Alterations in Thyroid Hormone Economy

in Patients with Hydatidiform Mole

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A B S T R A C T Studies of several aspects of thyroid hormone economy have been conducted in 11 patients before and after removal of a molar pregnancy. Before evacuation of the mole, all patients demonstrated moderately to greatly elevated values for thyroidal ^{131}I uptake, absolute iodine uptake, and serum protein-bound-^{131}I. Values for serum PBI and serum thyroxine (T_4) concentration were consistently and often greatly increased, averaging more than twice those found in normal pregnancy and three times those in normal controls. On the other hand, the maximum binding capacity of the T_4-binding globulin (TBG) was variably affected, and ranged between the values found in normal controls and those found in normal pregnancy. Values for the absolute concentration of free T_4 in serum were, on the average, only moderately elevated, since the proportion of free T_4 was moderately low, although not as low as in normal pregnancy. Sera of patients with molar pregnancy contained high levels of thyroid stimulating activity, as assessed in the McKenzie mouse bioassay system. The stimulator displayed a more prolonged duration of action than that of TSH and did not reveal a major immunological cross-reactivity with either human or bovine TSH, differing in the latter respect from the chorionic thyrotropin of normal human placenta. Abnormalities in iodine metabolism were rapidly ameliorated after removal of the molar pregnancy, and this was

associated with the disappearance from serum of the thyroid stimulator.

Despite the foregoing evidence of thyroid hyperfunction and hypersecretion of T_4, patients with molar pregnancy were neither goitrous nor overtly thyrotoxic. They did display, however, high values of the urinary pigment/creatinine ratio, a possible indication of the presence of a hypermetabolic state.

It is concluded that in patients with molar pregnancy, thyroid function and T_4 secretion are stimulated, often greatly so, by an unusual thyroid stimulator which is demonstrable in the blood and which is probably of molar origin. The nature of the stimulator, as well as the reasons for both the variability of the increase in TBG which occurs in molar pregnancy and the lack of frank thyrotoxicosis, remain to be clarified.

INTRODUCTION

Hydatidiform mole is the most frequent tumor of trophoblastic tissue; its frequency, geographical distribution, and pathogenesis have recently been reviewed (1). Dowling, Ingbar, and Freinkel first called attention to the occurrency of striking abnormalities in several aspects of thyroid hormone economy in patients with molar pregnancy (2). They found marked increases in serum PBI, PB^{131}I, butanol-extractable ^{131}I, thyroidal ^{131}I uptake, and absolute iodine uptake (AIU)[1] in one or more of

[1] *Abbreviations used in this paper:* AIU, absolute iodine uptake; HCG, human chorionic gonadotrophin; P/C, urinary pigment/creatinine ratio; T_3, triiodothyronine; T_4, thyroxine; TBG, T_4-binding globulin; TBPA, thyroxine-binding prealbumin; TCA, trichloroacetic acid.

three patients studied. More recently, Kock, Kessel, Stolte, and Leusden confirmed the elevation of PBI in six patients, and found that the resin uptake of ^{131}I-labeled triiodothyronine (T_3), assessed in two patients, was in the same range as in normal pregnancy. An ^{131}I-tracer test performed in one patient indicated a very high thyroidal ^{131}I uptake, conversion ratio, and thyroidal ^{131}I-secretion rate (3).

In all of the foregoing studies, the patients with molar pregnancy were apparently not thyrotoxic. Therefore it seemed important to ascertain whether the iodinated materials being synthesized in the thyroids of seemingly euthyroid patients with molar pregnancy were in fact thyroxine (T_4), whether the increase in serum PBI represented an increase in T_4 or other iodinated materials, and to what extent, if any, the increase could be attributed to an increase in the activity of serum thyroxine-binding globulin (TBG), analogous to that which occurs in normal pregnancy (4, 5). The present report describes the findings obtained in 11 patients in whom several aspects of thyroid hormone economy were studied before and after termination of a molar pregnancy. As a possible index of metabolic state, measurements were also made of the urinary pigment/creatinine ratio (6–8). Studies of thyroid hormone concentration and binding in the serum of nine other patients are also presented.

METHODS

Patient material and general procedures. All patients were studied at the Hospital de Gineco Obstetricia, numero dos, Centro Medico Nacional, Mexico City. Control ob-

TABLE I
Clinical Data of Patients with Molar Pregnancy§

Patient	Age	Obstetric history		Date of LMP	Date of evacuation	Urinary HCG*
		G	P			
						IU/24 hr
4	23	4	2	5-10-67	10-03-67	1,024,000
8	22	4	3	6-10-67	9-28-67	1,024,000
10	24	4	3	4-15-67	10-06-67	1,024,000
12	33	4	3	7-17-67	10-24-67	512,000
14	30	12	8	6-17-67	11-10-67	512,000
17	32	7	6	9-09-67	12-27-67	512,000
25	17	1	0	12-28-67	3-27-68	512,000
27	26	5	4	1-12-69	4-10-68	1,024,000
29	23	2	0	12-17-68	5-11-68	1,024,000
32‡	35	11	9	3-09-68	5-28-68	1,024,000
34	22	2	1	12-27-68	6-11-68	1,024,000

* Urinary HCG levels in normal pregnancy averaged 20,000 IU/24 hr.
‡ Patient evacuated of both a fetus and molar tissue.
§ G, gravidity; P, parity; LMP, last menstrual period.

servations were made in members of the staff of this institution. Pertinent clinical data concerning the patients with molar pregnancy are presented in Table I. The diagnosis of molar pregnancy was based on clinical findings and on the demonstration of a high titer of human chorionic gonadotrophin (HCG) in the urine. In all patients studied, the diagnosis was ultimately confirmed at curettage. Generally, delivery was induced by means of oxytocin about 48 hr after the diagnosis was made, and completeness of delivery was ascertained by subsequent dilatation and curettage. During the 48-hr period before induction, the following routine was adopted. Each patient underwent physical examination for signs of thyroid abnormality. At 0 and 24 hr, blood was drawn, and the serum obtained was frozen for later use in the several analyses to be performed. A portion of urine was obtained and was analyzed immediately for both urinary pigment (urochrome) and creatinine. A tracer dose of ^{131}I was administered and its uptake in the thyroid measured 24 hr later. A concomitant urine collection was made for measurement of the 24-hr excretion of [131]I. Portions of these urine collections were stored frozen for subsequent measurement of stable iodine concentration. A portion of serum obtained 24 hr after ^{131}I administration was employed for determination of the serum PB[131]I concentration.

At 1 and 4 wk after delivery of the molar tissue, and in a few cases more frequently, additional blood and urine samples were obtained. The 24-hr thyroidal uptake of ^{131}I was repeated in four patients at 1 wk, and in six at 4 wk postdelivery.

The nonpregnant control group consisted of nine Mexican females of an age range and socioeconomic status similar to that of the patients. All denied taking any medication. The pregnant controls consisted of hospital outpatients with uncomplicated pregnancy, patients undergoing normal labor, and the same patients at 3 and 6 days *post partum.*

Analytical techniques. The PB[131]I concentration in serum was determined by a standard trichloroacetic acid (TCA) precipitation technique. 2 volumes of 10% TCA were mixed with 3 ml serum containing added stable iodide (100 μg/ml). The precipitate was centrifuged and washed three times with 2 volumes of 5% TCA. The washed precipitate was dissolved in 20% NaOH for counting and its radioactivity was compared with that in a portion of the administered dose. Except where otherwise specified, the percentage of free T_4 in serum was determined by the equilibrium dialysis method of Ingbar, Braverman, Dawber, and Lee (9). Serum PBI was measured in duplicate in the authors' laboratories by an auto-analyzer technique. For samples in which values in excess of 20 μg per 100 ml were found, analyses were repeated using a 2-fold dilution of serum. Serum T_4 concentration was measured by the radioligand–binding displacement method of Murphy and Pattee (10), and urinary iodine by the method of Benotti and Benotti (11).[2] The maximum binding capacities of serum TBG and thyroxine-binding prealbumin (TBPA) were assessed by reverse flow filter paper electrophoresis employing glycine-acetate buffer, pH 8.6 (12). Urinary pigment concentration was measured directly in centrifuged specimens at 420 mμ in a Coleman Junior Spectrophotometer (Coleman Instruments, Maywood, Ill.), and urinary creatinine concentration was measured at 495 mμ after addition of alkaline picrate reagent. Details of these methods and the calculation of the urinary pigment/

[2] Measurements were made in the Boston Medical Laboratory, Boston, Mass.

TABLE II
Thyroid Function in Patients with Molar Pregnancy

Patient	131I uptake			24 hr PB 131I		Urinary/ iodide excretion			Absolute iodine uptake		
	Predelivery	Postdelivery		Predelivery	Postde-livery	Predelivery	Postdelivery		Predelivery	Postdelivery	
		1 wk	>4 wk		>4 wk		1 wk	>4 wk		1 wk	>4 wk
	% dose/24 hr			*% dose/liter serum*		*µg/24 hr*			*µg I/24 hr*		
4	60	—	23	0.29	—	94		91	191	—	36
8	50	—	—	—	—	51		—	571	—	—
10	67	—	—	0.17	—	148		—	1478	—	—
12	83	—	34	0.23	0.067	40		177	1386	—	135
14	73	—	—	0.29	—	92		—	247	—	—
17	65	—	32	0.46	0.090	—		—	—	—	—
25	—	47	27	—	—	—	100	131	—	90	148
27	74	53	36	0.82	0.03	253	74	182	2144	1027	33
29	62	26	27	0.13	—	88	173	128	195	42	36
32	41	29	—	0.48	—	5250	2215	—	15960	764	—
34	56	—	—	—	—	288	—	—	328	—	—
Mean ±SE	63 ±3.9			0.36 ±0.079		700 ±569			2500 ±1698		

creatinine ratio are presented elsewhere (13). The uptake of ^{131}I by the thyroid was determined in the radioisotope laboratory of the Hospital General, Centro Medico Nacional, through the courtesy of Dr. A. Cuaron.

Values for the 24-hr absolute thyroidal iodine uptake (AIU) were calculated as the product of the daily urinary iodine excretion and the quotient of the thyroidal ^{131}I uptake and the 24-hr urinary ^{131}I excretion.

Human pituitary TSH was measured in unextracted serum by a specific radioimmunoassay, modified from that of Odell, Rayford, and Ross (14). The serum samples were also assayed for a placental thyrotropin in a radioimmunoassay for beef TSH using a double antibody technique described previously (15). A thyrotropic substance extracted from normal human placentas cross-reacts in this beef immunoassay (16). Bioassay for thyroid stimulating activity in unextracted serum samples was performed by a modification of the McKenzie mouse bioassay method (16). Each mouse was injected intravenously with 0.5 ml serum, 4–6 mice per sample. To ascertain whether the biological activity in serum could be neutralized by anti-TSH antibody, samples of serum were reacted for 18 hr at 2°C with an antibody to human pituitary TSH. The amount of antibody employed was that which produced complete neutralization of a quantity of human TSH comparable in biological activity to that of the serum sample.

Statistical analyses were conducted employing Student's *t* test, as described by Snedecor and Cochran (17).

RESULTS

Indices of thyroid function (Table II). For the laboratory in which the studies were done, the mean value for the 24-hr thyroidal uptake of ^{131}I in nonpregnant euthyroid women was 35 ±6.5% (mean ±SE), range 10–50%. In 10 patients with molar pregnancy studied before delivery, the 24-hr uptake of ^{131}I averaged 63 ±4.0% (range 41–83%), a highly significant increase ($P <$ 0.001). Values of 50% or more were observed in 8 of the 10 patients. The lowest value (41%) was seen in

patient 32 who may have been exposed to excess iodide, since urinary iodine excretion was greatly increased. In the seven patients studied after evacuation of the mole, ^{131}I uptake declined rapidly, reaching values within the normal range by the time of first postdelivery measurement (1–4 wk).

Values for the 24-hr urinary excretion of ^{131}I and ^{127}I were measured during the 24-hr period in which thyroidal ^{131}I uptake was being determined so that absolute rates of thyroidal iodine uptake could be calculated. One patient (No. 32) was found to have a greatly elevated value for urinary iodine (5250 µg/24 hr). That this was not due to external contamination is strongly indicated by the finding that the urinary iodine excretion 1 wk after delivery of the mole was 2215 µg/25 hr. If values in this patient were excluded, mean iodine excretion in the remaining eight patients was 125 µg/24 hr, ranging between 40 and 288 µg/24 hr. Except in patient 32, cited above, no consistent or pronounced change in urinary iodine excretion occurred after delivery of the mole.

The calculated value of the AIU in patient No. 32 was exceedingly high (15,960 µg/24 hr) as a result of persistence of a 41% thyroidal ^{131}I uptake in the face of an apparent great increase in urinary iodine excretion. Within 1 wk after delivery of the mole, AIU had decreased to 764 µg/24 hr. In the remaining eight patients, values for AIU averaged 817 µg/24 hr and ranged between 191 and 2144 µg.[3] In the five patients in whom appropriate observations were possible, delivery of the mole was followed within 1–4 wk by a striking reduction in thyroidal AIU.

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[3] Values for the normal AIU in Mexico City have not been determined; however, in the continental United States, reported average normal values have ranged between approximately 70 and 120 µg/day (2, 18, 19).

TABLE III
Concentration and Physical State of Thyroxine (T_4) in Serum in Normal and Molar Pregnancy (First Series)

Group or patient	Serum PBI	Serum T_4 Measured	Serum T_4 Calculated*	Serum free T_4 % total T_4	Serum free T_4 ng/100 ml‡	Maximum T_4-binding capacity TBG	Maximum T_4-binding capacity TBPA
	μg/100 ml	μg/100 ml		% total T_4	ng/100 ml‡	μg T_4/100 ml	
Molar Pregnancy (11)§							
4	14.5	17.5	22.3	0.020	4.5	35.2	213
8	22.5	—	34.6	0.062	21.4	23.0	207
10	20.8	20.0	32.0	0.032	10.2	32.0	244
12	20.0	27.0	30.8	0.039	12.0	32.3	246
14	12.7	—	19.4	0.036	7.0	23.0	200
17	18.0	—	27.7	0.046	12.7	30.0	249
25	22.0	—	33.8	—	—	17.5	250
27	36.0	46.0	55.4	0.044	24.4	17.0	206
29	23.0	34.0	35.4	0.018	6.4	27.0	198
32	17.6	—	27.1	0.050	13.5	27.5	150
34	22.0	—	33.8	0.044	14.9	42.5	200
Mean ±SE	20.8 ±1.8	29.9 ±5.2	32.0 ±2.8	0.039 ±0.004¶	12.7 ±2.0¶	27.9 ±2.3	215 ±9.2
Postdelivery 1 wk (7)							
Mean ±SE	8.5 ±1.0‖		13.1 ±1.5				
Postdelivery 4 wk (7)							
Mean ±SE	6.2 ±0.7‖		9.5 ±1.1	0.047 ±0.006	4.1 ±0.7	20.6 ±2.9‖	278 ±7.9‖
Normal pregnancy 1st trimester							
Mean ±SE	8.5 ±4‖(6)		13.1 ±0.6	0.038 ±0.007	5.2 ±1.2(4)	31.4±2.4(5)	232 ±12(5)
Normal pregnancy 2nd trimester							
Mean ±SE	8.7 ±0.5‖(16)		13.4 ±0.8	0.036 ±0.003¶	4.7 ±0.6(7)	36.7 ±2.7(16)	260 ±13¶(16)
Normal pregnancy 3rd trimester							
Mean ±SE	9.1 ±0.3‖(18)		14.0 ±0.5	0.034 ±0.001¶	4.8 ±0.3(7)	42.4 ±1.9‖(18)	253 ±16¶(11)
Normal pregnancy Term							
Mean ±SE	8.8 ±0.2‖(5)						
Postpartum 1 wk (5)							
Mean ±SE	8.1 ±0.2‖						
Nonpregnant controls							
Mean ±SE	6.0 ±0.2(9)		9.2 ±0.3	0.059 ±0.008	5.5 ±0.5(7)	18.8 ±0.7(9)	242 ±12(9)

* Calculated as PBI/0.65.
‡ Based on % total T_4 and calculated serum T_4 concentration.
§ Numbers in parentheses indicate number of patients.
‖ Significantly different from molar pregnancy preevacuation group, $P < 0.05$.
¶ Significantly different from nonpregnant group, $P < 0.05$.

Values of the PB^{131}I at 24 hr were above the upper limit of normal (0.1% dose/1) in all of the eight patients in whom this measurement was made before removal of the mole, averaging 0.37% dose/1 (range, 0.13–0.82). In the three patients studied after removal of the mole, values for PB^{131}I had undergone a striking decrease into the normal range by 4 wk postdelivery.

Thyroid hormone in the blood. Results of analyses for serum PBI are shown in Table III. The mean PBI in nonpregnant Mexican women was 6.0 ±0.2 μg/100 ml. The increase in serum PBI normally associated with uncomplicated pregnancy was evident in specimens obtained during all three trimesters (4, 5). This increase

was maintained for at least the first 6 days postpartum. In patients with molar pregnancy, the mean serum PBI concentration was 20.8 ±1.8 μg/100 ml, 2–3 times that found in normal pregnancy. After delivery of the mole, the PBI dropped rapidly during the first wk, and was normal within a month. T_4 concentration was measured in serum obtained from five patients. As shown in Table III, the serum T_4 concentration in all was greater than that expected in normal pregnancy (10), averaging 29 μg/100 ml (range 17.5–46.0). Measured values for serum T_4 agreed reasonably well with values calculated by dividing the PBI by 0.65 , the fractional mol wt of iodine in T_4. In this group of patients, serum T_4 concentrations

measured directly averaged 82% of values calculated from the PBI.

Values for the maximum T_4-binding capacity of serum TBG in nonpregnant Mexican women (18.8 ±0.7 μg T_4/100 ml) were in the range reported by others for American women, and the usual marked increase in T_4-binding capacity associated with normal pregnancy was observed (Table III) (4, 5). In patients with molar pregnancy, an increase in the T_4-binding capacity of TBG was often, although not invariably, observed, but only occasionally to the level seen in normal pregnancy. Values ranged between 17–43 μg T_4/100 ml serum. In individual patients with molar pregnancy, no correlation was observed between the degree of increase in the PBI and the maximum T_4-binding capacity of TBG. Normal values were restored by 4 wk after delivery of the mole. The T_4-binding capacity of TBPA in the serum of patients with normal pregnancy was not significantly different from that obtained by the same technique in normal nonpregnant controls (242 ±12 μg/100 ml). Values in molar pregnancy (215 ±9.2 μg/100 ml), however, were significantly lower than those found in normal pregnancy ($P < 0.05$). Within a month after evacuation of the mole, the T_4-binding capacity of TBPA was somewhat higher than normal.

Radioautographs of paper electrophoretograms obtained during analyses for TBG and TBPA revealed no binding of T_4 in zones associated with other proteins, aside from albumin.

The percentage and absolute concentrations of free T_4 in the serum of patients with normal and molar pregnancy are also shown in Table III. In accord with earlier observations, the percentage of free T_4 in serum was significantly decreased during normal pregnancy (9, 20). The absolute concentration of free T_4 in serum, however, was only slightly, and not significantly, lower than normal. In molar pregnancy, the mean percentage of free T_4 (0.039 ±0.004) was reduced to about the same degree as in normal pregnancy. However, owing to the very marked increase in serum PBI, the absolute concentration of free T_4 in the serum of patients with molar pregnancy (12.7 ±2.0 ng/100 ml) was significantly higher than normal (5.5 ±0.5 ng/100 ml, $P < 0.05$). In patients with molar pregnancy, values for the per cent and absolute concentration of free T_4 had returned toward or into the normal range by 4 wk after delivery.

Concern was felt about the accuracy of some of these measurements, in particular the free T_4 analyses, since the equipment used in Mexico was not entirely adequate and strict regulation of the bath temperature during the equilibrium dialysis was difficult to attain. Furthermore, samples of serum were frozen for varying lengths of time before shipment to the United States for determi-

nation of PBI and serum T_4; in one instance considerable delay in delivery was encountered. Thus, it seemed possible that deterioration of the samples may have influenced the data. Therefore, samples of serum were obtained subsequently from nine additional cases of molar pregnancy admitted to the same hospital in Mexico (series 2). These were frozen until shipment within a month to the United States, where free T_4 analyses were performed under well standardized conditions. The concentrations of PBI and T_4 in serum and the maximum T_4-binding capacity of TBG were also measured. For comparison, similar studies were performed on samples of serum from eight nonpregnant women and eight women with normal pregnancies. The results obtained are shown in Table IV. Values obtained for serum PBI and the T_4-binding capacity of TBG were very similar to those obtained in the original series of samples (Table III). In this group of specimens, however, a closer agreement was noted between measured values of the serum T_4 concentration and those calculated from measurements of the serum PBI, the former averaging 95% of the latter. Again, in patients with molar pregnancy, values for the serum PBI (19.2 ±2.0 μg/100 ml) were approximately twice those found in patients with normal pregnancy (7.9 ±0.4 μg/100 ml). In this group of specimens, the percentage of free T_4 was measured by the dilution method of Oppenheimer, Squef, Surks, and Haver (20), and values were generally lower than those obtained by the previous technique. Normal values for the percentage of free T_4 averaged 0.028 ±0.001. A significant decrease was found in sera obtained from patients with normal pregnancy (0.021 ±0.001). Values in patients with molar pregnancy varied widely, the mean (0.024 ±0.0018) falling between that obtained in nonpregnant controls and that obtained in normal pregnancy. As a result of the very high values for serum PBI (and serum T_4), calculated values for the absolute concentration of free T_4 averaged more than 3 times the average of values found in normal controls and more than twice the average of those found in normal pregnancy.

Urinary pigment/creatinine ratio. Values of the urinary pigment/creatinine (P/C) ratio were approximately twice normal in patients with uncomplicated pregnancy. In molar pregnancy, the ratio was, in turn, increased to values more than twice those found in normal pregnancy (Table V). The ratio fell rapidly during the 1st wk after evacuation of the mole, and by 4 wk was not significantly different from normal.

Thyroid stimulator in serum. Bioassay according to a modified McKenzie technique revealed the presence of high concentrations of a thyroid stimulator in the sera of all patients examined before evacuation of the mole (Table VI). There was no detectable thyrotropic

TABLE IV

Concentration and Physical State of Thyroxine (T_4) in Serum in Normal and Molar Pregnancy (Second Series)

Group or patient	Serum PBI	Serum T₄		Serum free T₄		Maximum T₄-binding capacity of TBG
		Measured	Calculated*			
	μg/100 ml	μg/100 ml		% total T₄	ng/100 ml‡	μg T₄/100 ml
Molar pregnancy (9)§						
1	28.8	39.0	44.3	0.020	8.8	24.5
2	16.4	24.0	25.2	0.024	5.9	19.7
3	25.6	42.0	39.4	0.022	8.7	30.0
4	16.4	21.0	25.2	0.029	7.1	21.0
5	12.0	13.5	18.5	0.016	2.9	29.2
6	17.6	25.0	27.1	0.023	6.2	19.6
7	12.8	16.5	19.7	0.019	3.7	29.7
8	24.8	45.0	38.2	0.032	12.3	27.5
9	18.4	30.0	28.3	0.030	8.6	26.0
Mean ±SE	19.2 ±2.0‖	28.9 ±3.8	29.5 ±3.0‖	0.024 ±0.002	7.1 ±1.0‖	25.2 ±1.4¶
Normal pregnancy (8)						
Mean ±SE	7.9 ±0.4		12.4 ±0.6	0.021 ±0.001	2.6 ±0.2	34.2 ±1.2
Nonpregnant controls (8)						
Mean ±SE	5.1 ±0.2		7.9 ±0.3	0.028 ±0.001	2.1 ±0.1	18.1 ±1.0

* Calculated as PBI/0.65

‡ Based on per cent total T₄ and calculated serum T₄ concentration.

§ Numbers in parentheses indicate number of patients in group.

‖ Significantly different from both pregnant and nonpregnant groups $P < 0.001$.

¶ Significantly different from pregnant group, $P < 0.001$, and from nonpregnant group, $P < 0.005$.

activity in bioassays of unconcentrated serum from 15 American and 3 Mexican women with normal pregnancy. In the assay, blood was obtained from test animals at 2 and 8 hr after administration of the test serum. TSH potencies reported in Table VI are based upon the response at 2 hr relative to the International Bovine Thyrotropin Standard because the slope of the thyroid

TABLE V

Urinary $\dfrac{Pigment}{Creatinine}$ Ratio in Normal and Molar Pregnancy

Group	No. of patients	$\dfrac{Pigment}{Creatinine} \times 100$
Nonpregnant	12	153 ±11*
Pregnant		
12–18 wk	10	219 ±10
20–34 wk	10	263 ±22‡
36 wk	17	330 ±17‡
Molar pregnancy	9	571 ±140§
Postdelivery (1 wk)	5	294 ±32
Postdelivery (4 wk)	4	208 ±33

* Mean ±SE.

‡ Significantly different from nonpregnant group.

§ Significantly different from mid- and late-pregnant groups.

stimulator in the serum of patients with hydatidiform mole was parallel to that of the bovine standard (21). The dose-response curve of the International Human TSH Standard is much flatter than that for bovine TSH in this bioassay (16) and would have given higher values than those reported had responses in these sera been related to the human standard curve. In specimens obtained before delivery from 6 patients in the initial group of 11, thyrotropic activities were greatly elevated and ranged between 110 and 500 μU/ml. In sera obtained as soon as 2 days after evacuation of the mole, no thyroid stimulator could be detected in the bioassay. The thyroid stimulator present in preevacuation sera differed from TSH in that a greater response was evident at 8 hr than at 2 hr. The ratio of responses at 8 and 2 hr ranged between 0.91 and 1.44, suggesting a longer duration of activity than that of pituitary TSH, for which the 2 hr-response regularly exceeds the 8-hr response (15). The thyroid stimulating activity in the predelivery serum of patient 27 was unaffected by prior incubation with antibody to human TSH (Table VII).

Radioimmunoassays for human TSH revealed values within the normal range in specimens obtained both before and after delivery of the mole (Table VI). Unlike the thyroid stimulating material that has been extracted from normal human placentas (15), the thy-

TABLE VI

Bioassay and Immunoassay for Thyroid Stimulator in Serum Samples from Patients with Molar Pregnancy before and after Delivery

Patient No.	14 Pre	14 Post	17 Pre	17 Post	25 Pre	25 Post	27 Pre	27 Post	29 Pre	29 Post	32 Pre	32 Post	34 Pre	34 Post
Interval from day of delivery (day)	−4	22	−20	2	—	−4	−0.5	3	—1	3	−0.5	4.5	−4	—
r, 2 hr*	3.90	1.25	1.40	0.95	—	1.17	3.58	1.12	3.75	1.01	2.55	0.99	3.79	—
±SE	±0.72	±0.12	±0.12		—	±0.24	±0.45	±0.11	±0.72	±0.07	±0.40	±0.06	±1.03	—
r, 8 hr	5.61	1.14	1.86	1.35	—	1.16	4.32	1.43	4.45	1.12	3.26	1.09	3.50	—
±SE	±1.10	±0.04	±0.36		—	±0.35	±0.36	±0.22	±0.88	±0.33	±0.54	±0.20	±0.37	—
8 hr/r 2 hr	1.44	0.91	1.33	1.42	—	0.99	1.21	1.28	1.19	1.11	1.28	1.10	0.92	—
Thyroid stimulator bioassay (μU/ml)	500‡	U§	110	U	—	U	430	U	460	U	260	U	470	—
Immunoassay H-TSH‖ (μU/ml)	11.6	7.2	8.8	—	—	9.3	9.3	8.1	11.5	8.0	5.6	6.7	5.8	—

* Blood ^{125}I at 2 hr or 8 hr after injection of sample into mice, divided by blood ^{125}I before injection. For controls injected with buffer, r was 1.09 ±0.07 at 2 hr and 1.31 ±0.10 at 8 hr.
‡ Based on the 2-hr response to the International Bovine Thyrotropin Standard.
§ Too low to be measured.
‖ Normal range is <1.8–12 μU/ml.

roid stimulator present in the sera of patients with molar pregnancy did not cross-react in the radioimmunoassay for bovine TSH. Studies performed in the second group of nine sera revealed the presence of high titers of a thyroid stimulator similar in the temporal characteristics of its action to that found in the earlier group. Positive responses were obtained in sera from seven of nine patients. The two patients in whose sera the stimulator could not be detected were those in whom the PBI was the least increased. Very high titers of stimulator in patients 2, 3, and 8 (2000, 1200, and 1300 μU/ml, respectively) permitted dilution of the sera for use in four point bioassays. These assays confirmed that the slope of this thyroid stimulator was parallel to that of the bovine standard.

DISCUSSION

Although previous reports have demonstrated the occurrence of thyroid hyperfunction in patients with molar pregnancy, the number of patients examined was few, and the tests performed were scattered. Hence, it was not possible to conclude whether the thyroid hyperfunction and the associated increase in serum PBI which had been described truly represented hypersecretion of thyroid hormone, particularly since clinical evidence of thyrotoxicosis was lacking. The present study comprises the largest number of observations in the largest number of patients with this disorder yet reported. While the study clarifies certain questions raised by earlier reports, others remain unanswered, and new questions have been raised.

The present observations in 11 patients studied before delivery of the mole have demonstrated consistent and marked thyroidal hyperfunction, as evidenced by greatly elevated values of the thyroidal ^{131}I uptake. That such hyperfunction was not the result of iodine deficiency was indicated by the finding of essentially normal, or occasionally increased, values for the daily urine iodine excretion, and ultimately by the demonstration that daily absolute iodine accumulation in the thyroid was increased, often greatly so. Values for thyroidal ^{131}I uptake were well into the range found in patients with thyrotoxicosis, and those for AIU were unusually high, even for patients with thyrotoxicosis (18, 19). More-

TABLE VII

Failure of Neutralization of the Bioassay Response to the Molar Thyroid Stimulator by Antibody to Human TSH

Substance	Response ratio ±SE* 2 hr	Response ratio ±SE* 8 hr
Control (buffer)	1.10 ±0.08	1.36 ±0.19
Human TSH standard 0.21 mU	2.75 ±0.45	1.92 ±0.22
Human TSH standard 0.21 mU + anti H-TSH	1.01 ±0.07	1.03 ±0.08
Serum of patient 27, 0.25 ml	2.48 ±0.31	2.64 ±0.39
Serum of patient 27, 0.25 ml + anti H-TSH‡	2.39 ±0.45	2.69 ±0.56

* Blood ^{125}I at 2 hr or 8 hr after injection of sample into mice, divided by blood ^{125}I before injection.
‡ Sample was incubated with 0.1 volume 1/100 antibody to human TSH for 18 hr at 2°C before assay. Samples without antibody were diluted in same way.

over, the consistent occurrence of abnormally high values for the $PB^{131}I$ concentration in serum provides evidence that the increased quantities of iodine accumulated by the thyroid were being rapidly secreted in an organically-bound form. Finally, analyses of serum by the highly specific technique of radioligand binding displacement indicated that most, if not virtually all, of the protein-bound iodine in the serum was comprised of T_4. Taken together, the foregoing findings provide strong evidence that in patients with molar pregnancy there occurs a pronounced increase in the secretion of T_4.

Several of the changes in certain aspects of thyroid hormone economy which occur in molar pregnancy resemble superficially those seen in normal pregnancy. These include the increase in thyroidal ^{131}I uptake and serum $PB^{131}I$ (22–25); in patients with molar pregnancy, however, these indices tended to be more markedly increased. Of greater importance is the fact that in normal pregnancy AIU is increased little if at all (25), and T_4 secretory rate, as judged from peripheral T_4 disposal, is normal (26). In the present patients with molar pregnancy, AIU was consistently increased, often well into the range found in thyrotoxicosis, while evidence for hypersecretion of T_4 is present, as cited immediately above. Finally, values for serum PBI and T_4 concentration in molar pregnancy were usually much greater than in normal pregnancy.

The far higher values for serum PBI and T_4 in molar than in normal pregnancy cannot be explained by a greater increase in T_4-binding by TBG in the former state. Indeed, on the average, values for the maximum binding capacity of TBG were lower than in normal pregnancy, although somewhat higher than in the non-pregnant female. Moreover, among patients with molar pregnancy, no correlation between values of the serum PBI and the T_4-binding capacity of TBG was evident. Values for the latter varied widely, some falling within the normal range and others within the range found in normal pregnancy. In these patients measurements of urinary estrogen excretion just before induction of labor, published in detail elsewhere (27), revealed no correlation between the binding capacity of TBG at that time and the excretion of either total estrogens or estrone and estradiol, which are far more potent than estriol in inducing an increase of TBG (28).

In view of the variability in the T_4-binding capacity of TBG and the evidence that the increased serum T_4 concentration was at least partly the result of enhanced secretion of the hormone, and not solely secondary to an increase in TBG, it is not surprising that no clear correlations were evident among the T_4-binding capacity of TBG, the T_4 concentration, and the proportion of free T_4 in the serum. Over the range which obtains in a variety of primary disorders of either thyroid function or hormonal binding, the proportion of free T_4 in serum is inversely correlated with the concentration of unsaturated binding sites on TBG (29). Such correlations were difficult to seek in the present series of patients, because in the majority of instances the total serum T_4 concentration exceeded the maximum concentration of T_4-binding sites on TBG, often greatly. In such cases, it would be expected that the proportion of free T_4 would be substantially increased, and it seems anomalous that such was not the case; values in such cases being increased only moderately, if at all. Previous studies have suggested, however, that the extent of increase in the proportion of free T_4 that occurs when binding sites on TBG are saturated depends upon the availability of binding sites on TBPA (9, 30, 31). Thus, for the group of patients with molar pregnancy as a whole, the relatively low values for the proportion of free T_4 in serum, despite very high values of the serum T_4 concentration, may have resulted from the modest increase in the T_4-binding capacity of TBG and the presence of nearly normal values for the binding capacity of TBPA.

With few exceptions (15, 32–34), previously reported cases of trophoblastic tumor which demonstrated thyroid hyperfunction or marked increases in serum PBI were peculiar in that they failed to display signs or symptoms of frank thyrotoxicosis. A similar paradox was evident in the present series of patients, who appeared clinically euthyroid and yet presented multiple lines of evidence suggesting hypersecretion of T_4. The presence of apparently normal concentrations of TSH in serum before evacuation of the mole does not, as might be expected, provide conclusive evidence against the presence of thyrotoxicosis in view of difficulty in distinguishing between normal and subnormal levels of TSH, and the possibility that any TSH actually detected might represent a weak cross-reaction between high concentrations of the molar thyroid stimulator and human TSH (21, 32, 34). Unfortunately, conventional indices of peripheral metabolic state, such as basal metabolic rate, could not be measured. Since random urine collections could be obtained, however, measurements of the urinary P/C ratio could be made. The pronounced increase in this ratio which occurred in patients with molar pregnancy would suggest that the patients were indeed hypermetabolic, since the ratio has been shown to increase in association with hypermetabolism of either thyroidal or extrathyroidal origin (6–8). Nevertheless, so little is known of other factors which may influence this ratio that this cannot be considered conclusive evidence that the patients were hypermetabolic, let alone thyrotoxic.

The explanation for the absence of frank thyrotoxico-

sis in most patients with molar pregnancy, despite evidence of pronounced hypersecretion of T_4, is unknown, but may relate to the magnitude of the increase in free T_4 concentration in their serum. In patients with the usual varieties of hyperthyroidism, the absolute concentration of free T_4 in serum is usually quite elevated as a result of increases in both the total concentration of T_4 in serum and the proportion which is free. In most series, the absolute concentration of free T_4 in hyperthyroid patients averages about 5 times the normal value (9, 20, 29, 30); in the patients with molar pregnancy, a smaller increase in the concentration of free T_4 was generally seen, since the proportion of free T_4 was usually subnormal. Small to moderate increases in the serum concentration of free T_4 in the absence of thyrotoxicosis are also seen in some patients with non-thyroidal illness (9, 20), as well as in patients receiving replacement doses of synthetic thyroxine (35).[4] Hence, it may be, as earlier studies have also suggested (36), that patients can tolerate moderate increments in the availability of T_4 to tissues without developing frank thyrotoxicosis.

Although several questions concerning the changes in thyroid hormone economy which occur in molar pregnancy, such as the reasons for the variability in TBG or the absence of frank thyrotoxicosis, remain to be answered, it seems quite clear from the present data both that the intense thyroid hyperfunction seen in patients with molar pregnancy results from the highly active thyroid stimulator present in their serum and that the stimulator itself originates in the molar tissue. Consonant with this view is the rapid amelioration of thyroid hyperfunction and the often precipitous decrease in serum PBI associated with rapid disappearance of the stimulator from the serum after removal of the mole (Fig. 1). The nature of the unusual stimulator is uncertain. Bioassayable thyroid stimulating activity has been detected in serum or tumor from several patients with trophoblastic neoplasms (21, 32, 37, 38). Most germane to the present observations are the reports of Hershman and Higgins (32), who studied two patients with molar pregnancy, both of whom were thyrotoxic. Thyroid stimulating activity was found in both serum and extracts of tumor from one patient. Like the stimulator found in the present patients, it failed to demonstrate major immunologic cross-reaction with human TSH. Moreover, unlike normal chorionic thyrotropin, it failed to cross-react with bovine TSH. Gel filtration studies suggested that the thyroid stimulator in molar tissue has a higher molecular weight

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[4] In the studies cited (35), the concentration of free triiodothyronine (T_3) was also moderately increased. No data concerning the proportion or concentration of free T_3 in the serum of patients with molar pregnancy are available.
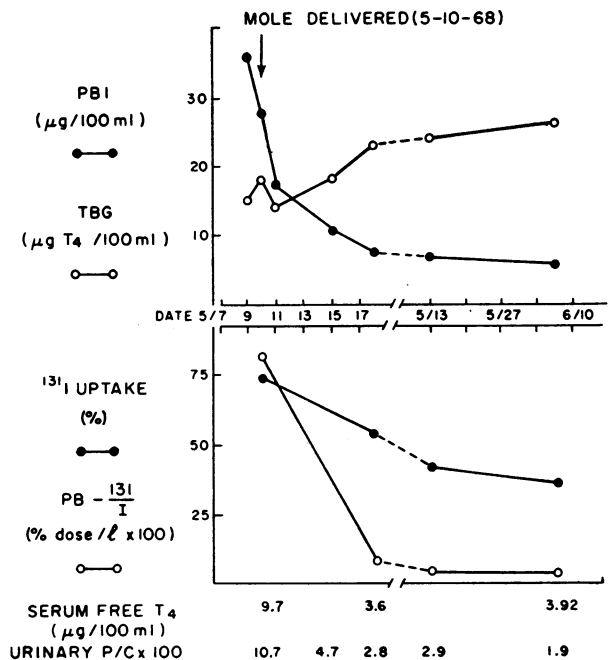

FIGURE 1 Thyroid function in a patient with molar pregnancy. Age 26, gravida 5, para 4, L. M. P. 2-12-67.

than bovine or human TSH or normal chorionic thyrotropin (21, 32). The material may be related to the thyrotropic substance found by Burger in commercial preparations of human chorionic gonadotropin obtained from the urine of normally pregnant women (39). This, too, appears to have a higher molecular weight than bovine TSH (39), and fails to cross-react immunologically with bovine or human TSH (21). Thus, it seems possible that the same thyroid stimulator that is secreted abundantly in patients with molar pregnancy may be secreted in small amounts by the normal human placenta.

ACKNOWLEDGMENTS

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