

tion that an early action of thyroid hormones is stimulation of oxidative phosphorylation (3). Since this process occurs on the inner mitochondrial membrane, the present evidence suggests a direct action of thyroid hormones on mitochondria independent of nuclear involvement. The model of direct thyroid hormone action on mitochondria (15) in no way negates the concept of other more sustained if somewhat delayed effects mediated by the nucleus (16); such effects might include increased synthesis of messenger RNA directing the synthesis of inducible proteins. This pathway would presumably account for thyroid hormone stimulation of adenosine triphosphatase, which apparently entails formation of additional "sodium pump" units in the plasma membrane (17).

Previous reports (18) have implicated the mitochondria as a target of thyroid hormone action. While some reported effects might entail enhanced protein synthesis mediated by either nuclear or mitochondrial DNA, the effects we have observed (3) would appear to be too rapid for such a mechanism. Future studies may indicate the mechanism whereby interaction of the hormone with the postulated inner membrane receptor may activate oxidative phosphorylation.

KENNETH STERLING
JOHN H. LAZARUS
PETER O. MILCH
TOSHIRO SAKURADA
MILTON A. BRENNER

Department of Medicine, Columbia University College of Physicians and Surgeons, and Protein Research Laboratory, Veterans Administration Hospital, Bronx, New York 10468

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The Fanconi Syndrome in Basenji Dogs: A New Model for Renal Transport Defects

Abstract. *The renal defects resulting in a Fanconi syndrome were seen in eight Basenji dogs by measuring renal clearance and in vitro amino acid and sugar uptake and performing histopathologic evaluations. Renal tubular handling of glucose, phosphate, sodium, potassium, uric acid, and amino acids was abnormal, and in vitro uptake of labeled lysine, glycine, and α-methyl-D-glucoside by renal cortical slices was impaired. Histopathology was normal except for enlarged nuclei in some renal tubule cells. These Basenji dogs, which may be genetically affected, represent a likely model for idiopathic Fanconi syndrome in humans.*

The Fanconi syndrome in humans is a constellation of abnormalities associated with renal defects of tubular reabsorption; it is manifested by excessive urinary loss of glucose, amino acids, phosphate, bicarbonate, sodium, potassium, and water (1-3). The etiology may be unknown or idiopathic, but frequently the disorder is associated with a variety of inherited diseases (such as cystinosis, tyrosinemia, Lowes syndrome, and hereditary fructose intolerance in children) or with acquired conditions (such as multiple myeloma, amyloidosis, drug intoxication in adults). Such widespread derangement of renal tubular cell transport function could result from disordered membrane structure or abnormal cell metabolism, but as yet, the underlying mechanisms are unknown.

An animal model exists for Fanconi syndrome in which maleic acid is injected into dogs (4-6) and rats (7-9). We have characterized a spontaneous renal

tubular disorder in eight dogs of the Basenji breed; this disorder resembles human idiopathic Fanconi syndrome. Our studies, which include physiological assessment of renal function, in vitro uptake of a sugar and amino acids by kidney cortex slices, and pathological evaluation of the kidney, indicate this disorder is a new model of deranged active renal transport.

Clinical signs first appear in adult Basenjis of both sexes and progress to renal failure after months or years. These signs, similar to those in humans with Fanconi syndrome, include polydipsia, polyuria, dehydration, weight loss, and weakness. Profound glycosuria and hypotonic urine are present in the absence of diabetes mellitus. Plasma electrolytes are normal, but arterial blood gas values in affected dogs suggest a moderate metabolic acidosis.

Renal clearance studies were performed on seven affected and three nor-

Table 1. Renal handling of solutes by normal and affected Basenji dogs. One week prior to clearance studies, all dogs were fed a standard diet. None of the dogs were volume depleted or debilitated by routine clinical evaluation. Glucose, phosphate, and sodium values are expressed as the fractional reabsorption of the filtered load \pm standard error (S.E.). The potassium value for normal animals is the mean ratio of urinary excretion to filtered load. Potassium values for affected dogs and the uric acid values are expressed as the mean \pm S.E. ratio of urinary excretion to filtered load. Numbers in parentheses indicate the number of animals in each group.

Solute	Normal	Affected	P
Glucose	99.6 \pm 0.2 (3)	71.7 \pm 8.1 (7)	<.01
Phosphate	91.6 \pm 4.2 (3)	61.3 \pm 6.2 (7)	<.01
Sodium	97.4 \pm 0.6 (3)	90.0 \pm 3.1 (7)	<.05
Potassium	0.2 (2)	2.53 \pm 0.77 (7)	
Uric acid	0.34 \pm 0.08 (3)	0.95 \pm 0.11 (4)	<.01

mal Basenjis (10). Glomerular filtration measured by creatinine clearance (11) was normal in five affected dogs and was slightly decreased in two. The tubular reabsorption of glucose, phosphate, and sodium was diminished, and potassium and urate excretion was elevated (Table 1) (11). The tubular maximum (T_m) for glucose reabsorption was low in three affected dogs infused with glucose. The renal clearance of amino acids was abnormal in all seven dogs. Six dogs had a generalized aminoaciduria with reabsorption of 50 to 95 percent of filtered amino acids (Table 2); normal dogs reabsorb 96 to 100 percent of filtered amino acids. One dog had increased cystine excretion and reabsorptive defects characteristic of canine cystinuria (10). Urinary excretion of albumin was moderately increased over controls.

In vitro uptake of radioactive lysine and glycine, two amino acids whose tubular reabsorption was diminished, and α -methyl-D-glucoside, a nonmetabolizable model for the glucose transport system, was measured in renal cortical slices obtained at biopsy (12-14). This technique has been used to show a defect in dibasic amino acid uptake by the kidney in human cystinuria patients (15). Uptake of the three substrates was measured and expressed as the ratio of the number of counts per minute per milliliter of intracellular fluid to that in the medium (distribution ratio) (Fig. 1) (16). A distribution ratio greater than 1 indicates active transport and reflects a concentration gradient, since there is little or no substrate metabolism during the in-

Table 2. Reabsorption of amino acids in a dog with Fanconi syndrome. The mean \pm standard error of the fractional reabsorption are given for three collection periods of a clearance experiment. Normal dogs absorb 96 to 100 percent of filtered amino acids.

Amino acid	Reabsorption (%)
Threonine	52 \pm 0.7
Proline	64 \pm 2.5
Glycine	31 \pm 3.1
Alanine	68 \pm 1.8
Valine	82 \pm 1.9
Methionine	69 \pm 1.4
Phenylalanine	80 \pm 1.5
Lysine	68 \pm 2.9
Arginine	95 \pm 1.5

cupation. The ability to take up the three compounds was impaired in slices of Basenji kidney. Since α -methyl-D-glucoside transport resides primarily at the luminal brush border membrane (5), these data reflect defective brush border function. The early decrease in uptake suggests that influx of the compounds into the cell may be slow and differs from the kinetic alterations in the maleic acid-induced Fanconi syndrome, where influx into cells is not altered but efflux is accelerated (7, 9).

The histology of renal tissue of affected dogs without renal failure was normal by light microscopy, except for enlarged nuclei in some tubular cells. The significance of this is unknown. Renal biopsies of dogs that progressed to renal failure revealed a nonspecific nephritis with glomerular atrophy and interstitial fibrosis. Four dogs suddenly died from renal failure; they were found

to have acute papillary necrosis that probably resulted from prolonged dehydration, electrolyte loss, and acidosis.

The Fanconi syndrome in the Basenji breed may be genetically determined, since several cases occurred in related individuals in our study and in a previous report of glycosuria in the breed (17). Although these animals do not emanate from one family, the examination of pedigrees of affected dogs indicated one or more common ancestors within three to five generations. The Basenji in Europe and North America in the past 20 years have a common genetic base of approximately 12 dogs. The exact mode of inheritance of the disease is unknown.

These dogs present a disorder similar to the human idiopathic Fanconi syndrome without cystinosis and show the renal defects of glucose, phosphate, sodium, potassium, uric acid, and amino acid reabsorption. The bicarbonate wasting and hypercalcuria seen in the human disease has not been characterized in these dogs, nor have hypokalemia and stunting. Nevertheless, this canine disease appears to be a useful model for investigating transport abnormalities of Fanconi syndrome in humans since it represents a spontaneous model for this group of defects. These dogs are also suitable for studies on the etiology of renal transport defects in the whole kidney, renal cortical slices, and isolated cell membranes.

K. C. BOVEE, T. JOYCE

Department of Clinical Studies, School of Veterinary Medicine, University of Pennsylvania, Philadelphia 19104

R. REYNOLDS, S. SEGAL

Division of Biochemical Development and Molecular Diseases, Children's Hospital of Philadelphia, and Departments of Medicine and Pediatrics, School of Medicine, University of Pennsylvania

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11. Renal clearance was performed under light anesthesia of sodium pentothal and 1.5 percent halothane. A maintenance infusion of creatinine and mannitol was given to ensure adequate urine flow. Urine specimens were collected from the ureters to allow accurate and complete collec-

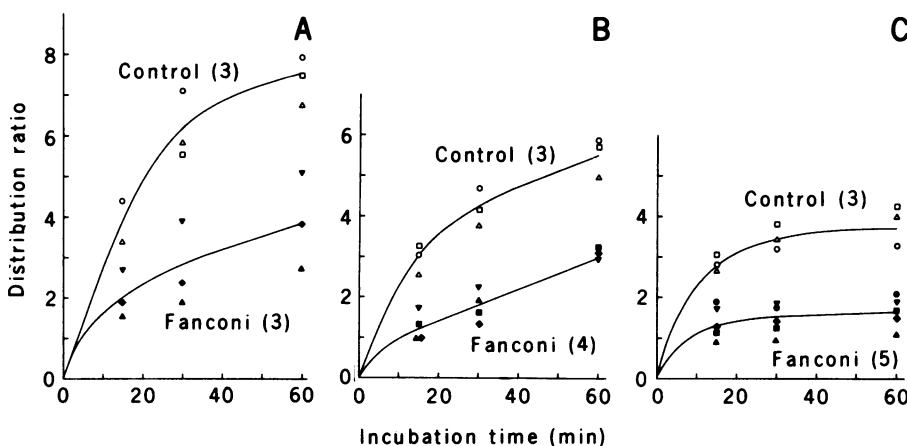


Fig. 1. Time course of accumulation of (A) L-lysine (0.065 mM), (B) glycine (0.065 mM), and (C) α -methyl-D-glucoside (2.065 mM) by Basenji kidney cortex slices. Two cortical slices totaling 5 mg from surgical biopsies were incubated in a Dubnoff metabolic shaker at 37°C in 30-ml plastic flasks containing 2 ml of Krebs-Ringer bicarbonate buffer (118.5 mM NaCl, 4.75 mM KCl, 2.53 mM CaCl₂, 1.2 mM KH₂PO₄, 1.2 mM MgSO₄, and 25 mM NaHCO₃), pH 7.4, with 0.1 μ Ci of labeled substrate per milliliter, in an atmosphere of 95 percent O₂ and 5 percent CO₂. Uptake is designated by the distribution ratio, the ratio of the number of counts per minute per milliliter of intracellular fluid to the number of counts per minute per milliliter of medium, using tissue water and inulin spaces in the calculation. The different symbols represent individual dogs. The numbers in parentheses indicate the number of animals from which samples were taken. In all cases, the difference between normal and affected animals was significant ($P < .001$).

- tion. Conventional clearance formulas were used, and solutes in urine and plasma were measured (10). The clearances of creatinine and inulin were identical when compared in affected dogs.
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Molecular Conformation of a Halogen-Free Thyroxine Analog: 4'-Methoxy-3,5,3'-trimethyl-L-thyronine N-Acetyl Ethyl Ester

Abstract. *The molecular conformation of the halogen-free thyroxine analog 4'-methoxy-3,5,3'-trimethyl-L-thyronine N-acetyl ethyl ester has been determined by x-ray diffraction techniques. The unsubstituted parent compound, trimethylthyronine, has significant biological activity in rat thymocyte tests when compared with the thyroid hormone 3,5,3'-triiodo-L-thyronine (T₃). Although no activity data are available for the analog studied, it is presumed to be inactive because of the 4'-methoxy blocking group. The observed conformation of this structure is similar to that found for the natural hormone T₃. The 3'-methyl group is distal, the overall conformation is cisoid, and the diphenyl ether conformation is twist-skewed. The results of this diffraction study show that methyl substituents are capable of maintaining the thyroxine conformation required for hormonal activity; they suggest that iodine enhances hormone-protein binding because of the electronic effects it produces either by alteration of molecular charge distributions or by direct charge-transfer interactions with the serum or nuclear binding proteins.*

Since the thyroid hormones thyroxine (T₄) and 3,5,3'-triiodo-L-thyronine (T₃) are the only naturally occurring iodinated compounds known to have vital biochemical activity, it is presumed that iodine plays an essential role in hormone activity. Some investigators have proposed that enhanced thyroid activity may be related to the ability of iodine to (i) achieve and maintain a specific hormone conformation or (ii) direct electronic interactions such as charge-transfer or electron donor effects in molecular associations. The observed antigoitrogenic activity (1-7) of such nonhalogenated thyroxine analogs as 3,5,3',5'-tetramethylthyronine (Me₄) and 3,5,3'-trimethylthyronine (Me₃) implies a distinction between these proposed functional roles of iodine and throws light on their significance for hormonal activity.

The results of studies measuring thyromimetic activity and protein binding affinities of these methylthyronines suggest that the role of iodine in functional activity and protein binding can be differentiated. In rat thymocyte activity tests (5) the methylthyronines had 23 percent of the activity of T₃, and in tadpole assays (4) they had 15 percent of the activity of T₄, but in rat antigoiter tests Me₃ had only 2 to 3 percent of the activity of T₄ (4). In studies of binding affinities for the serum protein thyroxine binding globulin, the methylthyronines were even weaker (6). The relatively high ac-

tivity in the thymocyte tests and the extremely low serum protein binding affinities suggest that these methyl derivatives cannot be transported, but do have intrinsic biological activity, implying the need for iodine in hormone transport but not activity.

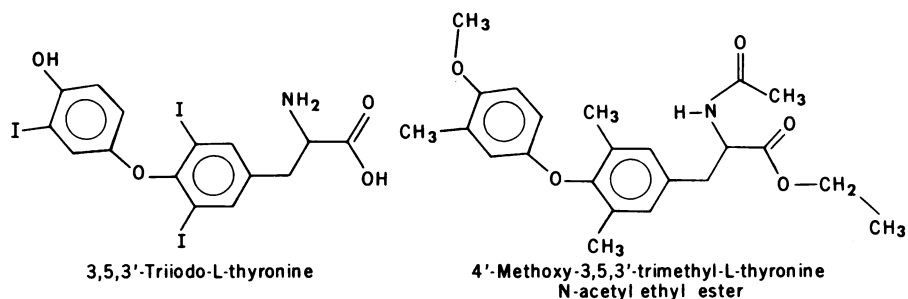


Fig. 1. Comparison of T₃ with the trimethylthyronine analog.

Table 1. Conformation of thyroactive compounds.

Structure	3' Conformation	Overall conformation	ϕ^*	ϕ'^{\dagger}	Reference
4'-Methoxy-3,5,3'-trimethyl-L-thyronine N-acetyl ethyl ester	Distal	Cisoid	-103°	25°	
3,5,3'-Triiodo-L-thyronine (T ₃)	Distal	Transoid	116°	-21°	(12)
3,5,3'-Triiodo-L-thyronine methyl ester (T ₃ Me)	Distal	Cisoid	-104°	32°	(13)
3,5,3'-Triiodothyroacetic acid	Distal	Transoid	92°	-1°	(18)
3,5,3'-Triiodo-L-thyronine H ₂ O HCl	Proximal	Transoid	90°	-11°	(19)
3'-Isopropyl-3,5-diiodo-L-thyronine HCl H ₂ O	Proximal	Transoid	90°	-27°	(20)
3,5,3'-Triiodothyropropionic acid ethyl ester	Proximal	Cisoid	-89°	-10°	(21)
4'-Methoxy-3,5,3'-triiodothyropropionic acid methyl ester	Proximal	Cisoid	-101°	21°	(22)

* ϕ = C5-C4-O4-C1'. † ϕ' = C4-O4-C1'-C6'.