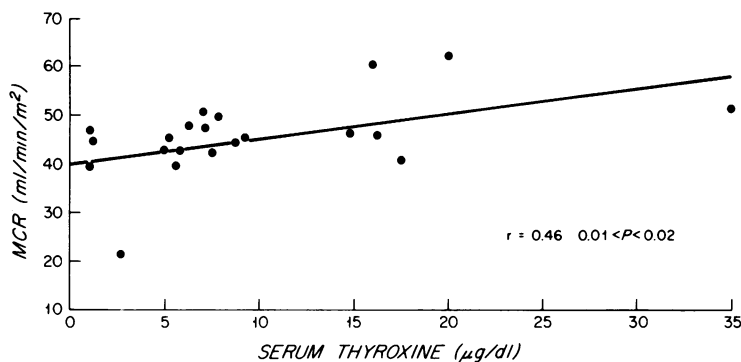‡‡ Patient taking L-thyroxine, 0.2 mg/d.

FIGURE 3   Positive correlation of PRL MCR with serum T_4 concentration ($r = 0.46$, $P < 0.02$).

for all the subjects studied. The infusate, which was prepared with only peak III material, characteristically contained a small amount of void volume (peak I) material ($\leq 10\%$), but the major form ($\geq 90\%$) was native (peak III) PRL (Fig. 6A). 5 min after starting the infusion, the amount of peak I material had increased to 20–30% of that total radioactivity, and a small amount of peak II PRL had appeared (Fig. 6B). After 180 min, a peak corresponding to ^{125}I had appeared. At this time, the area under the curves of peak I, peak III, and ^{125}I were approximately equal. In addition, 10–20% of the radioactivity was peak II material (Fig. 6C). There was no apparent difference in elution pattern between those samples chromatographed at 4°C and at room temperature. After gel chromatography of infusate and serum, the eluted peaks were immunoprecipitated with the following results (expressed as percent total counts bound): peak I = 10–30%; peak II = 0%; peak III = 65–90%; and ^{125}I = 0%. Thus, the peaks of radioactivity that developed over time in the serum (Fig. 6C) had the same elution positions and

immunoprecipitation characteristics as peaks that naturally occurred in the course of iodination with lactoperoxidase (Fig. 5A). Because the amount of radioactivity in serum was too low to be analyzed further, peaks I, II, and III from radioiodination were studied by SDS-PAGE. For these studies, it was assumed, but not proven, that peaks I, II, and III that arose during the infusion were qualitatively similar to peaks obtained during iodination.

SDS-PAGE of labeled PRL (Fig. 7A–C). After denaturing PAGE, >90% of labeled peak I was converted to peak III (Fig. 7B). There was no difference in the pattern when the sample was incubated in 1% β-mercaptoethanol. Before SDS-PAGE, peak I counts were 30% immunoprecipitable, but counts eluted from gel slices after SDS-PAGE (i.e., "peak III"), were 72% immunoprecipitable, similar to the immunoprecipitability of native peak III seen in Fig. 7A. After SDS electrophoresis of peak II ^{125}I-PRL, 30% of the counts migrated in the peak III position but were only 20% immunoprecipitable. The majority of peak II material repeatedly resolved into two peaks (IIa and IIb) after PAGE (Fig. 7C). Furthermore, peaks IIa and IIb from peak II samples that were incubated in 1% β-mercaptoethanol were slightly retarded compared with those resulting from peak II incubated in SDS alone. β-mercaptoethanol incubation did not affect the gel position of PRL standard (Fig. 7A) or cytochrome c (data not shown).
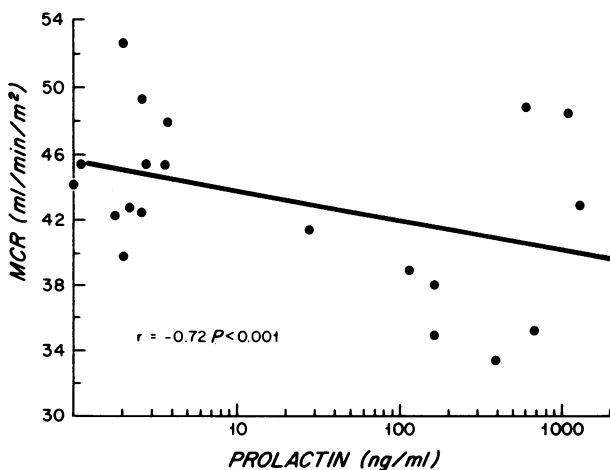
DISCUSSION

In this study, the constant infusion to equilibrium method of Tait (1) was employed to calculate the MCR of PRL in humans. Values for men and women were similar, and there was a highly significant correlation with body surface area. The MCR values obtained are virtually identical to the 77.5±4.7 ml/min calculated by Davis and Borger (11) for ewes weighing 48–71 kg. A positive correlation between MCR and body surface area has been noted for other pituitary hormones (4, 8, 9). The mean daily calculated PRL

FIGURE 4   Negative correlation of PRL MCR with logarithm of serum PRL concentration ($r = -0.72$, $P < 0.001$).

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TABLE III
Dopamine Infusion

Subject[*]	Age	Sex	Surface area	PRL	PRL MCR	PRL MCR/m²	Control PRL MCR/m²
			m²	ng/ml	ml/min	ml/min/m²	
1	45	F	1.4	4.0	70	44	48
7	28	M	1.86	4.0	95	51	40
8	25	M	2.04	5.4	84	43	46
26	26	F	1.47	25	72	49	41
Mean±SD						47±6	45±5

[*] Subject number from Tables I and II.

PR of 349 μg/d is similar to the data of Wartofsky et al. (16) who calculated a daily PRL PR of 197 μg/d using an indirect method. Because the pituitary PRL content is normally only 100–200 μg (17), it follows that the daily pituitary turnover of PRL is extremely rapid. Although only a fraction of the pituitary content of GH (17) or TSH (18) is secreted per day (6, 9), kinetic data similar to our own for LH have been obtained by Kohler et al. (6), who estimated that the daily pituitary LH turnover is once or twice per day.

The PRL PR that we have reported in this study should be viewed somewhat cautiously. A change in serum levels from basal to twice basal (i.e., from 2 to 4 ng/ml) would be reflected by a doubling of the calculated production rate, because PR = circulating concentration × MCR. For PRL, GH, and other hormones whose levels are not static, the only "true" daily PR data would be those derived from integrated serum concentrations over a 24-h period (5). In our studies, the PRL PR was calculated on the basis of one single serum PRL sample, drawn between 8:00 and 10:00 a.m. Nevertheless, these results are similar to those obtained when a mean daily serum PRL concentration, derived from 24-h secretory profiles, was used in the calculations. In subject 8, the PRL PR calculated on the basis of a single PRL value was 212 μg/d per m², although it was 227 μg/d per m² when the mean 24-h serum PRL concentration was used in the calculations. In subject 1, the change in calculated PRL PR from 318 to 325 μg/d per m² was even more trivial. Similar calculations have been made for the PR of GH in females, calculated either on the basis of a pooled 4-h sample (0.81 mg/d) (4) vs. an integrated 24-h serum concentration (0.85 mg/d) (5). Thus, the true daily PRL PR is probably slightly higher than the PR that we have reported, and this difference is probably related to the sleep-entrained rise in serum PRL levels (19).

Our results indicate that although PRL MCR was elevated in hyperthyroidism, it was not significantly different from normal in hypothyroidism. This is consistent with previous work showing variable effects of thyroid status on the MCR of other pituitary hormones (2, 8, 9). The severity and duration of the underlying thyroid disease are important, as illustrated by the significant correlation between serum T_4 levels and PRL MCR (Fig. 3). Davis and Borger (20) found a significant decrease in PRL MCR in the thyroidectomized lamb, and it is possible that our results would be comparable to theirs had we studied more patients and performed serial measurements in the same patient. Additionally, the hypothyroid group was older than the control group, a fact that could have contributed to the minor decrease in the MCR of these patients.

When compared with normal controls, the six hyperthyroid patients had a slight, but statistically significant, increase in mean PRL MCR, as well as a significantly higher mean basal PRL level. The calculated daily PRL PR was therefore higher than normal, in large part because of the higher basal PRL levels rather than the minor elevations in PRL MCR.

Our finding of increased PRL PR in hyperthyroidism is contrary to the commonly held notion that thyroid hormone administration results in decreased PRL secretion (in parallel to its effects on thyrotropin secretion). Although in vitro studies have shown that PRL secretion by pituitary cells is decreased after exposure to thyroid hormone (21–23), doses of T_4 in one study (21) were clearly supraphysiologic (1–10 μM), and other studies, which have used rat pituitary tumor cell lines (GH_1 and GH_3), have employed T_3, but not T_4, for testing (22, 23). In vivo, it has been shown that PRL levels do not change significantly after acute T_4 administration (24). However, in studies of PRL dynamics in chronic hyperthyroidism, normal or even slightly elevated basal PRL levels have been observed in many patients, although the data have not been emphasized or presented in detail (25–28). In three reports (25–27), basal levels of PRL in hyperthyroid patients were equal to or higher than control, but the differences were not statistically significant. In a fourth study (28), the mean basal PRL of 12±6.9 ng/ml was significantly higher than control values 4.3±4.9 ng/ml
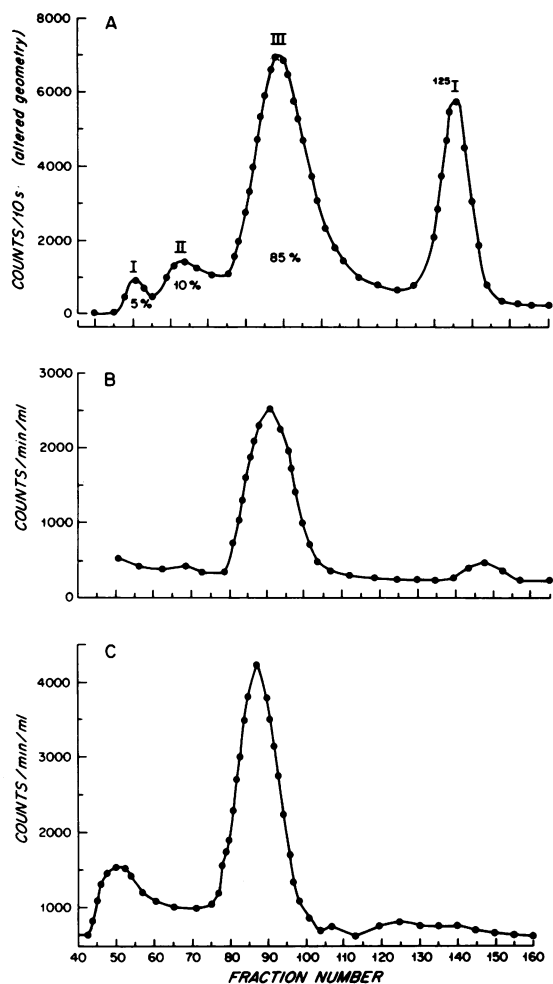
FIGURE 5 (A) Sephadex G-100 chromatograph (1.5 × 90-cm column) of a typical iodination of PRL by lactoperoxidase (13). 1-ml fractions were collected and counted. V_0 (void volume) = 45 ml, V_t (total volume) = 140 ml. Three protein peaks are evident: peak I (void volume, Kav (partition coefficient) = 0), peak II (molecular weight ≅ 44,000, Kav = 0.2), and peak III (molecular weight ≅ 23,000, Kav = 0.4, native PRL). (B) Sephadex G-100 chromatograph of peak III (~20,000 cpm) PRL incubated in 0.05 M $NaPO_4$ buffer, pH = 7.4, for 4 h at 37°C. There is no conversion of peak III to peak I. (C) Sephadex G-100 chromatograph of peak III (~20,000 cpm) PRL incubated in fresh human serum for 4 h at 37°C. There is some conversion of peak III to peak I, but there is no spontaneous deiodination.

FIGURE 6 (A) Sephadex G-100 chromatograph of infusate. Approximately 20,000 cpm were applied to 1.5 × 90-cm column. Peak III (native) ^{125}I-PRL is the predominant species. (B) Sephadex G-100 chromatograph of 4 ml of serum drawn 5 min after infusion began. Both peak I and II material have increased. (C) Sephadex G-100 chromatograph of 4 ml of serum drawn 180 min after infusion began. A striking increase in peaks I and ^{125}I are noted.

($P < 0.05$). In addition, elevation of basal serum PRL levels has been reported in hyperthyroid rats (29).

Several recent studies have shown that hyperthyroid patients have elevated serum levels of total and free estradiol (30, 31). Because estrogens are potent stimulators of PRL secretion in vivo (32) and in vitro (33), our observations of increased PRL PR in hyperthyroidism may be attributable to hyperestrogenemia. Elevation of GH PR has been observed in hyper-
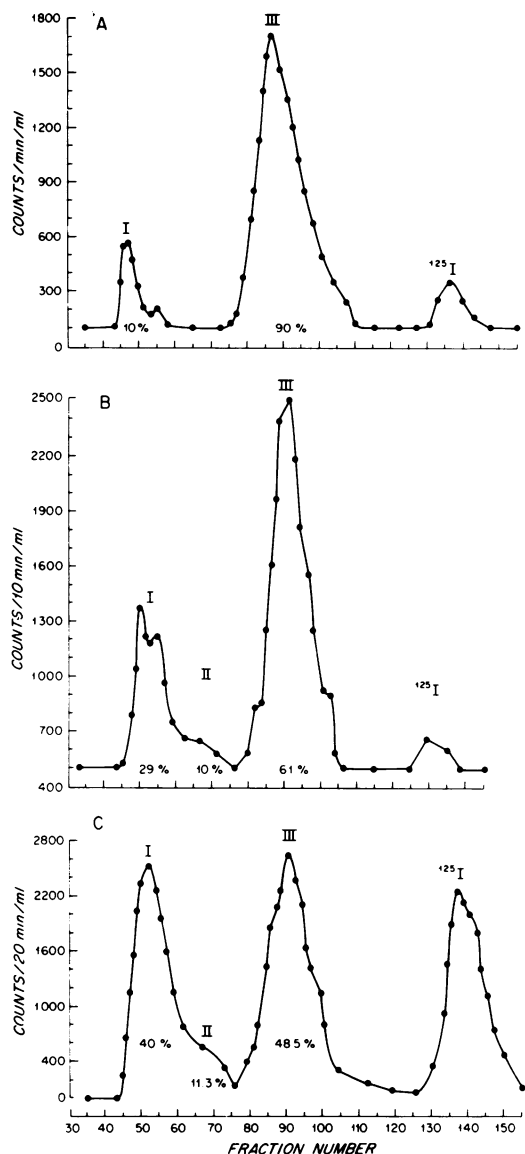
thyroidism (2) and could possibly be mediated in part by a similar mechanism. Although the data are clearly statistically significant, the number of patients studied is small. Further in vivo and in vitro studies are necessary before this phenomenon can be fully understood at the cellular level.
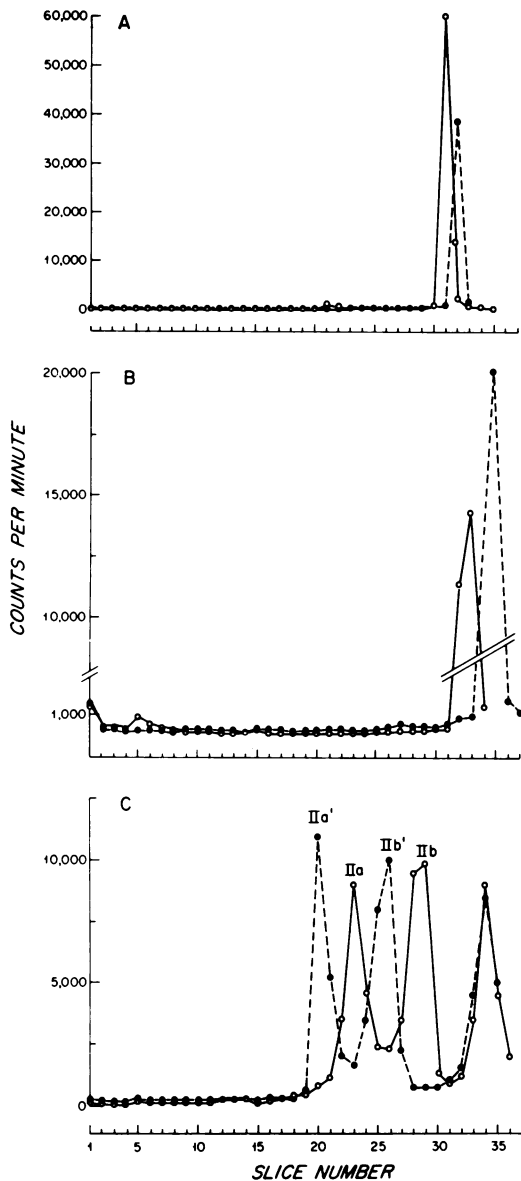
In contrast to the increased basal PRL PR in hyper-

FIGURE 7 Denaturing SDS-PAGE of peak III (A), peak I (B), and peak II (C) labeled PRL with (– – –) or without (———) 1% β-mercaptoethanol. Peak III is unaltered after PAGE (A). Peak I (B) is completely converted to peak III by SDS alone. Immunoprecipitability of peak I counts increased from 30 to 72% after SDS-PAGE. Peak II (C) is composed of material that migrates in the peak III position, as well as two other peaks (IIa and IIb), which are more complex.

thyroidism, our data show that peak PRL after TRH is decreased significantly in hyperthyroid patients. This observation correlates with in vivo (25) and in vitro (23) studies showing suppressive effects of thyroid hormone on TRH-mediated PRL secretion. These effects may be explained by an absolute decrease in TRH receptor number as a result of exposure to

thyroid hormone (23). Thus, it is possible that thyroid hormones have a dual effect, indirectly or directly increasing basal PRL secretion, while simultaneously decreasing the PRL response to TRH stimulation. These two effects are not mutually exclusive, and may well be exerted at separate loci.

In hypothyroidism, basal PRL PR was found to be higher than controls, but the difference was not statistically significant. The mean PR of 420±332 μg/d approximates the 307 μg/d calculated by Wartofsky et al. (16), using an indirect method. This is consistent with reports of basal elevations of PRL in some, but not all, hypothyroid patients (34). The duration and severity of the hypothyroid state is a likely explanation for the variability in the reported results (35). Peak PRL after TRH was markedly elevated in our patients, in keeping with previously published data (25).

The PRL MCR in hyperprolactinemic patients was significantly lower than control subjects. Saturation of PRL metabolic pathways by high hormone concentrations is one possible mechanism that would explain our findings. Although GH MCR in acromegalic patients was found to be no different from normal by two groups (2, 4), it was lower than controls in three patients studied by Thompson et al. (5). A recent study in the rat demonstrated that in vivo binding of ^{125}I-GH to putative hepatic GH receptors was decreased by simultaneous administration of unlabeled GH (36).

Dopamine and dopaminergic agonists have been demonstrated to inhibit PRL release from the pituitary gland, in vitro (37) and in vivo (38). However, none of the in vivo studies has determined whether an increased PRL MCR could be partly responsible for the decline in serum PRL concentration, although this has been postulated by one group of workers (39). Certainly the augmented renal and splanchnic blood flow produced by dopamine (40) could increase the metabolism of many substances. Indeed, an increase in GH MCR has been noted in acromegalics treated with the dopaminergic agonist bromocriptine (41). However, the data from our four subjects who underwent dopamine infusions did not support this hypothesis. The mean PRL MCR, before and during dopamine were essentially the same, whereas PRL serum concentrations and PRL PR declined dramatically, confirming the potent effect of dopamine as an inhibitor of pituitary PRL release.

PRL (42), GH (43), and placental lactogen (44) are known to occur in heterogeneous forms. For PRL, heterogeneity has been found in the circulation (44, 45), in cerebrospinal fluid (46), in amniotic fluid (47), as well as in media from pituitary glands cultured in vitro (48). These forms have gel elution profiles on Sephadex G-100 that are characteristic: (a) "Big-

Big" (peak I) PRL, which elutes in the void volume and comprises ~0–5% of total PRL immunoactivity, (b) "Big" (peak II, Kav = 0.2) PRL, which has a molecular weight of ~44,000 and represents 10–20% of immunoactive PRL, and (c) native PRL (peak III, Kav = 0.4), which accounts for 80–90% of the total immunoactive PRL. The immunologic and biologic potencies of these three species are virtually identical by radioimmunoassay and radioreceptor assay (48), although decreased receptor binding by peaks I and II has recently been reported (49). Although there are differences in the relative amounts of the three PRL species under various clinical circumstances, the precise biological significance of the heterogeneity of PRL remains to be elucidated.

Three species of PRL are also observed when PRL is iodinated with chloramine T or lactoperoxidase (50, 51). Although the three species have Kav that are identical to those seen with unlabeled PRL (50), they differ substantially from unlabeled peaks in their binding to anti-PRL antisera. Only 20–30% of peak I, essentially no peak II, and 70–90% of peak III counts are bound (50, 51).

In the present study, gel chromatography of infusate and serum ^{125}I-PRL yielded results that have a direct bearing on the interpretation of MCR data for PRL, and possibly for other polypeptide hormones as well. Infusate was almost completely homogeneous, consisting of ≤90% peak III material. However, within 5 min, peak I and II material had begun to accumulate, and at 180 min the area of peak I was almost as great as the area of peak III. These larger, new forms were products of clearance and should not have been included in the MCR determination of native, infused (peak III) PRL. However, MCR calculations rely on immunoprecipitable hormone. For the reason that 20–30% of all the immunoprecipitable counts were, in fact, peak I iodoprolactin, and because this peak accounted for 30–40% of the total protein counts at equilibrium, the immunoprecipitable counts that were a result purely of peak III were only 80% of that which was calculated. If, on the average, 20% of immunoprecipitable counts were the result of "contamination" with aggregation products, then a revised MCR would have been 20% greater than estimated above.

However, PRL exists as multiple forms in serum, and large species (peaks I and II) may comprise up to 40% of the total circulating immunoactive hormone (45). Because these forms display parallelism with authentic PRL (peak III) in the PRL radioimmunoassay (48), it must be concluded that these forms are measured routinely in determinations of serum PRL concentrations. If only native (peak III) PRL were considered in PR calculations, then serum concentrations would have to be reduced by up to 40%. This maneuver would counterbalance the corrected increase in PRL MCR, and the calculated PR value would remain essentially unchanged.

Similar data on the metabolic products of ^{125}I-bovine PRL and ^{125}I-GH have been reported by others (52, 53), who point out that these results are also compatible with the hypothesis that peak I hormone, contaminating the infusate, may have a very slow MCR, and thus appear to build up relatively higher levels in serum with time. That peak I is not an artifact of elution at 4°C (46) is supported by a similar profile obtained when serum was chromatographed at room temperature.

The molecular nature of the heterogeneous forms of labeled and unlabeled PRL is unclear. In the electrophoretic studies outlined above, we showed that labeled peak I PRL was almost totally converted to immunoactive peak III using denaturing conditions. This suggests that covalent linkages are not involved in the structure of this larger species. Approximately 30% of labeled peak II PRL migrated to the position of peak III using both nonreducing and reducing conditions, in agreement with the recently published data of Garnier et al. (49). However, the immunoprecipitability of this peak III material was greatly diminished, compared with native peak III. It can be inferred from these data that labeled peak I PRL is a noncovalently bound aggregate species, and although peak II also contains some peak III material, its nature is more complex.

It should be recalled that these studies were performed using peaks of iodoprolactin that were generated during iodination. Although each peak has a great deal of similarity with peaks isolated from serum with respect to molecular weight and immunogenicity, it is possible that the iodination peaks that we studied by electrophoresis represented entirely different molecules than those that arose in vivo.

The large ^{125}I peak seen in the 180-min elution profile (Fig. 6C) deserves comment. It was assumed, because ^{125}I-PRL and unlabeled PRL had similar rates of disappearance in the dog, that deiodination occurred on a molecule for molecule basis, pari passu with hormone clearance. Although this is probably correct in the case of the dog for the first 20 min, data beyond that time are lacking, as a result of undetectable levels of unlabeled hormone in the serum. It is, therefore, possible that deiodination occurred later, as a phenomenon independent of clearance. Because only radiolabeled hormone can be measured, a falsely high value for the MCR would be the result. We have attempted to rule out in vitro deiodination as a source of error by demonstrating that it did not occur when infusate was incubated in fresh human serum for 4 h at 37°C.

Validation of the constant infusion method requires

that equilibrium be reached and that labeled and unlabeled hormones be metabolized identically. The present study has examined in detail a third potential source of error: heterogeneous radioactive species that arise during infusion and that are partially immunoprecipitable. Whether these forms are true products of metabolism or nonspecific serum aggregations is unclear. Although corrections can be made to compensate for this phenomenon, such maneuvers do not elucidate the significance or physiological consequences of circulating hormonal pleomorphism.

Of great importance is the fact that high molecular weight immunoactive species normally exist in human serum, and, in some pathological states (PRL-secreting tumors), these larger forms may comprise a greater fraction of total PRL than in normal subjects (49, 54, 55). Because of the variability in circulating PRL heterogeneity, the PR that is calculated will not necessarily reflect the PR of the predominant circulating PRL species in any given individual.

It is evident from the preceding discussion that chromatographic analysis of iodinated serum species, with concomitant immunoprecipitation studies, should be an integral part of all MCR determinations performed with iodinated polypeptides. These studies are necessary to determine the extent to which "nonnative" forms of a hormone arise and immunoprecipitate, and the extent to which MCR and PR calculations are affected. In addition, all studies should include data that confirm not only identical disappearance rates for labeled and unlabeled hormones, but also data demonstrating the absence of deiodination of labeled hormone under in vitro conditions. These caveats apply to all hormones that display heterogeneity in serum, and it should be emphasized that MCR and PR data under these circumstances are at best only approximations of in vivo events.

ACKNOWLEDGMENTS

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