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Effects of Orally Administered Sodium D-thyroxine upon Pregnancy Progression in Euthyroid Rabbits and its Effect Upon the Newborn

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EFFECTS OF ORALLY ADMINISTERED SODIUM D-THYROXINE UPON PREGNANCY PROGRESSION IN EUTHYROID RABBITS AND ITS EFFECT UPON THE NEWBORN

BY

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GENERAL INTRODUCTION

There is a wealth of experimental data, from both animal and clinical research endeavors, that point to a direct causal relationship between elevated serum cholesterol levels, concomitant atherosclerosis, and degenerative cardiovascular disease.

Many authorities believe that prophylactic treatment of idiopathic hypercholesterolemia will prevent atherosclerotic plaque formation which almost always precedes clinical arteriosclerosis and subsequent degenerative heart disease.

There has been intensive research investigation initiated to provide a pharmacologic agent that will lower elevated serum cholesterol levels in mammals including humans.

Several hypocholesterolemic drugs are being tested at the present time, one of the most promising is the dextro-rotatory isomer of thyroxine. This study was conducted not only to determine the effects of orally administered dextrothyroxine on pregnancy progression, but also to provide information for further investigations concerning alterations related to hypercholesterolemic pregnant animals.

Cardiovascular diseases are by far the leading cause of death in the Western World. Therefore, the need for safe and reliable drugs that might be employed in the prevention and/or treatment of cardiovascular alterations is abundantly clear.

Presently, within the United States, three hypocholesterolemic agents are being tested in massive clinical trials: nicotinic acid,

ethyl p-chlorophenoxyisobutyrate[1] and sodium D-thyroxine[2], of which D-thyroxine is the most promising. Lowering serum lipids (cholesterol, phospholipids, and triglycerides) in hyperlipemic individuals is rapid, predictable and relatively free from adverse side effects even after prolonged administration. The drug has been released for clinical medicine utilization in Canada, England, and Western Europe, but it has not been released by the Food and Drug Administration in the United States.

Little is known concerning D-thyroxine alterations related to pregnant or pediatric patients. Isolated literature reports demonstrating myocardial hypertrophy and apparently increased numbers and severity of angina attacks during investigations utilizing D-thyroxine have been reported. Therefore, the Food and Drug Administration has classified DT_4 as "experimental", which restricts its use to animal experimentation and closely supervised clinical trials.

Almost nothing is known concerning the alterations of D-thyroxine in relation to pregnancy progression or its effects involving newborn mammals and/or humans.

The possibility of congenital cardiac anamolies must, however, be considered in the light of previous research investigations which

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[1]CPIB ester, Ayerst Laboratories, New York, New York.

[2]CHOLOXIN, Baxter Laboratories, Morton Grove, Illinois.

indicate that cardiac hypertrophy may occur during D-thyroxine administration. It is hoped that these investigations coupled with the contemplated studies utilizing hypercholesterolemic animals may shed some light on possible cardiovascular alterations caused by D-thyroxine administration during pregnancy.

INTRODUCTION

The chemistry and physiology of the parent thyroid hormones 3:5:3':5'-tetraiodo-L-thyronine (LT_4) and 3:5:3'-triiodo-L-thyronine (LT_3) have been reviewed at length by Pitt-Rivers and Tata (1959). It is well known that thyroxine and the thyroxine analogues have many biologic activities in addition to their well known calorigenic effects. One such metabolic effect is the thyroid hormone influence on cholesterol synthesis and excretion producing a net reduction in circulating blood cholesterol levels. Early studies in this area have been reviewed by Duncan and Best (1958) and Boyd and Oliver (1960).

Natural thyroxine utilization employing euthyroid subjects for serum cholesterol reduction is not feasible because of its pronounced calorigenic activity in most cases. There is, however, a good rationale for studying the effects involving thyroxine analogues in relation to cholesterol metabolism. Duncan and Best (1962) wrote:

> If the cholesterol-lowering effect could be separated from the other effects of the thyroid hormones, they would become valuable tools in research on atherosclerosis and potentially useful in its prevention and treatment.
>
> It has been well established in recent years that molecular alteration of the steroid hormones can result in accentuation on one biologic activity and diminution of another. The observation that the activity of thyroxine relative to some of its analogues differs depending upon the physiologic effect observed indicates that some degree of dissociation of the various effects of thyroxine may also result from its modification. There is a possibility that some alteration of the thyroxine molecule might result in accentuation of cholesterol effects relative to calorigenic and other metabolic activities.

Literally hundreds of compounds have been investigated during the previous four years hoping to find a suitable hypocholesterolemic agent relatively free of idiosyncrasies and undesirable side reactions. The following is a partial list of most extensively tested thyroxine analogues relative to hypocholesterolemic properties:

3:5:3':5'-tetraiodo-L-thyronine (LT_4)
3:5:3':5'-tetraiodo-D-thyronine (DT_4)
3:5:3':5'-tetraiodothyroacetic acid (TETRAC)
3:5:3':5'-tetraiodothyroformic acid (T_4F)
3:5:3'-triiodo-L-thyronine (LT_3)
3:5:3'-triiodo-D-thyronine (DT_3)
3:5:3'-triiodothyroacetic acid (TRIAC)
3:5:3'-triiodothyroformic acid (T_3F)
3:5-diiodo-L-thyronine (LT_2)
3:5-diiodo-D-thyronine (DT_2)
3:5-diiodothyroacetic acid (DIAC)
3:5-diiodothyroformic acid (T_2F)

3:5:3':5'-tetraiodo-D-thyronine (DT_4) has been shown to be the most promising hypocholesterolemic thyroxine alalogue tested.

Intensive research efforts during the past five years have produced a volume of literature describing the results utilizing DT_4 administration in animal investigations and extended clinical (human) trials. A careful literature search and personal communications with active research workers (Kritchevsky; Tepper; Best; Ginger and Tucker) reveal, however, that almost nothing is known concerning DT_4 effects in relation to pregnancy progression and/or its alterations in mammalian newborn.

This lack of knowledge and isolated literature reports related to liver and heart weight alterations following DT_4 administration prompted these investigations.

LITERATURE REVIEW

Hypocholesterolemic Effects of Thyroxine: Hyperthyroidism and Cardiac Hypertrophy

The hypocholesterolemic effect of thyroxine (LT_4 and LT_3) in deranged thyroid states is a classic observation in clinical medicine. One mechanism responsible for the altered blood cholesterol content in thyroid pathology was elucidated in 1952 (Rosenman, et al.). Most authors now agree that LT_4 and LT_3 increase hepatic cholesterol degradation. It has also been suggested that LT_4 may act as a hypocholesterolemic agent by potentiating the normal adipose tissue response to free fatty acid (FFA) mobilizing agents (Tepperman, 1962).

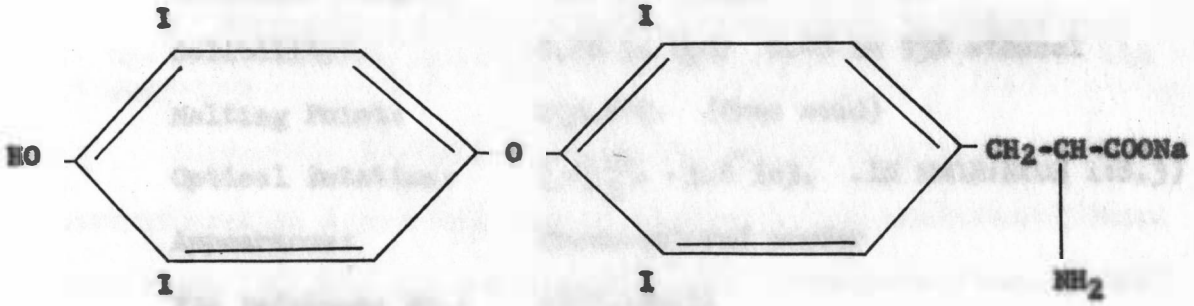
The correlation between myocardial hypertrophy and experimentally induced thyrotoxicosis was first reported in 1916 (Hoskins). It has since been shown many times that LT_4 administration will promote cardiac hypertrophy (Gross and Pitt-Rivers, 1953; Gemmill, 1953). Sandler and Wilson (1959) reviewed the literature concerning myocardial hypertrophy promoted by excess LT_4 administration. A pioneer study describing serum cholesterol and heart weight alterations in rats and mice employing thyroxine related compounds was published in 1960 (Cuthbertson, et al.).

Chemistry

The difficulty obtaining pure samples of D-thyroxine limited research investigations of any magnitude for several years. Ginger (1959) developed a process that effectively separated the D isomer from the naturally occurring racemic hormone.

The hormone (CHOLOXIN)[3] employed in this study was the sodium salt of dextrorotatory thyroxine chemically described as 3:5:3':5'-tetraiodo-D-thyronine. The chemical characteristics of the reference standard and the sample utilized during these investigations are listed in Table 1.

Sodium dextrothyroxine's structural formula, sometimes abbreviated NaD-T_4 is delineated below.



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[3]CHOLOXIN: Brand of Sodium Dextrothyroxine. Baxter Laboratories, Inc., (Flint Laboratories Division) Morton Grove, Illinois.

Table 1

Chemical and Physical Properties of a Reference Standard and Experimental Sample of 3:5:3':5'-tetraiodo-D-thyronine

A. Reference Standard	
Molecular Formula:	$C_{15}H_{10}O_4NI_4Na$
Molecular Weight:	798.884 (anhydrous salt)
Solubility:	0.2% in H_2O; 0.4% in 95% ethanol
Melting Point:	235-6°C.
Optical Rotation:	$[\alpha]_D^{25} = +5.5^\circ$ (C3, 1N NaOH:EtOH 1:2)
Appearance:	Cream-colored powder
B. Experimental Sample	
Molecular Formula:	$C_{15}H_{10}O_4NI_4Na$
Molecular Weight:	798.884 (anhydrous salt)
Solubility:	0.2% in H_2O; 0.4% in 95% ethanol
Melting Point:	235-6°C. (free acid)
Optical Rotation:	$[\alpha]_D^{25} = +5.0^\circ$ (c3, .1N NaOH:EtOH 1:8.3)
Appearance:	Cream-colored powder
FDA Reference No.:	1466-LBO-11
% H_2O	8.36
% I	62.6
% N	1.66

Pharmacology

Mechanism of Action: NaD-T_4

Kritchevsky stated the following based on available data in 1960:

> Data suggest that it is the increased rate of disappearance of cholesterol (excretion or catabolism) that accounts for the hypocholesterolemic action of thyroid active (DT_4 substances.... we can assume that thyroid active compounds exert their hypocholesterolemic effect by stimulation of the processes of cholesterol degradation and excretion. (1960)

Presently, after six years of intensive research involving thyroid hormone influences related to the chemistry of cholesterol biosynthesis and degradation this theory is still tenable. Best and Duncan (1966) described the following with reference to dextrothyroxine alterations on serum lipids:

> Dextrothyroxine is thought to act mainly by increasing the conversion of cholesterol to bile acids and thus hasten its excretion.

Supportive evidence has strengthened the theory, in that, dextrothyroxine apparently acts by shortening the cholesterol turnover time. Hoobler and associates (1959) demonstrated that control rats administered cholesterol-4-C^{14} excreted more than 90 per cent of the injected radioactive material in eleven and one-half days; rats receiving therapeutic doses of DT_4 excreted the same amount in seven days.

Cholesterol-lowering agents mode of actions, including the parent thyroid hormone and its analogs, have been reviewed extensively by Chiu (1961).

Absorption, Fate, and Excretion: NaD-T_4

Measurements of butyl-extractable iodine (BEI) concentrations and protein-bound iodine (PBI) in the serum of animals treated with sodium dextrothyroxine indicate that the drug was absorbed directly into the bloodstream from the gastrointestinal tract and was preferentially bound to the serum proteins. (CHOLOXIN Research Summary: 1965). Braverman (1964) noted that PBI levels alone were deceptive in establishing normal ranges because they depended upon the particular thyroid hormone employed. Butyl alcohol extraction was specific for thyroxine and chance contaminants are less likely to introduce error in this procedure when compared with the PBI method. The BEI technique also offers technical advantages as compared to the older distillation procedure.

A careful literature search and personal communications with research workers active in the thyroid metabolism area (Kritchevsky, et al.) indicated that almost nothing is known concerning the fate or excretion of sodium dextrothyroxine once it has entered the bloodstream of a pregnant animal. It has been known for years that mammalian placentas are freely permeable to thyroxine. Athyroid infants develop normally if there were adequate levels of circulating maternal thyroxine and the placenta was functional (Benson, et al., 1959). It has been suggested that D-thyroxine doses of 1 to 2 mg daily may be substituted for L-thyroxine in maternal thyroid

deficiencies, but the possible alteration and/or idiosyncrasies on the fetus when employing the synthetic isomer are completely unknown (Taylor, 1964).

A classic paper related to the excretion and physiological disposition of D- and L-throxine in the rat was published by Tapley, et al. (1959). Differences in distribution and half-life of radioactive I^{131} -tagged L- and D-thyroxine suggested, for the first time, a plausible mechanism of action explaining the differences in metabolic activity promoted by the two isomers in peripheral tissues.

In vivo investigations completed prior to the published work of Tapley et al. (1959) demonstrated that D-thyroxine reduced serum cholesterol levels in hypercholesterolemic euthyroid animals. But in contradistinction to the L isomer, such reductions occurred in the relative absence of the characteristic metabolic effects elicited by administration of the natural hormone. Depending on the bio-assay employed D-thyroxine has been said to be one-tenth to one-third as active as the L isomer (Garvin, 1962).

In vitro biochemical studies seemed to contradict clinical observations in that the two isomers proved equally effective promoting morphologic changes in mitochondria and 'uncoupling' oxidative phosphorylation reactions (Kritchevsky, et al., 1962).

Tapley, et al., attempted to explain the observed in vivo and in vitro differences by exhibiting unique distribution patterns and metabolic half-life differences between the two isomers. Using I^{131} labeled L- and D-thyroxine they proved that L-thyroxine was much more

widely distributed, especially in skeletal and cardiac muscle, and has a half-life over three times as long as the D form which was concentrated in renal and hepatic tissue.

Cholesterol-Lowering and Calorigenic Effects: NaD-T_4

One of the most unique and medically important physiological properties of D-thyroxine is its ability to reduce serum lipid levels without causing a significant rise in the basal metabolic rate. This effect, especially on cholesterol biosynthesis and degradation, has been studied in detail and is well documented (Tapley, et al., 1959; Oliver and Boyd, 1960; Chiu, 1961; Kritchevsky, et al., 1962; Soulairac, et al., 1963; Duncan and Best, 1966).

The anoxia survival time is one of the most useful physiologic tests utilized to measure the calorigenic activity of pharmacologic agents. In this test premedicated animals are placed in a closed container and their survival times are compared with a non-medicated control group. A mean reduction in survival time in minutes has been established using mice premedicated subcutaneously for seven consecutive days with one, two, four, and eight micro grams of L-thyroxine and 20, 40, 80, and 160 ug of D-thyroxine, respectively. The data is summarized in Table 2.[4]

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[4]Unpublished data on file in the Research and Development Department, Baxter Laboratories, Inc., Morton Grove, Illinois.

Table 2

The Effects of D- and L-Thyroxine Upon the Anoxia Survival Time of Mice Premedicated Subcutaneously for Seven Consecutive Days. (Each group represents 15 mice)

Control Survival Time = 151 minutes

Mean reduction of survival time in minutes	Micro grams LT_4	Micro grams DT_4	Mean reduction of survival time in minutes
10	1	20	25
35	2	40	60
64	4	80	68
70	8	160	73

Toxicologic Studies

The natural thyroid hormones and sodium salts of synthetic L-thyroxine have been investigated in depth both in laboratory animals and humans. Very little is known, however, concerning the physiological effects of D-thyroxine administered at relatively high dosage levels in either laboratory animals or humans. Comparing the effects of sodium D- and sodium L-thyroxine when administered chronically to rats and dogs Garvin wrote (1962):

> Although the purified hormone, sodium L-thyroxine, has been studied extensively in laboratory animals at physiologic doses ranging between 10 and 75 micrograms per kilogram of body weight, it has never been rigorously investigated at higher dose levels. Furthermore, the effects of large doses of the optical isomer, sodium D-thyroxine, are also unknown.

Because of its hypocholesterolemic effect most investigators administer the drug only in those therapeutic dosage ranges known to depress serum lipids without producing a calorigenic response. The approximate median lethal dose (ALD_{50}) for sodium D-thyroxine has been determined only in mice (1965):

> The drug was prepared as a solution in normal saline for intravenous administration or as a suspension in normal saline containing 10 per cent gum acacia for all other routes of administration.
>
> The ALD_{50} of intravenous sodium dextrothyroxine was in excess of 450 mg/kg at one and seven days after administration, and was more than eight times and 35 times that dose when administered orally and intraperitoneally respectively. (CHOLOXIN Research Summary: 1965)

The minimum lethal dose of DT_4 in the rabbit is unknown. It has been estimated, however, (Gesler: Personal Communication) to be around 10 mg/kg. The LD_{50} would be somewhat higher.

The correlation between myocardial hypertrophy and thyrotoxicosis has already been discussed. Oliver and Boyd (1959) first observed the incidence of anginal pain in patients being treated with certain thyroid analogues for hypercholesterolemia. They found that various tissues were affected, with regard to oxygen requirements, quite differently depending on the analogue utilized and the physical condition of the patient. They stated the following:

> In both hypothyroid and euthyroid patients, most of these analogues reduced the serum cholesterol without necessarily elevating the basal metabolic rate (B.M.R.). Nevertheless, in euthyroid patients with coronary heart disease several produced angina in the absence of any change in B.M.R., and this has been regarded as a sign of increased myocardial metabolism insufficient to be reflected in the overall measure of B.M.R. of all tissues.

Concerning angina, Cuthbertson and others (1960) suggested that the hypocholesterolemic thyroid analogues increased the myocardial oxygen requirements:

> ...and so might be expected to encourage cardiac anoxia and thus provoke anginal pain in patients with coronary disease.

In clinical investigations now in progress (1966) using D-thyroxine angina pectoris continues to be the idiosyncrasy promoting the most concern. Increased angina and other manifestations of myocardial pathology may be expected, however, when experimental groups have a pre-existing disease (hypercholesterolemia) which places them in a high risk category prior to treatment.

EXPERIMENTAL METHOD

Selection of Experimental Animals

Polytocous, virgin mammals were considered for use as experimental animals in this study. Several criteria were evaluated before rabbits were chosen in preference to other possible species. Additional studies evaluating DT_4 induced alterations in cholesterol metabolism during pregnancy will follow these investigations. Thus, not only placental type, and gestation duration, but also susceptibility to cholesterol-induced atherosclerosis were evaluated when considering the various species.

Canines and rats were eliminated as possible choices because both are normally resistant to experimentally induced hypercholesteremic hyperlipemia (Kritchevsky, 1959). The rabbit, however, is quite susceptible to pathological dyslipemias. Increasing dietary cholesterol levels will cause a rapid and predictable hypercholesterolemic response in rabbits but not canines or rats (Soulairac, 1963). Felines were eliminated because of their zonary rather than discoid and endothelial-chorial rather than hemoendothelial type placentation.

Rabbits utilized in this investigation were purebred, New Zealand White, virgin does weighing between three and three and one-half kilograms. The animals were ordered and received in a single shipment from one supply source. Clinical examination

demonstrated that each animal was alert, active and apparently disease-free. Purebred New Zealand White bucks were employed for breeding.

Experimental animals were individually housed in wire mesh hutches and maintained on a commercially available pellitized rabbit diet and mineral supplement (Table 3). Feed and water were provided ad libitum.

Treatment and Control Groups

Following a four day post-delivery observation period the animals were mated and randomly allocated to one of the two experimental groups or the control group each containing five does.

Control animals received 0.5 milliliter of diluent each day of pregnancy. Each animal in Treatment Group I received 0.5 ml of diluent during the first 15 days of gestation and two milligrams of NaD-T_4 in 0.5 ml diluent daily the last 15 days of pregnancy. Does in Treatment Group II received two mg of NaD-T_4 in 0.5 ml of diluent each day of pregnancy.

Animals in Treatment Group I were administered hormone diluent during the first 15 days of gestation because statistical analysis of possible heart and/or liver weight variations between the two treatment groups will be predicated on an adjusted control body weight standard.

The rabbit is a typical induced ovulator. Cervical stimulation during coitus results in neuronal LH release followed by ovulation in approximately 10 hours. Therefore, hormone or diluent

Table 3

Chemical Analysis of Hubbard Sunshine Rabbit Pellets 271 and Carey Mineral Supplement Spools With Salt

A. Hubbard Sunshine Rabbit Pellets 271

Guaranteed Analysis:

Crude protein, not less than	16.0%
Crude fat, not less than	3.0%
Crude fiber, not more than	13.0%

B. Carey Mineral Supplement Spools With Salt

Guaranteed Analysis:

Min. Phosphorus	(P)	2.760%
Max. Calcium	(Ca)	3.500%
Min. Calcium	(Ca)	2.600%
Min. Iron	(Fe)	0.270%
Min. Manganese	(Mn)	0.250%
Min. Copper	(Cu)	0.033%
Min. Cobalt	(Co)	0.010%
Min. Iodine	(I)	0.007%
Min. Zinc	(Zn)	0.005%
Max. Salt	(NaCl)	87.000%
Min. Salt	(NaCl)	84.000%

administration, calculated to begin on the first day of gestation, was administered the first 24-hour period following this approximate 10-hour delay.

Hormone Preparation: Oral Administration

The hormone employed in this study was supplied as a fine, cream-colored powder having the chemical and physical characteristics listed in Table 1 (Experimental Sample).

Hormone preparation, in a suitable media for oral administration, was unexpectedly difficult. Attempts to suspend the material in 10 per cent gum acacia solution as suggested by Gesler (Personal Communication)[5] were met with limited success even utilizing alkaline pH's. Experimentation with several polar and nonpolar solvents, at various pH's and in combination utilizing a number of different electrolytes proved equally unsatisfactory. The suggestion by Palmer (Personal Communication)[6] that the hormone be dissolved in hot ethanol adjusted to a moderately alkaline pH with sodium hydroxide produced a suitable media.

One hundred milliliter aquilots of the solution containing 4mg NaD-T_4/ml were prepared fresh each week. The hormone was supplied as a hydrate (8.36% H_2O). Therefore, a correction was made

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[5] R. M. Gesler, Ph.D., Director of Pharmacologic Research, Baxter Labs., Inc., Morton Grove, Illinois.

[6] Ivan S. Palmer, Ph.D., Station Biochemistry, South Dakota State University, Brookings, South Dakota.

for this moisture level when calculating the 4 mg/ml amount (4.3649 mg of hydrate in solution equals 4.0000 mg NaD-T_4/ml).

The hormone hydrate (.4365 gm) was placed in a 100 ml volumetric flask and 84 ml of ethanol added during continuous shaking. Sixteen milliliters of .1N NaOH were added slowly until the solution cleared. The temperature of the flask and contents was elevated from 20°C. to 75°C. and maintained until the hormone was completely dissolved. Once the hormone had dissolved the flask was sealed and slowly returned to room temperature.

Fifty ml aquilots of a solution containing 42 ml of ethanol and 8 ml of .1N NaOH were prepared fresh each week for administration to control animals throughout pregnancy and Group I experimental animals during the first 15 days of gestation.

By completely immobilizing a rabbit in a total body restraining cage quantities up to one ml could be orally administered by inserting a narrow, blunt tuberculin syringe into the oral cavity and expelling the contents. This technique eliminated many problems commonly associated with repeated passage of gavage tubes.

To reduce error possibility (oral leakage) and possible diluent induced gastric irritation the hormone solutions were prepared so that the desired daily dosage level was contained in one-half ml.

Collection of Data

Pregnant does near kindling were checked frequently. Newborn animals were removed from the nest and sacrificed (chloroform) immediately following parturition.

Data sheets identified by a treatment group or control number; individual doe number; litter size and kindling date were maintained for each litter. Each newborn animal in the litter was numbered, its total body weight recorded (to one-tenth of a gram) and then examined for externally apparent congenital anamolies. Lung flotation tests were employed to verify apparent stillbirth.

Dissection Technique

Hepatectomy - Cholecystectomy

Micro-dissecting surgical instruments employing a desk model magnifier were utilized in the dissection procedures.

Primary superficial incisions from sternal notch to umbilicus mid-ventrally and from a point one centimeter posterior to the xiphoid process bilaterally freed four skin flaps which were isolated and pinned. The abdominal muscles and peritoneum ventral to the liver were identified, transected and retracted. The liver and gallbladder were dissected and removed from the peritoneal cavity in six steps:

1. The dorsal border of the falciform ligament was identified at the umbilicus traced anteriorly to its diaphragmatic attachment, then transected with the round ligament (ligamentum teres) in its free edge.

2. The left hepatic lobe was partially retracted exposing the left triangular ligament. This ligament was transected at its attachment to the central diaphragmatic tendon and dorsal surface of the left hepatic lobe.
3. The coronary ligament was identified at its reflection from the dorsum of the right hepatic lobe and transected.
4. The hepatorenal ligaments were isolated, identified and transected.
5. The cholecystoduodenal ligament, surprisingly well developed in most of the animals, was transected freeing the liver except for vascular attachments which were then severed.
6. The gallbladder was removed from the inferior surface of the right lobe by blunt dissection.

The liver was removed from the body cavity, blotted on absorbent paper and weighed immediately on a Mettler balance sensitive to one ten-thousandth gram.

Cardiac Removal

The heart was removed from the thorax in six steps:

1. The thoracic cavity was opened by a transverse incision across the diaphragm at its midpoint.
2. The rib cage was transected from its inferior costal margin through the first rib bilaterally from a point on either side and about one and one-half centimeters ventral to the vertebral column.

3. The rib cage was elevated and parietal pleura and mediastinal contents freed from the internal, ventral, thoracic surface.

4. The sternum was freed at the Angle of Lewis bilaterally and the bony thorax discarded.

5. The mediastinum was incised and the pericardium dissected from the heart and great vessels.

6. All vascular connections were severed as close to the heart as possible and the organ removed from the body.

The excised heart was placed on a wax dissecting plate and the atria incised; blood clots were removed with micro-forceps. Each excised organ was then washed in 50 ml of a commercially available,[7] polyionic, physiological irrigating solution (composition: Table 4). Then it was drained and the epicardium blotted on absorbent paper before wet weights were recorded (to one ten-thousandth of a gram).

Dry weights were recorded to one ten-thousandth of a gram after desiccation in a vacuum oven (30 psi vacuum) for seven hours at 65°C. Livers from each litter were pooled, placed in specimen containers, sealed and quick frozen for tissue cholesterol analysis at a later date. Data on each animal is listed in the appendix.

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[7] TIS-U-SOL (Physiological Irrigating Solution) Baxter Laboratories, Inc., Morton Grove, Illinois.

Table 4

Chemical Composition of TIS-U-SOL, a Commercially Available Physiological Irrigating Solution

TIS-U-SOL*
(Physiological Irrigating Solution)

EACH 100 ml CONTAINS:

800	MG. Sodium Chloride, U.S.P.
40	MG. Potassium Chloride, U.S.P.
20	MG. Magnesium Sulfate $\cdot 7H_2O$, U.S.P.
8.75	MG. Sodium Phosphate, Dibasic $\cdot 7H_2O$, U.S.P.
6.25	MG. Potassium Phosphate, Monobasic
100	MG. Dextrose (HYDROUS), U.S.P.

MILLIEQUIVALENTS PER LITER:

137.6	mEq. Sodium
5.8	mEq. Potassium
1.6	mEq. Magnesium
142.3	mEq. Chloride
1.6	mEq. Sulfate
1.1	mEq. Phosphate

*Baxter Laboratories, Incorporated, Morton Grove, Illinois.

Data Analysis

An IBM model 1620 computer was employed for statistical analysis of data. Raw data punched on IBM cards for computer use included: treatment or control group number, doe number, fetal animal and total body weight, wet/dry heart weight and wet liver weight. Heart weights were recorded to one ten-thousandth gram and liver weights to one thousandth gram.

Raw means, standard deviations, and regression coefficients for fetal total body weight, wet/dry heart weights and wet liver weights were then computed and programmed. Using computed regression coefficients raw organ weights were then individually adjusted to a standard total body weight reference (51.92 grams).

Analyses of variance on adjusted organ weights was the statistical method used to interpret all final data. Statistical results are summarized in Table 5.

Table 5

Analyses of Variance for Wet/Dry Heart and Wet Liver Weight Data

Basis	Source	d.f.	Sum of squares	Mean square	"F"
Wet heart weight	Total	88	8.883		
	Treatment	2	.029	.015	7.69 **
	Error	85	.162	.002	
Dry heart weight	Total	88	.228		
	Treatment	2	.001	.0007	
	Error	85	.005	.00006	10.44 **
Liver weight	Total	88	921.423		
	Treatment	2	.062	.031	
	Error	85	20.336	.239	.13 NS

NS Not significant.
**P < .01.

DISCUSSION

Before the collection of experimental data began organs were removed from 23 newborn rabbits with body weights ranging from 34 to 94 grams. Several surgical approaches and dissection techniques were employed. The decision to use the standardized procedures outlined for cholecystectomy, hepatectomy, and heart removal in this study was empirical.

A polyionic irrigating solution (Table 4) was employed for flushing the heart chambers. It was felt that a polyionic, truly physiological, solution would be less likely to cause an abnormal fluid flux (wet weight error) than so called physiologic or normal saline.

All organ weights were recorded to one ten-thousandth gram. Liver weights were then rounded to one-thousandth of a gram for statistical analysis.

In order to compensate for expected variations in organ weights due to differences in total body weight between individual animals an adjusted body weight was calculated as a reference standard (51.92 grams).

Individual wet/dry heart and wet liver weights for each animal were then adjusted (increased or decreased) to this total body weight reference standard. This adjustment eliminated possible organ weight variation between treatment and control groups caused by individual animal total body weight differences.

The control and experimental groups each contained five does. All five control does kindled but each treatment group had a pseudo-pregnant doe. All animals were in apparent good health when sacrificed. Therefore, data was obtained from 34 control, 27 Treatment Group I, and 30 Treatment Group II animals.

It should be noted that relatively massive (2mg/daily) doses of dextrothyroxine were administered during this investigation to normocholesteremic animals. The maximum daily maintenance dosage of dextrothyroxine now recommended for adult, human, hypercholesterolemic patients with no clinical evidence of coronary artery disease is 8.0 mg. An equivalent daily dosage employing rabbits weighing two and one-half to three kilograms would be about .3 mg.

SUMMARY

Effects of orally administered sodium D-thyroxine upon pregnancy progression in New Zealand White, euthyroid rabbits and its effects upon their newborn were investigated. The experimental animals were maintained on a commercially available, pellitized rabbit diet and mineral supplement. Pregnant does were randomly allocated to one of two experimental treatments or the control group containing five animals per group.

Control animals received 0.5 ml of diluent each day of pregnancy. Treatment Group I animals were administered 0.5 ml of diluent during the first 15 days of pregnancy. Treatment Group II does received two mg of NaD-T_4 in 0.5 ml of diluent daily during pregnancy.

An IBM model 1620 computer was employed for statistical analysis of fetal data obtained from 34 control, 27 Treatment Group I, and 30 Treatment Group II animals. Parameters measured included fetal total body weight, wet/dry heart and wet liver weights. Raw means, standard deviations, and regression coefficients were computed and programmed. Utilizing computed regression coefficients raw organ weights were then individually adjusted to a standard total body weight reference (51.92 grams). Analyses of variance on adjusted organ weights was employed in the interpretation of final data.

Highly significant ($P < .01$) reductions in both wet and dry fetal heart weights were observed in treatment group animals compared to adjusted standards. There were no significant differences in wet liver weights between control and experimental animals and no increased doe lethality was observed.

Existing experimental data must be replicated and evaluated employing both pregnant and nonpregnant, normo/hypercholesterolemic rabbits and their newborn before definitive assessments can be elucidated concerning possible fetal response to DT_4 administration during pregnancy.

RESULTS AND CONCLUSIONS

Highly significant ($P < .01$) reductions in both wet and dry fetal heart weights were observed in treatment group animals compared to adjusted standards. There were no significant differences in wet liver weights between control and experimental group animals.

Observed reductions in both wet and dry heart weights were completely unexpected in the light of previous research work that demonstrated increases in heart weights when DT_4 was administered to euthyroid nonpregnant animals (rats). Further, unpublished data from a tetratogenic study utilizing DT_4 in pregnant rabbits carried out by a French firm showed an increased doe lethality at very high dosage levels (10 mg/kg) when administered orally from day 8 to day 20 of pregnancy. However, no alterations were noted in either mother or offspring when the drug was administered at a lower dose level during the same period of pregnancy (Gesler, Personal Communication).

There was a close correlation between total body weight and organ weight raw means. The possibility of error within or between treatment and/or control organ weights introduced by invalid or inconsistent dissection techniques is minimal. Detailed, sequential, and standardized dissection techniques were established prior to collection of experimental data and rigidly followed during each dissection procedure.

Incomplete separation and/or contamination of dextrothyroxine with its L-isomer could alter some <u>in vivo</u> physiologic responses expected to occur after administration of the pure D-isomer. The

chemical and physical characteristics of the hormone sample used in this investigation (Table 1) attest to its purity. However, minute L-isomer contamination of the experimental sample, if present, would have caused an increase rather than decrease in heart weight values. Gross contamination, which is most unlikely, would have altered stated dosage levels but such contamination would have affected observed heart weights only by decreasing, not increasing, their statistical significance.

Only purebred animals in excellent health were used for experimental or breeding purposes. Pregnant does were individually housed in wire mesh hutches and maintained in an optimal nutritional state throughout gestation. There is no evidence to suspect that genetic and/or nutritional deficiencies or environmental stress conditions contributed to observed morphologic alterations in DT_4 treated animals.

Previously reported increases in doe lethality following oral DT_4 administration during pregnancy did not occur in this investigation.

To the best knowledge of the author there is a complete literature void concerning data relating effects of DT_4 to pregnancy progression or its effects upon mammalian newborn in any species. After a careful literature search and personal communication with active research workers in this area it would appear that data obtained in this investigation is unique. Further, observed results

(fetal heart weight reductions) are in apparent variance with published data demonstrating increased heart weights following DT_4 utilization in nonpregnant adult animals.

Although unlikely, species differences may explain unexpected decreases in fetal heart weights observed following DT_4 utilization in pregnant rabbits. Therefore, until additional morphologic data concerning effects promoted by DT_4 utilization in pregnant and nonpregnant, normo/hypercholesterolemic rabbits and their newborn is available definitive interpretation of existing data is impossible.

Developmental idiosyncrasies could result from placental transfer of cholesterol degradation products such as bile acids. Investigations utilizing hypercholesterolemic animals could validate or discredit this assumption. However, the importance of such a mechanism is doubtful considering the highly significant reductions in fetal heart weights observed in this investigation when utilizing euthyroid, normocholesterolemic does.

It is reasonable to assume that DT_4, like parent thyroid hormones, passes freely through the placental barrier. The distribution and concentration of dextrothyroxine in fetal and/or placental tissue is unknown. Therefore, definitive studies, similar to those of Tapley, et al. (1959), using I^{131}-tagged levo- and dextrothyroxine are necessary to elucidate the distribution patterns and concentrations of both isomers in these tissues.

Replication of existing data will be evaluated with experimental results obtained employing pregnant and nonpregnant,

normo/hypercholesterolemic rabbits and their newborn before any definitive assessment will be made concerning possible fetal response to DT_4 administration during pregnancy.

Experimental data suggesting a causal relationship between ingestion of a therapeutic drug during pregnancy and fetal cardiovascular alterations must be evaluated in detail. Until such data is discredited or, if verified, proven species definitive the possibility that a similar cause-effect relationship exists in other species, including humans, must be considered.

At the present time, in those countries where DT_4 has been released for use in clinical (human) medicine, the drug is contraindicated during pregnancy and not recommended for use in children under 12 years of age. Until more is known concerning dextrothyroxine alterations in pregnant or pediatric patients such restrictions appear both justifiable and prudent.

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APPENDIX

Raw Data Tables I Through XIII

Each table contains the following data on each doe and newborn animal:

DOE:

1. Treatment or control group number.
2. Individual doe number.
3. Kindling date.
4. Litter size (number of newborn animals).

NEWBORN:

1. Total body weight (to one-tenth gram).
2. Wet liver weight (to one ten-thousandth gram).
3. Wet heart weight (to one ten-thousandth gram).
4. Dry heart weight (to one ten-thousandth gram).
5. Viable or stillbirth.

RAW DATA TABLE I

Treatment # - Control
Animal # - I
Kindling Date - 9/16/66
Litter Size - 5

Animal #	Body Wt.	Liver Wt.	Heart Wts. Wet	Heart Wts. Dry	Viable Birth	Stillbirth
1	51.8	3.2513	.3515	.0495	x	
2	38.9	2.3695	.2993	.0436	x	
3	64.9	3.6623	.3700	.0616	x	
4	46.1	2.6783	.2761	.0439	x	
5	53.2	3.1643	.3129	.0501	x	

RAW DATA TABLE II

Treatment # - Control
Animal # - II
Kindling Date - 9/15/66
Litter Size - 6

Animal #	Body Wt.	Liver Wt.	Heart Wts. Wet	Heart Wts. Dry	Viable Birth	Stillbirth
1	68.4	3.9676	.3781	.0631	x	
2	65.7	2.7603	.3426	.0554	x	
3	60.7	3.3813	.3173	.0490	x	
4	55.5	3.1781	.2554	.0413	x	
5	70.9	2.9475	.3711	.0609	x	
6	65.7	3.4613	.3185	.0498	x	

RAW DATA TABLE III

Treatment # - Control
Animal # - III
Kindling Date - 9/18/66
Litter Size - 7

Animal #	Body Wt.	Liver Wt.	Heart Wts. Wet	Heart Wts. Dry	Viable Birth	Stillbirth
1	54.3	4.1603	.3161	.0519	x	
2	53.3	3.1838	.3107	.0501	x	
3	54.0	3.7183	.2989	.0452	x	
4	46.8	2.9043	.2620	.0403	x	
5	51.6	2.9323	.2741	.0410	x	
6	46.0	2.7748	.2774	.0411	x	
7	56.9	4.4206	.3286	.0490	x	

RAW DATA TABLE IV

Treatment # - Control
Animal # - IV
Kindling Date - 9/19/66
Litter Size - 7

Animal #	Body Wt.	Liver Wt.	Heart Wts. Wet	Heart Wts. Dry	Viable Birth	Stillbirth
1	34.2	2.4243	.3243	.0423	x	
2	41.1	2.5238	.3543	.0474		x
3	38.2	2.4863	.2940	.0522	x	
4	45.3	2.7345	.3048	.0519	x	
5	44.2	2.7188	.3241	.0580	x	
6	41.3	2.2263	.2769	.0432	x	
7	42.3	2.4840	.2762	.0462	x	

RAW DATA TABLE V

Treatment # - Control
Animal # - V
Kindling Date - 9/22/66
Litter Size - 9

Animal #	Body Wt.	Liver Wt.	Heart Wts. Wet	Heart Wts. Dry	Viable Birth	Stillbirth
1	51.6	3.2880	.3637	.0521	x	
2	54.0	3.6064	.3940	.0700	x	
3	47.1	3.3216	.4067	.0754	x	
4	54.8	3.5543	.4130	.0740	x	
5	53.9	3.9884	.3832	.0669	x	
6	59.6	4.0063	.4470	.0780	x	
7	62.8	3.7189	.4087	.0738	x	
8	57.7	3.3392	.4062	.0732	x	
9	53.7	3.2913	.3107	.0537	x	

RAW DATA TABLE VI

Treatment # - I
Animal # - I
Kindling Date - 9/25/66
Litter Size - 6

Animal #	Body Wt.	Liver Wt.	Heart Wts. Wet	Heart Wts. Dry	Viable Birth	Stillbirth
1	58.9	3.7676	.4173	.0562		x
2	64.7	3.1958	.3601	.0579	x	
3	53.9	2.8244	.2480	.0395	x	
4	45.2	2.0278	.2357	.0379	x	
5	57.9	3.0903	.2643	.0424	x	
6	23.6	1.1472	.1939	.0285	x	

RAW DATA TABLE VII

Treatment # - I
Animal # - II
Kindling Date - 9/22/66
Litter Size - 6

Animal #	Body Wt.	Liver Wt.	Heart Wts. Wet	Heart Wts. Dry	Viable Birth	Stillbirth
1	60.0	3.2693	.3268	.0510	x	
2	56.3	3.3981	.3341	.0491	x	
3	50.6	2.8973	.2782	.0446	x	
4	43.9	2.3437	.2540	.0391	x	
5	50.6	2.7618	.3180	.0447	x	
6	52.3	2.8228	.3295	.0480	x	

RAW DATA TABLE VIII

Treatment # - I
Animal # - III
Kindling Date - 9/24/66
Litter Size - 6

Animal #	Body Wt.	Liver Wt.	Heart Wts. Wet	Heart Wts. Dry	Viable Birth	Stillbirth
1	61.9	4.6087	.3108	.0520	x	
2	55.7	3.9250	.3612	.0524	x	
3	48.1	3.1025	.2727	.0422	x	
4	54.3	2.5446	.3185	.0559	x	
5	56.9	4.7386	.2845	.0488	x	
6	32.8	1.7845	.1820	.0296	x	

RAW DATA TABLE IX

Treatment # - I
Animal # - IV
Kindling Date - 9/23/66
Litter Size - 9

Animal #	Body Wt.	Liver Wt.	Heart Wts. Wet	Heart Wts. Dry	Viable Birth	Stillbirth
1	40.5	3.0175	.2091	.0313	x	
2	39.1	3.0283	.2372	.0390	x	
3	39.9	2.7838	.2291	.0331	x	
4	45.5	3.3827	.3031	.0455	x	
5	38.7	2.7664	.2079	.0297	x	
6	49.3	4.2337	.3120	.0473	x	
7	43.9	3.3098	.2363	.0368	x	
8	47.7	4.3242	.2904	.0456	x	
9	39.2	2.8016	.2603	.0399	x	

RAW DATA TABLE X

Treatment # - II
Animal # - I
Kindling Date - 9/18/66
Litter Size - 5

Animal #	Body Wt.	Liver Wt.	Heart Wts. Wet	Heart Wts. Dry	Viable Birth	Stillbirth
1	--	--	--	--		x
2	42.4	2.7975	.2879	.0488	x	
3	48.5	3.1178	.3051	.0542	x	
4	44.1	2.7266	.3033	.0490		x
5	--	2.4303	.4005	.0685		x

RAW DATA TABLE XI

Treatment # - II
Animal # - II
Kindling Date - 9/19/66
Litter Size - 10

Animal #	Body Wt.	Liver Wt.	Heart Wts. Wet	Heart Wts. Dry	Viable Birth	Stillbirth
1	54.8	3.1533	.2860	.0451	x	
2	74.5	4.2823	.4260	.0708	x	
3	65.4	3.8493	.3472	.0524	x	
4	68.8	4.4146	.3637	.0539	x	
5	57.8	3.3983	.3847	.0594	x	
6	61.7	3.1613	.3973	.0629	x	
7	66.9	4.3373	.4257	.0663	x	
8	71.6	4.5707	.4371	.0718	x	
9	45.9	2.4413	.2970	.0460	x	
10	39.9	2.4320	.2665	.0422	x	

RAW DATA TABLE XII

Treatment # - II
Animal # - III
Kindling Date - 9/19/66
Litter Size - 8

Animal #	Body Wt.	Liver Wt.	Heart Wts. Wet	Heart Wts. Dry	Viable Birth	Stillbirth
1	37.9	2.1951	.2221	.0356	x	
2	34.0	1.7835	.1895	.0296	x	
3	54.0	3.4088	.2784	.0459	x	
4	52.0	2.8452	.2841	.0423	x	
5	51.5	2.9583	.2751	.0470	x	
6	57.8	3.5902	.3141	.0520	x	
7	52.8	3.0664	.2579	.0418	x	
8	41.3	3.2432	.2756	.0461	x	

RAW DATA TABLE XIII

Treatment # - II
Animal # - IV
Kindling Date - 9/19/66
Litter Size - 7

Animal #	Body Wt.	Liver Wt.	Heart Wts. Wet	Heart Wts. Dry	Viable Birth	Stillbirth
1	56.9	2.6095	.3673	.0598	x	
2	50.0	3.3468	.3371	.0444		x
3	59.2	3.7478	.3333	.0502	x	
4	51.1	3.4033	.3377	.0500	x	
5	51.4	3.1909	.2470	.0361	x	
6	52.9	3.5403	.3270	.0507	x	
7	51.8	3.2563	.2937	.0487	x	