Submitted in accordance with the requirements of the University of Khartoum for the Degree of

Master of Veterinary Science

By

SAFIK EL TAFIH ISRAHIM
(B.V.Sc., Khartoum)

December, 1982
CHAPTER I

CHAPTER II

REVIEW OF LITERATURE

1. Relation of iodine to the thyroid gland 0
2. Thyroid hormones 7
3. Clinical importance of thyroid hormones 8
4. Calorimetric effects of thyroid hormone 9

Effects of thyroid hormone on vitamin metabolism 10
Effects of thyroid hormone on protein metabolism 11
Effects of thyroid hormone on carbohydrate metabolism 12
The effects of trimethyloxanthine (T3)-induced hyperthyroidism, and of carbimazole-produced hyperthyroidism on lipid metabolism, histology of vital organs and serum constituents in Mabini goats were studied.

Groups of goats were given T3 and carbimazole by the oral route daily for 24 days at doses of 0.250, 0.50 and 0.75 mg/goat and 60 and 30 mg/goat respectively.

T3 treatment decreased the serum, liver and heart triglyceride, cholesterol and phospholipid concentrations. These changes were accompanied by an increase in the activity of lipo-protein lipase (LPL) in the heart and abdominal aorta.

Carbimazole treatment at a dose of 30 mg/goat significantly increased the serum triglyceride, liver cholesterol and heart phospholipid. In both cases used, carbimazole significantly increased the liver and heart triglyceride concentration and the activity of HDL was not affected by carbimazole treatment.
Although T3 significantly decreased high density lipoprotein cholesterol, no significant effects on triglycerides.

Serum total protein, albumin, globulin, sodium and potassium concentrations as well as RBC and HGB counts,

HCV and haemoglobin contents were not affected by either treatment.
The study of thyroid disease has been the focus of many investigators. This is of particular interest especially if we think about the possible alterations in serum lipids and the theoretical risk factor of cardiovascular diseases that are known to develop with hyperlipemia.

A better understanding of altered thyroid function in man, no doubt necessitates the establishment of an animal model in which altered thyroid function mimics that of man.

In the present study, groups of Nubian goats were rendered hypothyroid by triiodothyronine (T3) treatment or hypothyroid by carimamolo treatment with the following aims:

1. To find if the goat could be a good model for the study of altered thyroid function. The relatively longer life span of goats when compared to that of laboratory animals makes them particularly suitable for the study of consequent chronic complications like cardiovascular diseases.

2. To determine the nature of the altered serum lipid concentrations especially those of triglycerides and cholesterol in relation to the corresponding levels in the liver.
5. Effects of thyroid hormone on glucose and water metabolism

6. Effects of thyroid hormone on activities of enzymes involved in fatty acid and glyceride synthesis

7. Effects of thyroid hormone on fatty acid synthesis

8. Plasma triglyceride metabolism in thyroid disease

9. Effects of thyroid hormone on cholesteral metabolism

CHAPTER III

MATERIALS AND METHODS

1. Drugs

2. Experimental animals

3. Collection of serum

4. Extraction of liver and heart

5. Adipose-other vascular tissues
6. Histochemical methods

7. Chemical methods

7.1. Determination of serum transaminase activities

7.2. Determination of serum total cholesterol concentration

7.3. Determination of cholesterol concentration in liver and heart extracts

7.4. Determination of phospholipids concentration in serum and liver and heart extracts

7.5. Determination of triglyceride concentration in serum and liver and heart extracts

7.6. Determination of lipoprotein lipase activity in tissues

7.7. Determination of serum total protein concentration

7.8. Determination of serum albumin concentration

7.9. Determination of globulin concentration
7.1. Determination of serum sodium concentration
7.2. Determination of serum potassium concentration
7.3. Determination of high density lipoprotein cholesterol concentration
8. Histopathological methods

CHAPTER IV

BACKGROUND

1. Effects of TJ and carbenoxolone on serum lipid concentration
2. Effects of TJ and carbenoxolone on liver lipid concentration
3. Effects of TJ and carbenoxolone on heart lipid concentration
4. Effects of TJ and carbenoxolone on the activity of cardiac and skeletal lipoprotein lipase
5. Effects of T3 and carbimazole on high density lipoprotein (HDL) cholesterol concentration

6. Effects of T3 and carbimazole on serum CKT and ECT activities

7. Effects of T3 and carbimazole on the concentration of serum total protein, albumin and globulins

8. Effects of T3 and carbimazole on serum sodium and potassium levels

9. Effects of T3 and carbimazole on total BUN and HbA1c

10. Histopathology

CHAPTER V

DISCUSSION

CHAPTER VI

BIBLIOGRAPHY
The studies described in this thesis were carried out in the Department of Veterinary Clinical Studies of the University of Khartoum.

I express my gratitude to Mrs. E. E. Smith and T. M. James for the supervision and guidance of my experimental work.

I am especially grateful to Professor J. A. I. S. Al-Meini for constant help and encouragement during my studies. The drugs used in these studies were supplied by Professor J. M. Nasser of the Faculty of Medicine, University of Khartoum.

My thanks are also due to Miss Majid Taha, Miss Al-Diem Ali, and Mr. S. Ahmed for technical assistance. Mr. T. M. Al-Meini has typed the thesis rapidly and most efficiently.

This work was financed by the Sudan Academic Exchange Service (SAES) and administered by the Graduate College, University of Khartoum.

[Signature]
concentration of any metabolite in the blood is a function of its rate of entry in the blood (not synthesis or from food) and of its removal rate. For this reason, this specific aim will be achieved by correlating the activity of the enzyme lipoprotein lipase (LPL), an enzyme which is involved in the clearance of triglycerides as a test of the very low density lipoprotein (VLDL), with serum triglyceride values. Clearly, since VLDL particles are also composed of cholesterol, cholesteryl esters and phospholipids, an understanding of the metabolism of the VLDL will also throw light on the metabolism of these lipids.

3) To find if the altered thyroid function for a short period, would have any direct toxic effect on tissues. This is of interest especially with regard to心脏病 and to find out whether it would be effectively effective in ruminants. In addition, and especially for cardiomyopathy, we would like to know if the effect on lipid metabolism is secondary to its effect on the thyroid gland or to a direct action. For this
roten, histological examination and various other parameters were used to evaluate basic properties of 25 and cementum.
The principles involved in the measurement of thyroid function in normals are simple in spite of the occurrence of some practical difficulties. As in all states of disease, an intelligent clinical examination is necessary to indicate whether a disturbance of thyroid function is likely to be present. Thyroid function tests should be employed to confirm the diagnosis and estimate the degree of the abnormality. Among the factors to be considered are the possible enlargement of the thyroid gland, the metabolic rate, body temperature, the presence of obesity, symptoms, and mental reactions to stimuli.

In normals, the thyroid gland consists of two lateral lobes, one on each side of the trachea close to its junction with the larynx. The lobes are connected by an isthmus crossing the ventral surface of the trachea (Sierson, 1937). Microscopically, the thyroid gland is made up of numerous closed follicles. These
are lined with a single layer of low cuboidal epithelial cells and are filled with a viscid colloid. The colloid contains, combined with a protein, the specific thyroid hormone, thyroxine (Hewit and Cear, 1954). It is believed that the rate of secretion is varied by alterations in the blood supply and the anterior pituitary gland has an effect through the thyrotropic hormone which stimulates the thyroid gland (Dukes, 1960).

1. Relation of Iodine to the Thyroid Gland

The thyroid gland has a special affinity for iodine (Dukes, 1960). The average amount of iodine in the normal thyroid gland of mammals has been shown by Hewit, Cear and Sommerson (1956) to vary from 2.2 to 3.5 mg/g of iodine of dried gland.

Dukes (1960) suggested that, in many species, there exists an intimate relation between the amount of iodine in the thyroid gland and its histological appearance. The author mentioned that the iodine content varies directly with the amount of glandular colloid and inversely with the degree of hyperplasia of the gland. For example, when the iodine content remained 0.2% of the
dried weight of the thyroid gland, no hyperplasia was detected.

It is well known that, not all the iodine is present as thyroxine
and there is reason to believe that the thyroid gland serves as a
storehouse for iodine and as protection against its excretion
in excessive amounts (Duke, 1966).

2. Thyroid hormones

The secretion of the thyroid gland contains the hormone
thyroxine or T4, which has been isolated, analyzed and synthesized
(Kovak and Ocar, 1954; Thorpe, Bray and Drew, 1970).

The structural formula of the hormone is

\[
\begin{align*}
\text{HO} & \quad \text{O} \\
\text{I} & \quad \text{I} \\
\text{C} & \quad \text{C} \\
\text{NH}_2 & \quad \text{O} \\
\text{H} & \quad \text{H} \\
\end{align*}
\]

The compound is thus seen to be related to the amino
acid tyrosine, which is probably its precursor (Duke, 1966).
The chief protein, thyroglobulin, present in the thyroid follicles, contains thyroxine (Hawke and Coar, 1964). In addition to thyroxine, the thyroid gland contains the compound diiodotyrosine. This substance is physiologically inactive and is believed to be an intermediary compound in the synthesis of thyroxine (Dokes, 1960; Thorpe et al., 1970). The thyroid gland also contains the iodinated compounds triiodothyronine (T3) and moniodothyronine (Hawke and Coar, 1964). Both T3 and T4 are transported bound to thyroxine binding globulin (T.B.G.). It is well known that the concentration of T4 in the blood reflects thyroid function (Hawke et al., 1974; Dokes, 1960; Thorpe et al., 1970).

3. Clinical importance of the thyroid hormones

In hypothyroidism, the result of a deficiency of iodine in the food and water (Hawke and Coar, 1964) both T3 and T4 levels in the blood are depressed (Rupp, Potashkin and Castanow, 1967). However, these authors suggested that T3 level in the blood may remain within normal limits or that in hyper-
thyroidism, which is due to an over-function of the thyroid gland, 
T₄ and T₃ concentrations in the blood are raised.

T₄ concentration in the blood may be altered by changes in 
the binding capacity of the thyroid hormone binding protein. 
For example, the concentration in the blood of T₄ is elevated 
when the binding capacity increases during pregnancy and after 
the administration of oral contraceptives and estrogens (Rupp 
et al., 1967).

On the other hand, T₄ level in the blood is reduced when 
the binding capacity is decreased as in the cases of severe renal 
and hepatic failure, anticonvulsants therapy (Rupp et al., 1967) and 
administration of drugs such as phenylbutazone and salicylates 
dephenyloxylation that compete for binding sites of T₃,T₄. (Rupp 
et al., 1967).

4. Calorigenic actions of thyroid hormones

The effect of T₄ and T₃ on the basal metabolic rate 
(B.M.R) varies from one tissue to another. For example, Parker 
(1935) found an increase in the rate of oxidation in the liver.
and muscular tissue and a decrease in the rate of oxidation in
the nervous, spleen and brain.

The thyroid hormones cause uncoupling of oxidation and
phosphorylation (Pat, 1953). As a result of these mechanisms, a
portion of the energy produced during cellular oxidative processes
is lost as heat instead of being utilised for synthesis of high-
energy phosphorus compounds. The effect occurs only with high levels
of the thyroid hormone (Fith and Trotter, 1964).

5. Effects of thyroid hormone on vitamin metabolism

High levels of the thyroid hormone have been reported to
inhibit vitamin A synthesis from its precursors and may result in
vitamin A deficiency (Klein, Campbell and Fetz, 1959). The ad-
ministration of thiamine when the weight loss observed in
hypothyroidism (Gray et al., 1967).

6. Effects of thyroid hormone on protein metabolism

Small amounts of the thyroid hormone have negligible effects
while large amounts of the hormone have ectopic effects (Gray
of thyroid hormone on carbon dioxide exchange.

While increased absorption of glucose, glycogen, and other sugars from the gut was observed in the absence of the thyroid hormone, increased absorption of the sugars was observed in the presence of large amounts of the thyroid hormone (Shaw et al., 1967).

The thyroid hormone enhances glycogen breakdown and at the same time it increases gluconeogenesis and utilization of glucose by the tissues (Shaw et al., 1967).

Kaye and Nalband (1970) were able to increase gluconeogenesis in perfused livers from hypothyroid rats.
.9. Effects of the thyroid hormone on the activities of enzymes involved in fatty acid (FA) and glyceride synthesis

The influence of the thyroid hormone on fatty acid synthesis was studied in rats. The activity of acetyl co-A
carnitine and folic acid synthetases was increased significantly in the adipose tissue and markedly enhanced in the liver of thyroxine-treated rats (Cancemi and Burgy, 1973). The use of antibodies specific against rats liver fatty acid synthetase showed that the change in the activity of this multisubunit complex was due to activation in the amount of enzyme protein. At the molecular level, these results were attributed to an increase in the activity of N.A.D. dependent Era synthase (Nata, 1975).

Tritiated thyronine was found to increase the activity of acetyl co-A carboxylase and fatty acid synthetase from co-A in both liver and fat tissues in rats (Blumert and Sharfier, 1976).

The cell-free fractions of the heart derived from thyroxine-treated rats also showed enhancement of glyceride synthesis (Cancemi et al., 1973).

Myocardial triglyceride content was found to increase in hypothyroid animals (Brossier and Mitola, 1966).

Tritiated thyronine was found to enhance the heart micro-
comprised phospholipid and diglucol phospholipid formation in yasks (Rohe and Liu, 1976).

An increase in thyroxine fatty acid oxidation and lipid
biosynthesis in the heart and liver of rats was reported by
Aritchayas and Zappa (1962).

Increased liver biosynthesis of lipids results in an in-
crease in low density lipoprotein which occurs in the presence of
thyroid hormone excess (Mikilo and Kobal, 1971).

The plasma triglyceride concentration was reduced in
hypothyroid patients (Sandhufer and Brauerstain, 1960). Changes
in hepatic metabolism of the free fatty acid (FFA) and thyroid
status affect the hepatic output of triglyceride and very low
density lipoprotein (VLDL) (Loeser and Heidberg, 1979).

IV. Effects of thyroid hormone on fatty acid (FA) synthesis

It is well known that the thyroid gland of animals regulates
the hepatic metabolism of FA. In rats treated with the TSH extract,
the livers of hypothyroid mice produced more triglycerides
than those from hyperthyroid mice (Agus et al., 1975).
The hepatic oxidation of FA increases in hyperthyroidism (Hayman, 1966; Blumet, Gorin and Shafier, 1970), and at the same time the synthesis of fatty acids is stimulated (Spitera, Ador and Weinhouse, 1953).

In hyperthyroidism in rats, the increased serum FFA depresses fatty acid synthesis (Deininger, Ringrose, Angliss, Holstein and Wilcox, 1973).

Laugier (1957) suggested that the synthesis of fatty acids in the cell is regulated by the concentration of nicotinamide adenine dinucleotide phosphate (NAF) and that the reduction of NADP in thyroidic liver results in a decrease in the synthesis of FA.

11. Plasma triglyceride metabolism in thyroid disease

A linear positive correlation between the concentration and turnover rate of plasma triglyceride was observed in thyroid hyperfunction and euthyroid, but the efficiency of the removal of triglyceride from the circulation was improved in the thyroid hyperfunction (Hikida and Koketsu, 1976).
Deoxyribonuclease activity (DNA) was also observed to be above normal in thyrotoxicosis (Klemp, 1968).

In hyperthyroidism, in vivo, the synthesis of triglyceride was found to be normal, but the fractionation removal of both exogenous and endogenous triglyceride was reduced (Michl and Kepki, 1972). The same authors reported that the thyroid hormone influences both production and removal of triglyceride and suggested that, in hyperthyroid state, the main diminution is the increased synthesis of endogenous plasma triglycerides. This leads to an elevation of plasma concentration which is relatively slight, since the efficiency of triglyceride elimination is simultaneously increased in thyrotoxic condition. On the other hand, slight or moderate hypertriglyceridemia develops as a result of an impaired removal of triglyceride, and the elimination of exogenous fat particles decreases (Zich and Schafer, 1959). In hypothyroidism, there is an increase in the turnover rate and plasma concentration of LDL and glycerol (Sato and Thompson, 1955).

In hypothyroidism, changes in triglyceride metabolism
are not opposite to those in hypothyroidism. For example, Nikkila and Hakki (1972) showed that the hepatic triglycerides were not diminished in severe hypothyroidism in spite of the fact that plasma FFA concentrations were below normal.

12. Effects of thyroid hormone on cholesterol metabolism

In hypothyroidism, plasma cholesterol levels were reported to be 2.5 times higher than normal with an increase in both free and esterified forms of cholesterol (Dattir and Lain, 1990).

The total increase in cholesterol concentration takes place mainly in the low density lipoprotein (Malorn and Benin, 1963). The treatment of such individuals with thyroxine resulted in the return of plasma cholesterol concentration to normal values (Oliver and Bogan, 1960). However, in the presence of high levels of thyroid hormones in the plasma an increase in the synthesis of cholesterol was observed (Kurpa and Rober, 1963).

Cholesterol synthesis in the liver was suppressed by the administration of cholesterol (Touging, Steppard and Colow, 1953).

Prida (1952) and Welsh and Ture (1955) found a decrease
in the excretion of both cholesterol and other unspecific sterols in thyrotoxic animals.

Kitchener and Lyman (1956) maintained that the effect of thyroxine on cholesterol metabolism is mediated by its action on other endocrine glands such as the adrenal cortex and gonads.

Hypocholesterolemia and hypercholesterolemia associated with hypothyroidism and hyperthyroidism, respectively, were investigated by Putter and Man (1950), Saito and Lawrence (1964), Krichchevsky (1964) and Walton, Dykes and Davies (1965).
CHAPTER III

MATERIALS AND METHODS

1. Drugs

1.2. Triiodothyronine sodium

Triiodothyronine (T3, synthetic, 131I sodium) is absorbed from the gastrointestinal tract and is more readily bound to plasma proteins than thyroxine (Desai, 1956). Liothyronine is an active principle of the thyroid gland. It has been suggested that thyroxine becomes active only through conversion to liothyronine which is probably the final active substance of the thyroid hormone (Martindale, 1972). It is qualitatively similar to thyroxine in action but is much more potent and rapid in producing the effect. On cessation of treatment there is a rapid return to the normal metabolic state. Optimal was prepared by Smith Kline 

French Laboratories (B.K. and F.) in form of tablets, each containing 50 µg of triiodothyronine.
1.1.2. Carbimazole

Carbimazole (2-thio-4-thiobenzyl-2-thio-5-imidazoline-1-carboxylic acid CHN₂S₂O₃) and other thiocarbamides are absorbed from the gastrointestinal tract and are widely distributed throughout the body. These drugs readily cross the placenta and are excreted in the urine and milk (Doniach, 1953).

Carbimazole is soluble in water and it acts as an anti-thyroid substance which is known to suppress the formation of the thyroid hormone by reducing the uptake and concentration of inorganic iodine by the thyroid gland (Porteous, 1954).

Carbimazole (non-named) was prepared by Nicholas Laboratories Ltd., U.K. and obtained from the Ministry of Health, Merck, in form of tablets, each containing 5 mg of the active constituents.
2. Experimental animals

Experiment 1

Twenty clinically healthy male Syrian goats, 4 to 6 months-old, weighing 22 - 26 kg were divided into four groups of five each. The goats were received in pens at the Department of Veterinary Clinical Studies, University of Khartoum, and given free access to Lucerne and water.

Administration of triiodothyronine (T3)

T3 tablets were powdered, dissolved in distilled water and given by mouth daily for fourteen days at the following dose rates: 250 µg/goat (group 2), 400 µg/goat (group 3) and 500 µg/goat (group 4). Goats in group 1 served as unmedicated controls.

Experiment 2

Fifteen clinically healthy male Syrian goats, 4 to 6
month-old, weighing 21 - 27 kg were divided into three groups of five each. The goats were kept in pens at the Department of Veterinary Clinical Studies, University of Khartoum, and were fed on lucerne and water ad libitum.

Administration of carnitine

Carnitine was suspended in distilled water and given by drench daily for fourteen days at the following dose rates: 60 mg/goat (group 2) and 30 mg/goat (group 3). Goats in group 1 were used as controls.

The goats were weighed before the experiment started and every 4 or 7 days thereafter. The body weights were recorded and clinical examination was carried out on the experimental animals.

The goats were slaughtered on day 15 and tissues were collected for histopathological examinations, lipid extractions, and determination of lipoprotein lipase activity.
3. Collection of serum

Animals were bled in vacuo in containers containing ethylene-diamine-tetra acetic acid (EDTA) from the jugular vein on several occasions before being killed at suitable intervals afterwards. Part of the blood was centrifuged at 3500 r.p.m. for 15 minutes. The separated sera were collected and stored at -20°C pending analysis for various serum constituents. The other part of the blood was used for haematological investigations.

4. Extraction of liver and heart lipid

Immediately after slaughter and isolation of liver and heart were homogenised with cold 0.9% normal saline (6.0 ml/g of tissue). Total lipids in samples of liver and heart suspensions were extracted with chloroform-methanol 2:1 (V/V) according to the method of Folch, Lees and Sloane (1957) as follows:

1. To 1 ml of liver or heart suspension 6.5 ml of methanol were added and the mixture was kept for 5 minutes.
2. 13 ml of chloroform were added and the mixture was left overnight at room temperature.

3. 27 ml of isooctane (4.4%) were added, mixed and left overnight at room temperature.

4. The top aqueous layer was carefully aspirated and chloroform-lipid phase was then filtered through a small No. 1 filter paper to remove the precipitated proteins which were washed three times with 10 ml of chloroform.

5. The chloroform was evaporated to dryness and lipid precipitate was then brought to 10 ml with chloroform from which aliquots were used for the various lipid determinations.

6. 4.0 ml of isooctane was similarly extracted for the determination of lipid concentrations.

7. Aqueous-ether-macerated tissues

The aqueous-ether powder was prepared from the heart and skeletal muscles according to the methods of Korn (1955).
Immediately after the slaughter of goat, 2 g of tissues were
concentrated with cold redistilled acetone in a Waring Blender
for 3 minutes and filtered by the use of a Buchner funnel attached
to a suction pump. The resulting cake was blended again with
acetone, filtered and washed with ethyl ether. The final
powder was dried over calcium chloride in a desiccator and stored
at -20°C until used.

6. Haematological methods

6.2. Haemoglobin concentration (Hb)

The concentration was determined by the cyanmethaemoglobin
technique with a haemoglobin meter (Avan Electromedical Ltd.,
England). The method is based on the conversion of haemoglobin
tocyanmethaemoglobin by means of Lebed's solution (1.2 g
potassium cyanide, 0.2 g potassium ferrocyanide and 1 g sodium
bicarbonate per litre of distilled water). The haemoglobin con-
centration measured in g/100 ml was converted to ml/L.
6.2. Fasted cell volume (FCV)

Small samples of blood were centrifuged in a micro-haemocrit centrifuge (Hawksley and Sons Ltd., England) for 3 minutes. FCV per cent was read using the scaling instrument provided with the centrifuge.

6.3. Red blood cell count (RBC)

Red blood cells were counted using an improved Neubauer haemocytometer (Hawksley and Sons Ltd., England). Normal saline was used as a diluting fluid.

6.4. Total white blood cell count (WBC)

Total white blood cells were counted with an improved Neubauer haemocytometer (Hawksley and Sons Ltd., England). Rook’s fluid (15% glacial acetic acid buffered with gum agar wets) was used as diluent.
7. Chemical Methods

7.1. Determination of serum glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) activities

The activities of GOT and GPT were measured according to the method of Bittman and Freinkel (1957).

The principle of the method can be described as follows:

For GOT:

\[ \text{Aspartic acid} \rightarrow \text{-L-glutamic acid} \rightarrow \text{oxaloacetic acid} \rightarrow \text{glutamic acid} \]

For GPT:

\[ \text{Alanine} \rightarrow \text{-L-glutamic acid} \rightarrow \text{pyruvic acid} \rightarrow \text{glutamic acid} \]

2,4-Dinitrophenyl hydrazine was used as a colour reagent and the colour intensity of the resulting dinitrophenylhydrazones is proportional to the transaminase activity.
Procedure for GGT

1. 1.0 ml of proper substrate (0.02 M of DL-sketoglutaric acid + 0.66 g of mercapto acid + 20 ml of 1M NaCl adjusted to pH 7.5) was pipetted in a test tube and placed in a water-bath at 37°C to warm.

2. 0.2 ml of serum was added to the substrate in the test tube, mixed and returned to the water-bath.

3. Exactly one hour after adding the serum, 1.0 ml of colour reagent (0.0356 g 2,4-dinitrophenylhydrazine + 200 ml of 1M NaCl) was added, shaken gently and left at room temperature.

4. 20 minutes after the addition of the colour reagent 10.0 ml of 0.14M sodium hydroxide solution (6.3 g NaOH in 1.0 litre distilled water) were added and mixed by inversion.

5. Five minutes later, the transmission (%) was recorded against the wavelength of 520 millimetre using an EL Spectra (World Instruments Ltd., England).
6. Calculations: The activities of GOD in Sigma Model (3.5) were converted to International units (7.4).

Procedure for GOD

1. 1.0 ml of prepared substrate (0.1 ml of 1.75 g L-glutathione, 0.022 g of distilled water, 23 ml of solution adjusted to pH 7.5) was pipetted in a test tube and placed in a water-bath at 37°C to warm.

2. 0.2 ml of serum or standard (0.02 g of sodium pyruvate in 100 ml phosphate buffer made of 83 ml of solution A (14.2 g of K2HPO4 in 1000 ml of distilled water) and of solution B (13.6 g of KH2PO4 in 1000 ml of distilled water adjusted to pH 7.5) was added, simultaneously and placed in the water-bath at 37°C.

3. Exactly 30 minutes after adding serum, 1.0 ml of the colour reagent was added, mixed and the mixture was kept at room temperature.
4. 20 minutes after adding the colour reagent, 12.0 ml of 0.1M NaOH solution were added and mixed.

5. After 5 minutes the transmission (AT) was read against the wavelength of 520 millimicrons using the XE1 Spectrophotometer.

6. Calculation: The activity of GGT in Sigma Avondel (S.F) units was converted to international units (I.U.).

7.2. Determination of serum total cholesterol concentration

The concentration of serum total cholesterol was determined by the method of Bitkin, Zak and Boje (1969).

The principle of this method

Cholesterol in serum when dissolved in glacial acetic acid reacts with concentrated sulphuric acid in the presence of iron in typical alcohol reaction to produce red coloured products entirely cholesterylone and cholesterolanthracene ions.
Procedure

1. 0.5 ml of serum or cholesterol standard (200 mg/100 ml in glacial acetic acid) was placed in a test tube.

2. 5 ml of the colour reagent (PbCl₂ - H₂O, 0.5M) in alcohol free glacial acetic acid) were added, mixed, kept at room temperature for 10 minutes, and centrifuged at 3000 r.p.m. for 5 minutes.

3. The supernatant was transferred to the bottom of another test tube.

4. 3 ml of concentrated H₂SO₄ were added, mixed well and the transmission (YT) was read at a wavelength of 520 milli-microns.

5. Calculation: The concentration of the total cholesterol in serum in mg/100 ml was converted to mol/L.
7.3 Determination of cholesterol concentration in liver and heart extracts

Aliquots of the liver and heart cholesterol extracts were evaporated to dryness. 5.0 ml of the colour reagent (0.015 M HCl, 6 % HgO (w/v) in ethylene-free glacial acetic acid) were added and mixed and the total cholesterol content was determined as described for the serum.

7.4 Determination of phospholipids on liver in serum and liver and heart extracts

The concentration of phospholipids was determined by the method of Bartlett (1950).

Principle of the method

The method involved the oxidative digestion of lipid by perchloric acid and hydrogen peroxide for the liberation of inorganic phosphate. The inorganic phosphate reacted with
ammonium molybdate to yield phosphomolybdate ions which are
further reduced to give a blue colour.

Procedure

1. 1 ml of serum and of liver or heart extracts or standard
   \( \text{CH}_3\text{PO}_4 \) or \( \text{Na}_2\text{HPO}_4 \) \( 10 \text{ mg/ml} \) was placed in a test tube
   and the chloroform extract was evaporated to dryness in a
   water bath at 85°C.

2. 0.5 ml of perchloric acid (75%) was added and mixed.

3. 0.2 ml of iron-cupric perchlorate (5%) was then added, mixed and
   heated in an oven at 100°C for 30 minutes.

4. After removal from the oven, 1 ml of distilled water was
   added.

5. 2.5 ml of ammonium molybdate (2.5%) was added and mixed.

6. 1.2 ml of the colour reagent (sodium nitroprusside sulphonic acid
   0.05 g in 5% of sodium metabisulphite) and 0.2% sodium
sulphoal was added # added and the test tube was placed in
an oven at 100°C for 10 minutes.

7. The examination per cent was read at 680 millimicrons.

8. Calculation: The value of phosphoal was calculated in
mg/100 ml and the results were then converted to mmol/L.

7.5. Determination of triglyceride content in serum and
liver and heart extracts

The determination of triglyceride content in serum and liver and heart extracts was performed as described by Weir,

The procedure of the method

1. After extraction of lipids, 2 ml of aliquots or the standard
(trioblate, 3 mg/50 ml of chloroform) were placed in a
capped test tube containing 0.8 g silica gel,
2. 2 ml of chloroform were added to the test tube and centrifuged at 5000 r.p.m. for 5 minutes.

3. 1 ml of the upper supernatant solution was pipetted into another test tube and evaporated in a water bath at 85°C.

Solution

1. 4.2 ml of the working alcoholic KOH solution (2.50 g of 36.5% KOH in 200 ml of redistilled 95% ethanol) was added, mixed thoroughly and placed in an oven at 100°C for 30 minutes for saponification.

5. 0.2 ml of 5.0 N HCl was added.

6. 3 ml of petroleum ether were added, mixed and left to stand for five minutes.

7. The upper petroleum ether layer was removed by suction and the remaining layer was evaporated by placing the tube in a water bath at 70°C and then left to stand at room temperature.

8. 1 ml of 0.02M HCl was added, mixed thoroughly and left to stand for 10 minutes.
9. 5.2 ml of 5% NaOH was added, mixed and kept for 5 minutes.

10. 3 ml of the colour reagent, chromotropic acid 1.5% were added and vortexed.

11. The tube was then placed in an oven at 100°C for 30 minutes.

12. The test tubes were removed from the oven and allowed to cool at room temperature. 0.2 ml of 10% formaldehyde was added and mixed well.

13. Changes in the scale readings of the pH Meter were recorded at 570 millivolts, and results were converted to pH.

14. Determination of mineralogical elements in tissues

Eggs as an example:

The source of the eggs was the chicken egg-cooper producer prepared from the antibiotic and heart samples.
Extraction of Eggshell Membrane

100 mg of the dried eggshell powder were extracted
with 2 ml of 10\% \(\text{NH}_4\text{OH}\) buffer pH 8.6, according to the method
described by Korn (1955). The extract was centrifuged at
2000 rpm for 10 minutes and the supernatant was used
immediately as the enzyme source.

Enzyme Activity

The enzyme activity was proposed as described by
Selby et al. (1970). To 1 mg of eggshell Tri (1-\(\alpha\)) clan
(specific activity 50 mCi/mg code 1, 2, 3, 4, radioc.
mochlancy purity 99\% obtained from Radiochemical Centre,
Buckinghamshire, England) contained in 1 ml benzene was added to
150 mg cold trypsin dissolved in benzene and the mixture was
overmixed to ensure uniformity of a trypsin. The following
substrates were added to the mixed trypsin:

1. 3 ml of fresh goat serum
1. 0.5 ml of 1% bovine serum albumin dissolved in Tris buffer and adjusted to pH 8.0.

2. 0.5 ml of triton X-100.

3. 7.2 ml of 0.2 M tris buffer, pH 8.2.

The mixture was placed in an ice bath and sonicated for 5 minutes in the sonicator (Ultrasonic Disintegrator M X-2, diameter 9.5 cm and ratio 5:1). The resulting milky solution was used as the enzyme substrate.

**Incubation conditions**

Enzyme extract (0.2 ml) was added to 0.8 ml of the substrate in glass-stoppered test tube and the mixture was incubated at 37°C in a shaking water bath for 60 minutes.

**Extraction of precipitated fatty acids**

The reaction was terminated by addition of...
1. 4 ml isopropanol - 3N H$_2$SO$_4$ mixture (v:v 1:1).

2. 2 ml of distilled water and 5 ml of hexane.

The mixture was shaken thoroughly for 10 minutes.

The upper hexane layer was removed with a Pasteur pipette and 5 ml of this hexane layer were mixed with 1 ml of 0.1N KOH.

The released fatty acids were extracted into the lower KOH phase.

**Radioactivity counting**

1 ml of KOH phase was dissolved slowly in 30 ml of instagel (8 g/L ppo (2,4-diphenylloxazole)-0.1 g/methyl-pyron (2,4-bis 4-methyl-5-phenyl-oxazolyl) benzene) in a counting vial which was capped and shaken for 20 minutes. The radioactivity in the released fatty acids was measured in a liquid scintillation counter (Nuclear Enterprises Ltd., England (NPL 132-2 photomultiplier mode M 1672 (linear), M 4671 (logarithmic) and M 4671 (model)). The enzyme specific activity was defined as the number of disintegrations of the TS released by one milligram of the enzyme protein in one hour.
7.7. Total protein concentration

Sera total protein concentration was determined by the use of the biuret reagent as described by [Michaelis and Bernhard, 1945]. The principle of the method is based on the reaction of the protein with copper sulphate in the presence of sodium hydroxide. 8-Mmol-lactate (inhibitor salt), was used to keep in solution, the formed copper hydroxide which gives the blue colour.

Changes in the scale readings of the 622 spectrophotometer were recorded.

Calculation: The concentration of sera total protein was calculated in g/100 ml as follows:

\[
\text{Total serum protein} = \frac{\text{readings of test} - \text{readings of standard}}{x \text{ concentration of standards}}
\]

5.7.1. Serum albumin concentration

Serum albumin concentration was determined by the use...
of transcorval green reagent as described by Rockey (1965).

Procedure

1. A 0.2 ml of serum or standard (e.g. pure crystalline bovine albumin in 100 ml of distilled water) was placed in a test tube and 4 ml of transcorval green reagent (0.176 g B.O.G., 2.5 ml 0.1M NaOH + 2.5 ml distilled water). The resulting solution: 6 ml of B.O.G. were added to 37.5 ml of 1M sodium citrate and 5.7 ml of citric acid on; volume made up to 1000 ml with distilled water. pH was adjusted to 6.8 and gently mixed.

2. Changes in the scale readings of the B.E. Spectra at 657 millimicrons were recorded and values were expressed in /10 ml.

7.3. Determination of serum globulin.

Serum globulin concentration was obtained by subtracting...
the value of serum albumin from the total serum protein concentration and recorded in g/100 ml.

7.10. Serum total protein concentration

The concentration of serum albumin was determined by a flame photometer (model Electroanalyser Ltd., England) as described by Verley (1967). The method is based on passing, under controlled conditions, a diluted serum solution diluted as a very fine spray in the air supply to a burner where the solution vaporizes and dissociates to give neutral atoms emitting light which is passed through a specific filter for sodium onto a photomultiplier cell. The amount of the current produced was read on a galvanometer scale. To form spray, compressed air was passed through an atomizer into which serum was drawn by a suction pump and then directed into the burner with its air supply. Air pressure was adjusted to 15 lb per square inch, and butane gas was used for the flame. Changes in the galvanometer readings were recorded and the results calculated using verbal
dilutions of stock standard solution of sodium chloride (100, 320, 108, and 350 \ microg/L). The results were then converted to
mEq/L.

7.11. Serum potassium concentration

The method used for the estimation of serum potassium concentration is based on the same principle of flame photometry as in the case of serum sodium. However, specific filter for potassium was used. The changes in the galvanometer readings were recorded and values estimated in mEq/L using serial dilutions of stock standard solution of potassium chloride (4, 6, 8, 12, and 16 mEq/L). The results were then converted to
mEq/L.

7.12. Determination of high density lipoprotein (HDL)
cholesterol

HDL cholesterol was determined after the precipitation of LDL, VLDL, and non-HDL cholesterol according to the method of
Warnick and Albers (1978).
1. To 1.0 ml of serum in a test tube was added 0.2 ml of the working horseradish peroxidase solution (2.6 ml of sodium horseradish peroxidase solution, 0.005 mg units/ml, mixed to 11.0 ml of 1.16 M NaCl solution), mixed thoroughly and incubated at room temperature for 10 minutes.

2. The test tube was then centrifuged at 10,000 r.p.m. for 30 minutes.

3. The supernatant was recovered for analysis of catalase and as previously described.

---

**Histopathological Methods**

Tissues for histopathological examination were immediately fixed in 10% formalin solution, embedded in paraffin wax, sectioned at 5 um thick and stained with hematoxylin and eosin (H & E).
The body temperature and weights of the goats and the daily doses of T3 and carbinsole they received are given in Tables 1 and 2.

There were no significant changes in body weights and temperatures in T3 or carbinsole-treated goats.

1. The effects of T3 and carbinsole on serum lipid concentration in male bullock goats

T3 treatment for 14 days resulted in a significant reduction in serum cholesterol concentration (Fig. 1). On the other hand, carbinsole treatment for a similar period of time did not produce significant changes in serum cholesterol concentration (Fig. 1).

The effects of the two drugs on serum triglyceride
Fig. 1. The effect of T3 and carbimazole treatment on total serum cholesterol concentrations in male Wistar rats.

Values given are mean ± S.E.M. of five animals.
Statistical significance is compared with the control values.
concentration are presented in Fig. 1. Serum triglyceride concentrations were significantly decreased by T3 treatment. Cumarinole at a dose rate of 60 mg/animal had no effect on serum triglyceride levels. However, when given to rats at the rate of 20 mg/animal, cumarinole caused a significant increase in serum triglyceride concentration. As with serum cholesterol and triglyceride levels, treatment of groups with T3 at doses of 250, 450, and 550 mg/animal resulted in a significant elevation in serum phospholipid concentration (Fig. 3). However, cumarinole treatment of rats at the doses used had no effect on serum phospholipid concentration (Fig. 3).

1. The effects of T3 and cumarinole on liver lipid concentration

At all doses tested, T3 caused a significant decrease in cholesterol levels in the liver (Fig. 4). Conversely, cumarinole increased liver cholesterol levels which reached significant values at the dose of 30 mg/animal (Fig. 4).

Although T3 treatment resulted in a decrease in liver
Fig. 3. The effect of T3 and cortisone treatment on serum phospholipid concentration in male Syrian hamsters.

Values given are means ± S.E.M. of five animals.

Statistical significance is compared with the control values.
Fig. 6. The effect of 2% indomethacin treatment on hepatic cholesterol concentration in male S auctions.

Values given are mean ± S.E.M. of five animals. Statistical significance is compared with the control values.
triglyceride levels, a significant reduction was only observed in goats which had received the highest dose of the drug, 550 μg of T3/animal. The group of goats receiving 550 μg of T2/animal showed a border line significance (Fig. 5). On the other hand, oestradiol at both doses used significantly increased hepatic triglyceride levels (Fig. 5).

A decrease in the liver phospholipid concentration was observed in goats which had been given T3 at a dose of 550 μg/animal; however, a significant reduction was evident in the group of animals which received 550 μg of T2/animal (Fig. 6). Hepatic phospholipid level was not determined in the group of goats which received 550 μg of T2/animal. At both doses used, oestradiol was ineffective in modulating liver phospholipid levels (Fig. 6).

4.3. The effect of T3 and oestradiol on hepatic lipid content

With increasing doses of T3, the hepatic cholesterol levels tended to fall; however, a significant decrease was only observed in the goats which received T3 at a dose of 550 μg/
Fig. 5. The effect of 1% and 2% cellulose treatment on
serum triglyceride concentration in male
Golden sows.

Values given are means ± S.E.M. of five animals.
Statistical significance is compared with the
control values.
Fig. 6. The effect of 

The effect of Fj and carbimazole treatment on 

hepatic phospholipid concentration in rats 

hepatic phospholipid concentration in rats 

hepatic phospholipid concentration in rats 

hepatic phospholipid concentration in rats 

Values given are means ± S.E.M. of five animals. 

Statistical significance is compared with the 

control values.
animal (Fig. 7). On the other hand, no effect on cardiac
cholesterol concentration was observed with cerivastatin treat-
ment (Fig. 1).

Triglyceride levels in the heart were significantly
decreased by T3 treatment at all doses used (Fig. 8), conversely,
cerivastatin treatment at two doses used, significantly increased
triglyceride levels in the cardiac tissue (Fig. 8).

T3 treatment at a dose of 400 μg/animal significantly
reduced the concentration of phospholipid in the heart. The
dose of 250 μg of T3/animal caused no change in the level of
cardiac phospholipid (Fig. 3). Phospholipid values were not
determined in the group of goats which received 500 μg of
T3/animal.

At a dose of 60 mg/animal, cerivastatin did not affect
the heart phospholipid levels. However, with the higher dose of
cerivastatin (90 mg/animal) significant increases in phospha-
lipid concentrations were observed (Fig. 9).
Fig. 7. The effect of T3 and cornflakoids treatment on carotid cholesterol concentration in male Nubian goats.

Values given are means ± S.E.M. of five animals. Statistical significance is compared with the control values.
Fig. 8. The effect of T3 and continuous treatment on thyroid gland size and activity in male Guinea pigs.

Values given are mean ± S.E.M. of five animals.

Statistical significance is compared with the control values.
Fig. 9. The effect of X and cadmium treatment on camelina phytolipid concentration in male tissue grains.

Values given are mean ± SEM of five plants. Statistical significance is compared with the control values.
The effects of T3 and coadministration on the activity of skeletal and cardiac lipoprotein lipase

In the heart tissue, T3 treatment caused a significant increase in the activity of lipoprotein lipase. The increase in enzyme activity was sharpest in the group of rats which had received 100 and 250 μg of T3/animal (Table 2). Although T3 also resulted in a steady increase in lipoprotein lipase activity in the skeletal muscle (Table 4), the increase was not as substantial as that observed in the heart tissue. On the other hand, carnitine treatment did not affect lipoprotein lipase activity either in the heart (Table 1) or skeletal muscles (Table 4).

In rats, T3 treatment resulted in a significant increase in HDL cholesterol concentration (Table 5), however, there was no difference in LDL concentration between the different doses used.
Table 3. Changes in the activity of lipoprotein lipase (LPL)
in cardiac tissues of SJ and noradrenaline-treated male
Brownian rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Hypothyroidism</th>
<th>Hyperthyroidism</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>UFP/1 mg enzyme\nprotein</td>
<td>UFP/1 mg enzyme\nprotein</td>
</tr>
<tr>
<td>I (control)</td>
<td>21.3</td>
<td>224.3</td>
</tr>
<tr>
<td>II</td>
<td>41.7</td>
<td>16.29</td>
</tr>
<tr>
<td>III</td>
<td>134.5</td>
<td>128.6</td>
</tr>
<tr>
<td>IV</td>
<td>395.1</td>
<td>(-)</td>
</tr>
</tbody>
</table>

(-), activity of LPL was not determined.
Table 4. Changes in the activity of lipoprotein lipase (LPL)
in the skeletal muscles of 53 and salsodose-treated male abaxian mice.

<table>
<thead>
<tr>
<th>Group No.</th>
<th>Hypothyroidism 10 μg/l mg enzyme protein</th>
<th>Hypothyroidism 20 μg/l mg enzyme protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>I (control)</td>
<td>38.9</td>
<td>38.3</td>
</tr>
<tr>
<td>II</td>
<td>38.7</td>
<td>148.6</td>
</tr>
<tr>
<td>III</td>
<td>75.3</td>
<td>92.6</td>
</tr>
<tr>
<td>IV</td>
<td>125.1</td>
<td>(-)</td>
</tr>
</tbody>
</table>

(-), activity of LPL was not determined.
Table 3. Serum high density lipoprotein (HDL) cholesterol percentage in T3 and carbenoxolone treated male patients. 

<table>
<thead>
<tr>
<th>Group (N)</th>
<th>Hypothyroidism (mg litre$^{-1}$)</th>
<th>Hypothyroidism (mg litre$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I (control)</td>
<td>$63.7 \pm 3.97$</td>
<td>$66.13 \pm 2.83$</td>
</tr>
<tr>
<td>II</td>
<td>$97.2^{***}$</td>
<td>$68.8 \pm 5.38$</td>
</tr>
<tr>
<td>III</td>
<td>$84.0 \pm 1.38 \ N.S.$</td>
<td>$57.71 \pm 2.33 \ N.S.$</td>
</tr>
<tr>
<td>IV</td>
<td>$91.0^{**}$</td>
<td>$(..)$</td>
</tr>
</tbody>
</table>

*Values given are means ± S.E.M. of five animals.*

**Statistical significance is compared with control values.**

*$p < 0.05, \quad **p < 0.01, \quad ***p < 0.001$

(-) Serum HDL cholesterol concentration was not determined.

N.S. Not significant.
No effect on HDL cholesterol level was observed with carnosol treatment at both doses used.

6. The effects of T3 and carnosol on serum HDL and LDL concentrations

Neither T3 nor carnosol treatment had any significant effect on serum HDL activity (Fig. 10) or on serum LDL activity (Fig. 11).

7. The effects of T3 and carnosol on the concentrations of serum lipids, albumin and fibrinogen

T3 and carnosol treatments had no significant effects on the serum concentrations of total protein (Fig. 13), albumin (Fig. 13) or fibrinogen (Fig. 14).

8. The effects of T3 and carnosol on serum motility and attachment assays

The concentration of serum medium was not affected by
Fig. X: The effect of T3 and carbimazole treatment on serum GGT activity in male thiamin gnotobes.

Values given are means ± S.E.M. of five animals.
Statistical significance is compared with the control values.
Fig. 11. The effect of T3 and carbimazole treatment on serum GGT activity in male New Zealand white rabbits.

Values given are means ± S.E.M. of five animals. Statistical significance is compared with the control values.
Fig. 12. The effect of 25 mg carbamazepine treatment on total serum protein concentration in male golden hamsters. Values given are means ± S.D. of five animals. Statistical significance is compared with the control values.
Fig. 13. The effect of 13 on spermatic testosterone on serum albumin concentration in male rabbits grade.

Values given are means ± S.E.M. of five animals. Statistical significance is compared with the control values.
Fig. 1. The effect of T3 and methimazole treatment on 
serum glucocorticoid concentration in adult vitiligo patients.

Values given are means ± S.E.M. of five animals.
Statistical significance is compared with the 
control values.
either T3 or carbimazole treatment (Table 6). Similarly, serum potassium concentration did not change by either treatment (Table 7).

3. The effects of T3 and carbimazole on total RBU and MBV must be assessed in combination and PCV values.

T3 and carbimazole treatments did not cause significant changes in the total number of RBC and WBC or in PCV or MBV content (Tables 8, 9, 10 and 11) when compared to the corresponding value in the control goats.

4. Histopathology

There were no significant lesions in T3-treated goats or in the control animals. Carbimazole treatment at a dose of 20 mg/goat produced fatty myeloid vacuolation of the hepatocytes and degeneration of the cells of some renal convoluted tubules.
<table>
<thead>
<tr>
<th>Group No.</th>
<th>Hypothyroidism (23 days)</th>
<th>Hypothyroidism (53 days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I (control)</td>
<td>122.4 ± 4.34</td>
<td>138.0 ± 3.6</td>
</tr>
<tr>
<td>II</td>
<td>144.92 ± 7.05</td>
<td>355.75 ± 1.25 N.S.</td>
</tr>
<tr>
<td>III</td>
<td>195.92 ± 26.74 N.S.</td>
<td>135.6 ± 6.8 N.S.</td>
</tr>
<tr>
<td>IV</td>
<td>226.35 ± 3.30 N.S.</td>
<td>(—)</td>
</tr>
</tbody>
</table>

Values given are means ± S.E.M. of five animals.

Statistical significance is compared with the control values.

(—), serum concentration of sodium was not determined.

N.S. not significant.
Table 7. Serum potassium concentration in mmol/L in T3 and carbimazole-treated male albino pups.

<table>
<thead>
<tr>
<th>Group No.</th>
<th>Hypothyroidism (T3 treatment) mmol/L</th>
<th>Hypothyroidism (carbimazole treatment) mmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>I (control)</td>
<td>2.37 ± 0.63</td>
<td>2.58 ± 0.38</td>
</tr>
<tr>
<td>II</td>
<td>4.68 ± 0.87 N.S.</td>
<td>3.1 ± 0.65 N.S.</td>
</tr>
<tr>
<td>III</td>
<td>4.11 ± 0.30 N.S.</td>
<td>4.25 ± 0.22 N.S.</td>
</tr>
<tr>
<td>IV</td>
<td>5.0 ± 0.2 N.S.</td>
<td>(-)</td>
</tr>
</tbody>
</table>

Values given are means ± S.E.M. of five animals.

Statistical significance is compared with the control values.

‘N.S.’: Not significant.

(-), serum potassium concentration was not determined.

N.S., not significant.
Table 8. Total WBC counts (x10^6/mm) in T3 and carbimazole-treated male Nissen goats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Hypothyroidism (T3 treated)</th>
<th>Hypothyroidism (carbamazepine treatment)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I (control)</td>
<td>12662.586</td>
<td>15 234.000</td>
</tr>
<tr>
<td>II</td>
<td>16 130.000</td>
<td>173 40000</td>
</tr>
<tr>
<td>III</td>
<td>25 125.016</td>
<td>11 790 000</td>
</tr>
<tr>
<td>IV</td>
<td>15 822 430</td>
<td>(-)</td>
</tr>
</tbody>
</table>

Values are given as means of five animals.

(-), WBC count was not determined.
Table 9. WBC count (x10^3/mm^3) in Tj and carbimazole-treated male tinia posts.

<table>
<thead>
<tr>
<th>Group</th>
<th>Hypothyroidism (TS treatment)</th>
<th>Hypothyroidism (carbimazole treatment)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I (control)</td>
<td>8.546</td>
<td>12.680</td>
</tr>
<tr>
<td>II</td>
<td>9.095</td>
<td>7.563</td>
</tr>
<tr>
<td>III</td>
<td>3.437</td>
<td>11.430</td>
</tr>
<tr>
<td>IV</td>
<td>5.529</td>
<td>(-)</td>
</tr>
</tbody>
</table>

Values are given as means of five animals.

(-), WBC count was not determined.
Table 1. Hemoglobin concentration (mmol/l) in T3 and carboxyglutared male Hidijin guinea.

<table>
<thead>
<tr>
<th>Group No.</th>
<th>Hypothyroidism (mg/100g)</th>
<th>Hypothyroidism (mg/100g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I (control)</td>
<td>4.90 ± 0.33</td>
<td>4.95 ± 0.61</td>
</tr>
<tr>
<td>II</td>
<td>4.76 ± 0.35 N.S.</td>
<td>4.76 ± 0.11 N.S.</td>
</tr>
<tr>
<td>III</td>
<td>5.73 ± 0.21 N.S.</td>
<td>5.72 ± 0.16 N.S.</td>
</tr>
<tr>
<td>IV</td>
<td>4.30 ± 0.28 N.S.</td>
<td>(—)</td>
</tr>
</tbody>
</table>

Values given are means ± S.E.M. of five animals.

Statistical significance is compared with control values.

N.S., Not significant.

(—), TH concentration was not determined.
<table>
<thead>
<tr>
<th>Group No.</th>
<th>Hypothyroidism (100% treated)</th>
<th>Hypothyroidism (control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I (control)</td>
<td>$26.91 \pm 3.63$</td>
<td>$31.70 \pm 6.74$</td>
</tr>
<tr>
<td>II</td>
<td>$30.40 \pm 1.11$ N.S.</td>
<td>$35.70 \pm 0.50$ N.S.</td>
</tr>
<tr>
<td>III</td>
<td>$29.47 \pm 3.59$ N.S.</td>
<td>$35.20 \pm 2.71$ N.S.</td>
</tr>
<tr>
<td>IV</td>
<td>$23.5 \pm 1.36$ N.S.</td>
<td>( - )</td>
</tr>
</tbody>
</table>

Values given are means ± S.E.M. of five animals.

Statistical significance is compared with control values.

(-), T37 values were not determined.

N.S. Not significant.
CHAPTER V

INTERPRETATION

The main objective of this study was to examine the effects of I) and carcinoma-induced hyperthyroidism and hypothyroidism respectively, on lipid metabolism in male Nubian goats. Our results have indicated that in I)-treated goats, there was a decrease in the serum HDL, LDL, triglycerides, cholesterol and phospholipids, and an increase in lecithin-cholesterol acyltransferase (LCAT) activity in cardiac and skeletal muscles. On the other hand, carcinoma treatment resulted in increased serum and hepatic lipid levels with no changes in the activity of LCAT in the heart and skeletal muscles. The hypothyroidism observed in I)-treated animals agrees with the report by Schlicker et al. (1968), Volle et al. (1971), Mikkelsen and Kjaer (1973) and Bloxley (1969). Under our experimental conditions, the hypothyroidism, especially hyperglycemia, at least in part, might have been due to an increased triglyceride removal from the circulation. Although we have only measured LDL activity in cardiac and skeletal tissues, it is likely that the
activity of the enzyme in other tissues is similarly increased. LPL is the enzyme thought to mediate the removal of circulating lipoprotein triglyceride. In many situations, changes in LPL activity are associated with opposite changes in plasma triglyceride. For example, the studies of Rosenblatt and Swerish (1975) and Kim and Malloy (1975) have demonstrated a decrease in adipose tissue LPL with estrogen, inferring an impairment in LPL-related triglyceride removal. Studies in men have consistently demonstrated a decrease in plasma post-heparin lipolytic activity during estrogen (Barlow and Swerish, 1975) and oral contraceptive therapy (Hawgood, Ng, and Hawgood, 1977). Several studies have also suggested that the deficient removal of circulating triglyceride plays an important role in the pathogenesis of diabetic hypertriglyceridemia (Hawgood et al., 1977; Yeaven et al., 1975).

A decrease in the adipose tissue LPL has been demonstrated in diabetic humans (Pykälä et al., 1975; Nikkila et al., 1977). The PPLA is composed of several lipolytic enzymes of different tissue origin. Kreuses et al. (1974) have shown that the hepatic triglyceride lipase (H.T.L.1) is heparin-releasable and it makes a significant contribution to plasma PPL. Nesi et al. (1980)
observed that the plasma release of H.T.O.I. from isolated liver parenchymal cells prepared from streptozotocin-diabetic rats was approximately half of that from control rats. Furthermore, the enzyme activity in the cells of these diabetic rats was lower than that of control rats. These results are attributed to synthesis and secretion of H.T.O.I. by the liver of streptozotocin-diabetic rats. More recently, Wolf et al. (1987) have shown that hypertriacylglyceridaemia in streptozotocin-diabetic rats is associated with a decreased output of triglycerides by the liver from these animals when perfused with relatively quantities of fatty acids in comparison with the liver from non-diabetic rats. These authors concluded that this hypertriacylglyceridaemia might have resulted, at least in part, from decreased peripheral utilization of triglycerides, consequent to decreased activity of LPL. Elevated plasma triglyceride levels frequently occur in patients with chronic renal failure as a result of long-term haemodialysis. In these patients, the activities of post-heparin plasma H.T.O.I. and PL were low; the former was significantly correlated with triglycerides in plasma (Appelbaum et al., 1979). Clearly, the modulation of LPL activity, irrespective of
the case, is expected to affect the serum triglyceride level specifically and other lipids classes generally. This is true if we regard that these lipids (triglycerides, cholesterol and phospholipid) are excreted as an integral moiety of lipoprotein which is really the case.

Contrary to what we found in I3-treated animals, in the animals dosed post, hypothyroidism, the I2 played a passive role in the development of hypolipemia. Other scientists have observed increased PHE in hypothyroid animals with decreased clearance of intravenously administered exogenous triglyceride (Hicklin and Kestel, 1972; Tulloch et al., 1973).

It is also possible that in I3-treated animals, the decreased output of triglyceride might have contributed to the hypothyroidism. In this regard, Keynes and Hales (1972) perfused livers from I3-treated rats (different doses and different duration of treatment) with 0.14 M free fatty acid (FFA) and observed a decreased output of triglyceride by the liver when compared to livers from untreated animals. The availability of FFA substrate to the liver is an important
factor, depending on the nutritional and hormonal status of the animal, in its metabolic pathway. This could be directed to produce of oxidation (triglyceride, cholesterol, or phospholipids) or to produce of synthesis (acids, triacylglycerols, and CoA). The FFA available to the liver in the intact animal can be derived from exogenous or endogenous sources (Steinberg et al., 1974). In animals in the fasted state, or on a fat-free diet, or stimulated under certain endocrine pathways, the major source of FFA is probably adipose tissue triglyceride. However, in the fed state, when neutral lipids are provided in the diet of as and animals, the primary source of FFA is probably dietary triglyceride (Steinberg, 1974). When and animals were fed
neutral fats of particular fatty acid composition, the plasma and FFA and hepatic triglyceride within a short time become enriched with the fatty acid-rich. It has been shown that the uptake of FFA by the liver is proportional to the concentration of FFA in the plasma, as is in or in the medium perfusing the isolated liver in vivo (Steinberg et al., 1974). The influence of the concentration of FFA uptake by the liver can be modulated by the rate of flow of the blood or perfusate and by the ratio of FFA
to albumin in the medulla (Sokol-Arinyo et al., 1974).

In intact animals, hyperparathyroidism is well documented (Koper and Reinberg, 1971). As noted previously, fatty acids have also been reported to be stimulated in hyperparathyroidism. Bissessar et al. (1972) have observed increased incorporation of (3H) acetate into fatty acids and increased activities of fatty acid synthetase and acetyl-CoA carboxylase by 100,000 g supernatant from livers of hyperparathyroid rats. Increased activities of fatty acid synthetase and acetyl-CoA carboxylase have also been reported by Sperber and Marks (1973). Moreover, these workers reported an increased incorporation of $\beta$-hydroxybutyric acid into fatty acids in the liver of intact hyperparathyroid rats. The stimulation of endogenous fatty acid synthesis by acetate in the liver from hyperparathyroid animals was also reported by Sperber et al. (1973). However, the phenomenon of an increased synthesis of endogenous fatty acids in hyperparathyroid rats is controversial. Hunt and Illiffe (1969) have observed a diminished incorporation of (1-14C) acetate into fatty acids by liver slices and 10,000 g supernatant fractions from hyperparathyroid rats.

The serum 1,25(OH)$_2$ vitamin D concentration was shown to be increased in
hypothyroidism (Makiela and Kashi, 1972; and cassette, 1973). This was possibly reflected by the production role of the thyroid hormones in the lytic response with out-of-hand animals, ACTH or glucagon (Kroege et al., 1976). All these mechanisms together via hyperphagia, increased lipogenesis, synthesis of fatty acids and increased lipoprotein in hypothyroid animals will probably result in an increased availability of EPA to the liver. Clearly, under our experimental conditions, EPA was most probably channelled into products of oxidation rather than those of esterification in T3-treated animals. The basal metabolic rate increases in hypothyroidism is well established and indicative of the oxidative pathways. The concentration of hepatic carbohydrates may be an important factor in regulating ketogenesis (Rose et al., 1969). Enhanced ketogenesis is associated with increased concentrations of carbohydrates in the liver under a variety of conditions. Glutamine is necessary for the entry of EPA to the mitochondria for subsequent oxidation completely into CO₂ or incompletely into ketone bodies. It is possible that hypothyroidism is associated with increased concentrations of carbohydrates; however, this needs to be further investigated. Conversely, hypothyroidism might
also stimulate the activity of hydroxyethylglucosylase, the rate-limiting enzyme of ketogenesis. Increased ketogenesis, regardless of the mechanism, would make less fatty acids available for triglyceride synthesis.

In carbamazepine-treated animals, hyperthyroidism, the activity of Cpe was not different from that in control rats, yet there was hypertriglyceridemia. Therefore, under our experimental conditions, the hypertriglyceridemia might have resulted from the increased output of triglyceride by the liver. This might be the result of an increased triglyceride synthesis and/or of a decreased oxidation of fatty acids. In this regard, Requena et al. (1976) reported that the changes in hepatic metabolism profile of thyroidectomized rats relative to that of pair-fed controls, appeared to indicate a decreased rate of fatty acid oxidation. In particular, the fall in content of long-chain acetyl-CoA derivatives and the lesser extent of acetyl-CoA, as well as the reliance for a more oxidized state of the mitochondrial compartment point to a decreased lipid oxidation. A direct role of carbamazepine in triglyceride synthesis and/or fatty acid oxidation cannot be excluded, however, by this study.
The other lipid classes in serum viz., cholesterol and phospholipid, were expected to somewhat follow the general trend of triglycerides. This is particularly true if we regard that these lipids are secreted together as lipoprotein particles especially very low density lipoprotein (VLDL) by the liver. The metabolism of fatty acids taken up by the liver varies, as previously mentioned, with the hormonal and nutritional status of the subject. The FFA taken up by the liver will be esterified primarily to triglycerides and to a lesser extent to phospholipids, diglycerides and cholesterol esters or will be oxidized to CO₂ and ketone bodies. The triglycerides in serum in the liver or is secreted into plasma as the major component of the VLDL in conjunction with other lipid particles and apoprotein components. When triglycerides is not secreted, for example, when blocked by various hepatotoxins or in several pathological states or when the maximal rate of triglyceride output has been exceeded because of a large available pool of FFA, triglyceride accumulation in the liver and proximal hepatic steatosis (Halberg et al., 1976). This may possibly the cause of the increases triglyceride, cholesterol and phospholipid concentrations in the liver of
carbimazole-treated animals. The presence of fatty cytoplasmic
vacuolization of the hepatocytes in carbimazole-treated goats is
in accord with the biochemical evidence of hypothyroidism. On the
other hand, the preferential deamination of FFA in the oxidative
pathway in T3-treated goats is made less available for triglyceride
synthesis and hence depressed hepatic levels when compared to con-
trol values. It would be extremely interesting to study the inter-
relationship of lipid metabolism with altered thyroid status as a
function of time and also after the cessation of T3 and carbimazole
treatments. This may show the onset of development of the lipid
metabolic status. Replacement therapy may also be investigated
during T3 and carbimazole induction of hyper- and hypothyroidism
to establish a therapeutic dose for the goat.

It is interesting to mention that T3 treatment in goats
resulted in increased HDL-cholesterol. We have no explanation for
this change at the present time.

Under our experimental conditions, neither T3 nor carbi-
mazole altered the blood cellular elements, Na, K, or protein
concentration in the serum of goats. Since our animals were
treated with both drugs for only 31 days, treatment for a prolonged period of time may have effects on these parameters.

We conclude that:

1. Carbenoxolone treatments, in most of the time, produced effects on lipid metabolism opposite to those of T3-treatment in rats.

2. The hypertriglyceridemia in carbenoxolone-treated animals, hypoglycemia, is primarily the result of increased triglyceride production by the liver, possibly as a consequence of increased hepatic microsomal synthesis of triglyceride.

3. The hypocalcemia in T3-treated animals, hypothyroidism, is the result, at least in part, of increased peripheral utilization. In addition, decreased production of hepatic triglyceride consequent upon increased oxidation of FFA might also be a contributing factor.

4. The parallel change in cholesterol and phospholipid with triglycerides suggests that these substances are secreted by the liver in a definite ratio possibly as VLDL particles.
5. The determination of serum lipid composition may be used as an aid for the diagnosis of thyroid disease in animals.

6. Induction of altered thyroid function in goats by 7,3 and carbimazole clearly indicates that this species of small ruminants may serve as a useful animal model for such diseases. The relatively longer life span of goats when compared to laboratory animals, makes it particularly valuable in studying thyroid disease complications such as the involvement of the cardiovascular system.


Journal of Biological Chemistry, 214, 1466.


phosphodiesterase, insulin and thyroid hormones. Molecular 
and Cellular Endocrinology, 3, 47 – 69.

related to fatty acid synthesis in liver and adipose 

Dukakis, D. (1953). In 'Metabolism'. Edited by the Pharmaceutical 
Society of Great Britain (1953), 5th edition. The 

Evans, R. H. (1964). Physiology of domestic animals, 4th 

acid turnover and total body $\text{CO}_2$ consumption in euthyroid


Lipids bei Störungen der schilddrüsen-Funktion des

Schulz, O. H., Andrae, S. C., Rehfeld, J. F. and James, E. S.
Lipid Research, 11, 60.

From J. B. Lippincott Company, Philadelphia, U.S.A.

Adler-Nissen, C., Enfalt, R., Samuel, B. and Polonsky, I.
(1974). Factors influencing the fatty acid uptake by
the isolated perfused rat liver. J. Biol. Chem., 249,
727 - 734.

Sylvestre, M. A., Medico, O. and Wandel, B. (1957). A study of
fatty acid metabolism and fatty acid synthesis in liver
slices of hypothyroid rats. J. Biol. Chem., 229,
713 - 719.

Lippincott, J. B. Lippincott, New York.


