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Iodinated Compounds and Thyroxine Binding to Albumin in Human Breast Milk

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Summary

Mature human milk samples from young healthy women on an equilibrated diet contained a mean of 81 ng/ml total iodine. Iodide represented a mean of 77% of the total iodine. Of the 22 ng/ml organic iodine, there was about 1 ng thyroxine and triiodothyronine and, after pepsin hydrolysis, up to 40% of organic iodine in monoiodotyrosine form. By electrophoresis, after incubation with radioactive thyroid hormone, we found an absence of binding on thyroxine-binding globulin, but thyroxine and triiodothyronine were bound to albumin with a maximal capacity 50 times higher than in human serum. All these differences did not favor transport of iodinated compounds from maternal serum to milk. In conclusion, milk iodide was taken up by newborn thyroid to make thyroid hormones.

Abbreviations

T_4, thyroxine
T_3, triiodothyronine

For the past 10 years, thyroid congenital screening has brought a new approach to the neonatal thyroid function, and thyroxine appeared as the most important feature. These considerations have been certainly related to the interest recently developed by breast milk studies. Sack *et al.* (19) had found a significant amount of thyroxine in human milk. These last 2 years, using two different sensitive methods, Mallol *et al.* (10) and Moller *et al.* (13) found that the thyroxine supplied by breast milk was inadequate for normal neonatal development.

Recently, we have noticed a very low content of thyroid hormones in human breast milk and determined its total iodine level (4). This study presents the chemical form of the iodine and the proteins contained in the human milk with the aim of comparing milk to serum.

MATERIALS AND METHODS

Pooled and individual mature breast milks were obtained from the Lactarium of the Institut de Puériculture of Paris for the neonates in the Hôpital des Enfants-Malades. The mothers were young, healthy, living in and around the city, and had an equilibrated diet. Some samples of commercial cow milks were used for comparison.

Fresh milk samples were used immediately or kept frozen at −18°C. For total iodine determination, the catalytic effect of iodine on the reduction of ceric ions by arsenious acid is used in a Technicon Autoanalyzer. The automatic micromethod was applicable in the range of 0.5 to 6 ng/0.1-ml sample. Milk was free of substance interfering with the reaction; the recovery of a stable amount of iodine added to the milk was complete. Iodide was determined either after 24-h dialysis against water and/or after shaking with an anion exchange resin (Bio-Rad AG 1-X2 type). Radioimmunoassays used were: T_4 (6), T_3 (18), both with curve sensitivity of 20 to 600 pg/assay, and thyroxine-binding globulin with a commercial kit (Behringwerke, Marburg, Germany). Radioactive T_4 and T_3 used for radioimmunoassays and for the different incubations had high specific activity (>1200 μCi/μg, Amersham, UK). Peroxidase was determined according to Fragu and Nataf (5). Protein determinations were performed after trichloracetic acid precipitation, albumin after ammonium sulfate separation by the method of Lowry *et al.* (9), and different hydrolysis with protease, trypsin, and pepsin (Sigma Chemical Co, St Louis, MO). Polyacrylamide gel electrophoresis (1) was used for detection of proteins after staining with Amido Schwarz (Merck). Paper electrophoresis was performed (15) to determine the maximal binding capacity of albumin for T_4 and T_3; fresh milk was incubated for 4 h at 37°C in the presence of radioactive T_4 or T_3 enriched with 0.5 to 300 μg/ml of stable hormone (Henning, Berlin, Germany) dissolved in 0.1 M Tris buffer, pH 8.35. Paper electrophoresis was then carried out in 0.1 M ammonium carbonate buffer for 16 h at 120 V. The pH was adjusted by bubbling 5% O_2 and 95% CO_2. This buffer allowed a good separation of albumin from other serum proteins binding T_4. Iodotyrosine determinations were performed with paper chromatography on hydrolyzed milk (details in Ref. 3). Chromatograms were developed in acetic acid/butanol/water (5:78:17 by vol) and the iodine content of 1 × 3 cm paper strips was measured. Special extractions and incubations are described in "Results."

Means ± SEM were calculated only for more than 10 different milk samples.

RESULTS

Total Iodine Contents. The mean of total iodine content of 68 human breast milk samples was 81.6 ± 5.0 ng/ml (range, 17 to 205 ng/ml) (Table 1). Total iodine in the lipid fraction (n = 10) after centrifugation for 15 min at 2500 × g was 5.9 ± 1.7% of total iodine (range, 0 to 13%). For comparison, the cow milk samples (n = 11) contained 152.1 ± 12.3 ng/ml total iodine (range, 99 to 201 ng/ml).

Iodide. In human milk, the mean iodide percentage of total iodine obtained after resin adsorption was (n = 48) 75.4 ± 2.0 (range, 45.6 to 93.9) and after dialysis (n = 35) 79.3 ± 1.9 (range, 50.8 to 96). Iodide in cow milk (n = 11) was 90.9 ± 0.9% of total iodine by resin (range, 88.2 to 95.8) and 90.8 ± 1.6 by dialysis technique (range, 76.0 to 94.9).

Organic iodine content. The difference between total iodine content and iodide content was calculated and was the mean of results from resin and dialysis techniques. The mean for human

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Table 1. *Comparison of the amount of total iodine, iodide, and iodo compounds in human breast milk and commercial cow's milk*

	Human milk	(*n*)	Cow's milk	(*n*)
Total iodine (ng/ml)	81.6 ± 5.0	(68)	152.1 ± 12.3	(11)
Iodide				
Resin	75.4 ± 2.0	(48)	90.9 ± 0.9	(11)
Dialysis (% total iodine)	79.3 ± 1.9	(35)	90.8 ± 1.6	(11)
Organic iodine (ng/ml)	22.2 ± 2.5		14.4 ± 2.1	
Thyroxine (ng/ml)	0.57 ± 0.08	(12)	Undetectable	
Triiodothyronine (ng/ml)	0.34 ± 0.04	(25)	0.76 ± 0.03	(10)
Monoiodotyrosine (ng/ml)	6–20	(7)	ND*	

* ND not determined.

breast milk was 22.2 ± 2.5 ng/ml with a range of 2.2 to 59.5 ng/ml. The mean organic iodine in cow milk was 14.4 ± 2.1 ng/ml.

Organic iodine composition. Thyroxine of human milk samples was measured with a very sensitive method directly and after ethanol extraction (2 volumes) and after delipidation. The mean (n = 12) was 0.57 ± 0.08 ng/ml (range, 0.28 to 1.60 ng/ml). There was no T_4 detected in cow milk. The recovery of radioactive T_4 from human milk incubated for 5 h at 37°C and centrifuged 15 min was 15.9 ± 1.4% of total activity (range, 8.2 to 23.4) in the lipid fraction.

Triiodothyronine in human milks was determined either directly or after ethanol extraction. Various amounts of T_3 (45, 68, 91, and 136 pg) were added to different milk samples; the recovery after direct determination was 93.8% (n = 7) and 90.9% (n = 6) after ethanol extraction. Curves with 20, 40, 60, 80, 100, and 120 μl of nonextracted milk were parallel to standard curve as were serial dilutions of the extracted samples from 30 to 150 μl. The mean T_3 obtained (n = 25) was 0.34 ± 0.04 ng/ml (range, 0.19 to 0.82 ng/ml).

The amount of T_3 in cow milk (n = 10) was 0.76 ± 0.03 ng/ml (range, 0.63 to 0.90 ng/ml). 20.6 ± 2.1% of the total radioactive T_3 incubated with human milk was recovered in the lipid phase (range, 14.6 to 33.9%).

The mean amount of total T_3 and T_4 in human milk was about 1 ng/ml of a total of 22 ng/ml of organic iodine. T_3 and T_4 were determined after hydrolysis. Pepsin and trypsin were ineffective. After protease hydrolysis, T_4 contents were the same as prior to this last step. The amount of T_3 after protease hydrolysis was irregular, due to analytical artifacts.

In order to evaluate stable iodotyrosine levels, we used paper chromatography. High organic iodine (40–50 ng/ml) in human milk was not able to migrate while iodine stayed at the origin. After pepsin hydrolysis (protease and trypsin were ineffective), ethanol extraction, chromatography, and stable iodine determination, iodo compounds were recovered at the R_F of monoiodotyrosine standard. Mean iodine from monoiodotyrosine was 2.9 ng (n = 7) corresponding to 6 to 20 ng/ml milk.

Proteins: Enzyme. In cow's milk, either pasteurized or sterilized, the proteins were spoiled and could not be used for comparison.

Amounts. Mean of total proteins in human milk samples (n = 16) was 10.1 ± 0.8 mg/ml (range, 6.8 to 19.2 mg/ml). By ammonium sulfate precipitation, the mean albumin (n = 11) was 5.8 mg/ml (range, 2.5 to 11.6 mg/ml) or 54.7% of total proteins. Thyroxine-binding globulin was too low to be measured, less than 0.2 mg/liter. The methods used to detect peroxidase in thyroid tissue showed no enzyme in human milk.

Binding. The mobility of milk proteins was determined after polyacrylamide gel electrophoresis. There was a large band corresponding to albumin, and sometimes another smaller band corresponding to the albumin dimer. If about 0.1 μCi radioactive T_4 was incubated for 2 h with these milk samples, 90% of the radioactive T_4 was bound to albumin and 10% to albumin dimer.

The maximum binding capacity of albumin was determined after incubations of human fresh milk samples with radioactive T_4 or T_3. On each electrophoresis paper sheet, there was a human serum sample as control and five samples of 50 μl milk incubated previously (see "Materials and Methods") with increasing amount of stable T_4 (0.2 to 1, 0.5 to 10, 10, 33, 100, and 166 μg and even 50, 100, 200, and 300 μg/ml) and of stable T_3 (0.05 to 1 and 1 to 50 μg, and 66, 200, and 300 μg/ml). The measured radioactivity on albumin spots varied from 76 to 91% of total radioactivity. The maximal binding of T_4 to serum standard is 4 μg/ml on albumin. With milk, the maximal binding of T_4 and T_3 was about 50 times higher than with serum and the binding was as high with T_3 as with T_4. The uptake of radioactive T_4, after 2-h incubation with human milk was temperature (4, 20, or 37°C) independent.

DISCUSSION

T_4 has been found in human milk in sufficient amounts to attenuate congenital hypothyroidism (2, 19). However, the methods used were inadequate. Some authors using either sophisticated analytical methods (10, 13) or different sorts of kits (11) recently showed that the T_4 content in human milk was very low. We have also used a radioimmunoassay which is 40 times more sensitive than the usual commercial kits and our results showed that breast milk contained less than 1 ng T_4/ml. We also showed that cow's milk samples, and consequently formula milk, were free of T_4. Few authors, besides Varma *et al.* (23), Sato and Suzuki (20), and Mizuta *et al.* (11) have determined T_3. In human milk, we found about 0.3 ng T_3/ml, close to the above values and nearly double in cow's milk (Table 1). Human milk was also different from cow's milk because of the absence of peroxidase of the thyroid tissue type, but there is probably one derived from leukocytes (12) and by the very small amount of casein.

T_4 could be derived from circulating maternal plasma and we have determined in the milk its characteristics and compared them to serum. The first information was the total iodine content. In studies concerning the mineral content of human milk (22), iodine was omitted. The means found in a Paris neighborhood population were 81 ng/ml, a value close to the total iodine serum content in euthyroid subjects. Iodine was mostly in an iodide form (77%) in human milk and, in serum free of interfering drugs, iodide is only about 1% of total iodine. Milk contained hormones corresponding to 1/80 of total iodine while serum had mostly thyroid hormones. Several groups working on animal milks (16, 10, 24), using radioactive iodine, have shown a different distribution between iodide, total iodine, and hormone, depending on the species. They usually found that the most abundant organic iodine compound was monoiodotyrosine. In human milk, organic iodine could migrate on paper chromatography after pepsin hydrolysis and in organic iodine rich milks about 40% was in monoiodotyrosine form. Its serum origin was however doubtful. Protease hydrolysis did not seem to increase T_4 content in human milk while it interfered with the T_3 radioimmunoassay, though larger amounts have been found (14). Some authors (2, 21) had noticed a larger uptake on the lipid milk phase. We have shown that the distribution of T_4 was more dependent on the volume of the lipid fraction and our mean value was not very different from the 90% recovery in the aqueous phase found by Moller *et al.* (13).

The second series of experiments concerned the eventual binding of the hormones by proteins. T_4 was not bound to a specific protein as in serum. This is different from the binding of milk cortisol (17). The amount of total proteins in mature milks was about ⅒ of the serum protein contents and these results agreed with previous report (7). The binding of thyroxine to milk proteins was different from serum binding, particularly because of the absence of a thyroxine-binding globulin. T_4 and T_3 might be bound to albumin, both with high capacity, and this phenomenon was temperature independent. By chemical methods, the albumin origin could not be determined. The very low amount of hormones could be transported from maternal serum. Mizuta *et al.* (11) assume that transfer from serum to milk is a simple diffusion, but they believe that the real mechanism is not elucidated. The monoiodotyrosine origin has to be different and one hypothesis is that it is formed by the lactating breast.

In conclusion, it would seem that the hormones in human milk are in a too low concentration to supply neonates with a normal thyroid function if the gland is deficient. The neonates are able to make their thyroid hormones from the iodine contained in the milk exactly as does the adult with his various intake.

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