the same time interval. The reasons for the higher uptake of α-iduronidase as compared to α-acetylglucosaminidase are not apparent. Since we used the same media for culturing fibroblasts as Bach *et al.* (*6*), this would not seem to account for the difference. Similar uptake and correction studies in which arylsulfatase A preparations were added to cultured fibroblasts from patients with metachromatic leukodystrophy were carried out by Porter *et al.* (*7*) and by Wiessman *et al.* (*8*). Unfortunately, neither group reported values for the cellular uptake of this enzyme.

The degree of metabolic correction is large at very low cellular concentrations of enzyme; 42 to 70 percent correction occurred at concentrations that were 2 to 5 percent of the normal average. This fact is encouraging when considering enzyme replacement therapy in patients with Sanfilippo disease type B.

It is assumed that the cellular uptake of α-acetylglucosaminidase is accomplished by pinocytosis because uptake of other proteins by cultured cells appears to proceed via this mechanism (*9*). Our studies with α-acetylglucosaminidase indicate that the chief limiting factor in the correction of the metabolic defect by enzyme replacement is cellular uptake of the enzyme. Pinocytosis stimulants, such as basic polypeptides, may be useful adjuncts in enzyme replacement (*9*) because they enhance (up to ten times) the uptake of added proteins by cultured cells.

It is not surprising that trials of intravenous plasma infusions (100 to 200 ml of fresh normal plasma) have been ineffective in patients with Sanfilippo disease (*10*). Although α-acetylglucosaminidase activity in human plasma averages 20 units per milliliter (*11*), the amount of enzyme which would be supplied by administering 100 to 200 ml of plasma is approximately 250 times lower than should be metabolically effective if one assumes that enzyme uptake, metabolic correction, and enzyme degradation in the patient's tissues are similar to those in cultured fibroblasts. Enzyme replacement therapy with α-acetylglucosaminidase in higher quantities appears to be warranted in Sanfilippo disease type B.

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Osmolar Control of Prolactin Secretion in Man

Abstract. *To study the effect of changing serum osmolality on serum prolactin concentration 11 volunteers were given oral and intravenous hypotonic and hypertonic fluids. Mean serum prolactin fell to 10.5 percent of baseline after oral water loading and to 15 percent of baseline after intravenous hypotonic saline infusion. Conversely, mean prolactin rose to 417 percent of baseline after intravenous hypertonic saline administration. The correlation coefficient of simultaneously determined serum prolactin and osmolality was highly significant* ($P < .001$). *Isoosmolar changes in extracellular fluid volume did not consistently affect the concentration of prolactin in the serum. Thus, prolactin may be involved in the physiologic regulation of osmolar balance and the kidney may be an important target organ for prolactin.*

The adenohypophysial hormone prolactin exists in many vertebrate species (*1*). Among the many functions of prolactin, a role in osmoregulation has been demonstrated in teleost fish (*2*). After hypophysectomy certain euryhaline fish species are unable to maintain blood osmolality when placed in fresh water. Osmoregulation is restored by prolactin administration (*2*). Furthermore, in the intact fish the prolactin-producing eta cells appear to be functionally more active in fresh water than in salt water (*3*), an indication that prolactin secretion is increased in fresh water enabling the fish to maintain blood osmolality.

The role of prolactin in osmoregulation in mammals has not been studied systematically. There is, however, evidence that the kidney may be a target organ for prolactin. Specific binding activity for prolactin has been described in kidney homogenates (*4*). In some patients with renal failure concentrations of prolactin in the serum are elevated (*5, 6*). Rats (*7*), heart-lung-kidney preparations from cats (*8*), and humans (*9*) respond to prolactin administration with marked renal retention of sodium, potassium, and water. These lines of evidence suggest that prolactin may play a role in osmoregulation in mammals.

Table 1. Prolactin concentrations during administration of hypotonic and hypertonic fluid.

Subject	Sex	Prolactin concentrations (ng/ml) at								
		0′	10′	30′	50′	60′	70′	90′	120′	180′
					Oral water					
J.R.	F	11		7		8		4	6	9
J.M.	F	6		3		<1		<1	4	3
A.R.	M	4		3		5		5	1	2
M.W.	F	4		1		<1			<1	<1
M.B.	F	5		<1		<1			<1	
G.P.	M	3		9		2			<1	<1
					Hypotonic saline infusion					
S.M.	F	21		9	17		17			
C.L.	F	17	9	3	10		8			
J.F.	M	5	2	<1	<1		<1			
B.M.	M	4	2	<1	<1		<1			
					Hypertonic saline infusion					
S.M.	F	17	17	15	27		19			
C.L.	F	2	25	20	14		5			
J.F.	M	2	3	4	<1		<1			
J.H.	F	5	5	12	5		10			

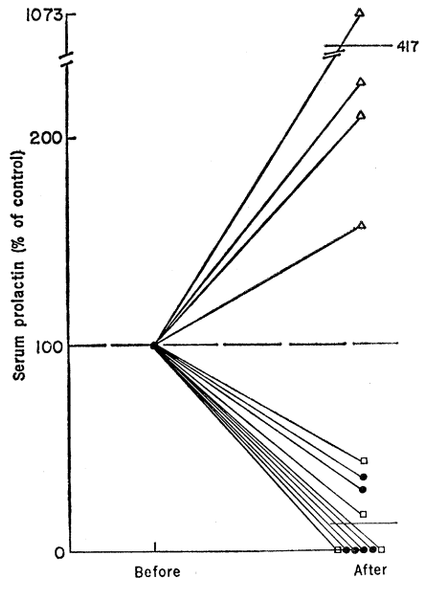

Fig. 1. Serum prolactin concentrations before and after oral and intravenous administration of hypotonic and hypertonic fluid. Samples obtained before fluid administration are considered 100 percent of the control. The maximum response during each test was used to calculate the after values in the percentage of the control; values less than 1 ng/ml are designated as 0 percent of the control. Oral water administration, circles; intravenous hypotonic saline administration, squares; and intravenous hypertonic saline infusion, triangles. The horizontal bars represent the mean percentage of baseline after administration of oral and intravenous hypotonic fluids (lower bar) and intravenous hypertonic fluids (upper bar).

In order to explore the effect of changing serum osmolality on endogenous prolactin secretion in man, we administered hypo- and hypertonic fluids to normal subjects. Eleven normal volunteers (four males and seven females), aged 18 to 36 years, in good health and taking no medications, were studied. Studies were begun between 8 and 9 a.m., after an overnight fast. Serum prolactin was assayed by a heterologous double antibody radioimmunoassay with the use of porcine [^{125}I]prolactin, antiserum to ovine prolactin, and human prolactin V-L-S No. 1 as a standard preparation (*10*). Except for the different standard which we used, the assay is similar to the one reported by Jacobs *et al.* (*11*). The minimum detectable amount of prolactin was 1 ng/ml. All values below 1 ng/ml were assigned a < 1 ng/ml value.

To evaluate the effect of decreasing serum osmolality on serum prolactin concentration, we administered water orally (20 ml of water per kilogram of body weight over 30 minutes) to six ambulating normal subjects (four females, two males). Blood was withdrawn by intravenous puncture at 0, ½, 1, 1½, 2, and 3 hours. As was reported (*12*), serum prolactin fell in most individuals by 1 hour and reached its nadir in all subjects within 1 to 2 hours of water loading (Table 1). In four subjects the serum prolactin decreased to undetectable amounts and in the other two to 28.6 and 34.5 percent of baseline (Fig. 1). After the water loading, mean serum prolactin was 10.5 ± 6.1 percent (mean ± S.E.) of baseline. All subjects excreted more than 70 percent of the administered water load within 4 hours.

In order to characterize further the effect of decreasing serum osmolality on serum prolactin concentration, we infused four subjects (two males, two females) with a hypotonic solution (0.45 percent saline) via intravenous cannula at 20 ml/kg over a 60-minute period. Blood was withdrawn through an indwelling venous cannula in the contralateral arm at 10- to 20-minute intervals, and urine was collected at 20-minute intervals. Serum prolactin fell more promptly after intravenous infusion of hypotonic solution than after administration of an oral water load, reaching the lowest point within 30 minutes (Table 1). The maximum decrease in serum prolactin correlated well with the maximum decrease in serum osmolality to less than 274 mosmole/kg in each subject. Prolactin fell to 43 and 17.3 percent of baseline in the two female subjects and to unmeasurable levels in the two male subjects (Fig. 1). After intravenous administration of hypotonic sodium chloride solution, the serum prolactin was 15 ± 8.8 percent (mean ± S.E.) of control value, a response comparable to that seen after oral water loading. All subjects responded with a brisk diuresis of more than 7 ml/min by the end of the infusion period and with a corresponding fall in urine osmolality to less than 80 mosmole/kg.

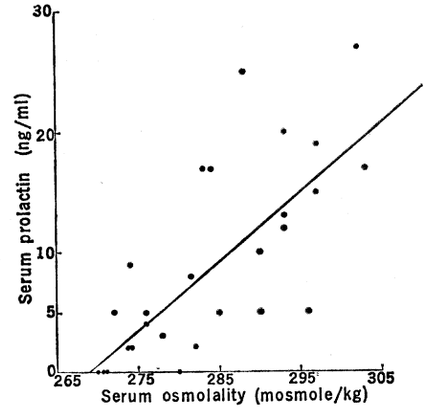

Fig. 2. Simultaneously determined serum prolactin and serum osmolality values before and at timed intervals during three hypotonic and three hypertonic saline infusions. The linear regression line was calculated from the formula $y = mx + b$, where the regression coefficient $m = .58$, and the y-axis intercept $b = -156$. The linear correlation coefficient was .732 which was highly significant ($P < .001$).

Having demonstrated that serum prolactin fell when serum osmolality was reduced by administration of oral or intravenous hypotonic solutions, we investigated the converse possibility, that serum prolactin would increase with an increase in serum osmolality. For this purpose 5 percent sodium chloride (3 ml per kilogram of body weight over 30 minutes) was administered via intravenous cannula to four normal subjects (three females, one male). Blood and urine were collected as outlined above. During the infusion, serum osmolality rose more than 10 mosmole/kg to exceed 290 mosmole/kg in each subject. Serum prolactin rose in each subject as shown in Table 1. In individual subjects, serum prolactin concentration paralleled the rise in serum osmolality with the maximum increase ranging from 158 to 1073 percent of baseline (Fig. 1); the mean (± S.E.) rise was 417 ± 190 percent. Urine volume decreased to less than 2 ml/min in each subject, with an appropriate rise in urine osmolality to more than 550 mosmole/kg.

The parallel relationship of simultaneously determined serum prolactin and serum osmolality before and at timed intervals during intravenous hypo- and hypertonic saline infusions is shown in Fig. 2. The correlation coefficient of simultaneously determined serum prolactin and osmolality at different times during the studies was highly significant ($P < .001$).

Since serum prolactin rose with hypertonic saline administration and fell with hypotonic saline infusion, it seemed unlikely that volume expansion per se resulted in the observed changes in serum prolactin concentration. To investigate the effect of isoosmolar volume expansion on serum prolactin concentration, two subjects were infused with isotonic saline (0.8 percent of body weight in kilograms) over 60 minutes. In order to evaluate the effect of isoosmolar volume contraction on serum prolactin

concentration, four subjects were given a potent diuretic (furosemide, 40 mg orally). During these studies the serum osmolality remained within 2 percent of the control value, and the serum prolactin concentration remained stable.

In man, changes in serum osmolality were accompanied by parallel changes in serum prolactin concentration. Administration of ovine prolactin to humans has been shown to result in a marked antidiuresis (*9*). Hypertonic saline infusion has been reported to cause a massive increase in serum prolactin in a single patient with a pituitary tumor and galactorrhea (*13*). We observed that hypertonic saline administration to normal subjects results in an increase in serum prolactin concentration which may facilitate the appropriate renal retention of water. Conversely, hypotonic saline administration resulting in suppression of endogenous prolactin secretion may facilitate the appropriate diuresis that occurs. The full significance of these observations in clinical medicine, especially the role of prolactin in volume-depleted and volume-expanded states, will require further investigation.

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Distal Conformation of the Thyroid Hormone 3,5,3′-Triiodo-L-Thyronine

Abstract. *In the crystal structure of the thyroid hormone, 3,5,3′-triiodo-L-thyronine, the 3′ iodine is observed in the* distal *position, away from the alanine-bearing ring of the thyroid hormone. This result had been anticipated from stereochemical and biological activity studies. However, previous observations of structures in which the 3′ iodine was* proximal *had cast some doubt on the stability of the 3′* distal *conformation. This observation suggests that the relative energies of the two conformers is similar and that perhaps the barrier to rotation is not as great as previously supposed since both the* distal *and* proximal *conformers have now been observed in the solid state.*

In the study of structural requirements for maximal thyromimetic activity, one of the most pertinent questions concerns the preferred conformation of the hormone triiodothyronine (T_3). Because the chemically identical 3′ and 5′ positions of the outer (β) ring of T_3 are not conformationally equivalent, as shown by Jorgensen and his co-workers (*1*), it is uncertain whether the preferred conformation of T_3 is with the 3′ iodine in the *distal* or *proximal* conformation. To verify the importance of this conformational feature, numerous structural analogs of thyroxine were synthesized (*2*) in an effort to determine the structural features that cause the stimulation or suppression of various physiological functions by thyroid hormones. From these stereochemical and biological activity studies, it appears that hormonal activity is greater for the *distal* orientation of the 3′ iodine. The requirement of a *distal* 3′ substituent for activity was further substantiated in a study of the binding of thyroxine-binding globulin to the thyroxine analogs 3,5-diiodo-2′,3′-dimethylthyronine and 3,5-diiodo-2′,5′-dimethylthyronine as test examples where either the *distal* or *proximal* conformation is locked by the steric hindrance of the 2′ substituents (*3*). This investigation showed that the *distal* analog had an almost twofold greater binding affinity for thyroxine binding globulin than the *proximal* form.

The studies of Kier and Hoyland (*4*) on molecular orbital energy calculations of trisubstituted thyronines suggest a perpendicular arrangement of the phenyl rings "locked in" by a considerable barrier to internal rotation. Their calculations do not show any significant preference for the *distal* or *proximal* form. Their results are not in agreement with the molecular orbital calculations made by Camerman and Camerman (*5*), who found that the total energy for the *proximal* T_3 is lower than that computed for the *distal* T_3. Earlier crystallographic verification of the mutually perpendicular arrangement of the two phenyl rings about the 120° ether linkage was first observed in the structure of diiodothyronine (*6*) and again in the structure of triiodothyronine HCl (*5*).

We report here a crystallographic observation of a triiodothyronine in which the 3′ iodine is in the *distal* conformation, as anticipated from stereochemical studies (*7*). The observation of the *distal* conformation in the crystal structure of 3,5,3′-triiodo-L-thyronine as well as in the crystal structures of 3,5,3′-triiodothyroacetic acid *N*-diethanolamine (1:1) complex (*7*) and 3,5,3′-triiodo-L-thyronine methyl ester (*8*) and the observation of the *proximal* conformation in the crystal structures of 3,5,3′-triiodothyronine HCl (*5*) and 3,5,3′-triiodothyropropionic acid ethyl ester (*9*) indicates that both forms are readily accessible in solution.

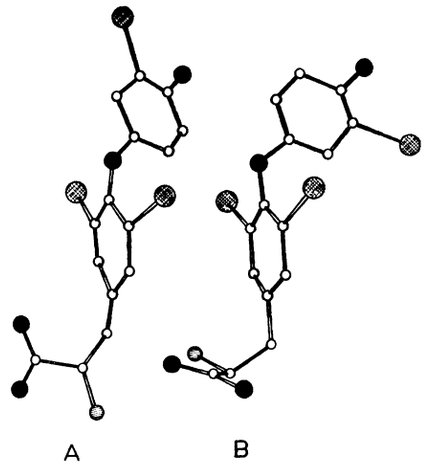

Fig. 1. Diagrams of triiodo-L-thyronine illustrating (A) the *distal* and (B) the *proximal* conformation.

Crystals of 3,5,3′-triiodo-L-thyronine were grown at room temperature from an ethanol solution containing an excess of salicylic acid. The crystal system is monoclinic $P2_1$, $Z=2$, with dimensions $a=13.891_4$ Å, $b=5.999_1$ Å,