THE EFFECTS OF ADDED IODINE AND FISHMEAL ON THE REPRODUCTION AND GROWTH OF MINK (*MUSTELA VISON*)

By

Ross Edward Jones Jr.

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ABSTRACT

THE EFFECTS OF ADDED IODINE AND FISHMEAL ON THE REPRODUCTION (MUSTELA VISON) AND GROWTH OF MINK

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A study was performed on mink (Mustela vison) to assess the adverse effects of 1) feeding various types of fishmeals on reproduction and growth and 2) feeding diets containing various levels of supplemental iodine on reproduction, growth, blood parameters and thyroid hormones. Rats were used in a screening test for the various fishmeal diets.

Young growing animals (both rats and mink) when fed fishmeal supplemented diets had depressed body weight gain. Adult rats fed diets supplemented with 40% fishmeal had no significant changes in their body weight or feed consumption. However, number of pups born alive, survival of pups to weaning and weaning weight were generally decreased in all rats fed fishmeal diets as compared to controls. Adult mink fed a diet supplemented with 30% fishmeal also had no significant changes in their body weights. The kits from those mink had significantly depressed birth weights and survival to weaning.

One group of mink were fed diets containing 0, 10 20, 40, 80 or 160 ppm supplemental iodine for eight months, long

term (LT), prior to mating. Another group of mink were fed diets containing 40, 80, 160 or 320 ppm supplemental iodine for one month, short term (ST), prior to mating. Both groups were fed their respective diets throughout gestation and weaning.

Adult mink on iodine treatment were not affected in their body weights or blood parameters. However, levels of approximately 100 ppm iodine tended to have detrimental effects on reproduction in the mink, including decreased number born, survival to weaning, weaning weight, and biomass of the kits. These changes were probably due to the placental transfer of iodine to the developing kits. Increased mortality and enlarged thyroid glands in the kits were associated with excessive iodine in the mink.

Tiiodothyronine (T_3) levels were slightly decreased at all treatment levels in all adults, fed the LT iodine-treated diets, and their kits. The T_3 values of kits from adults fed iodine supplementation for the ST were markedly decreased over all levels of iodine. Thyroxine (T_4) values in both adults and kits were decreased as iodine content in the diet increased. T_4-binding index in adults was generally inversely related to the adult T_4 values. However, T_4-binding index in the kits was directly related to the kit T_4 levels, especially in those from dams fed ST iodine diets. Reverse T_3 values generally declined in all adults and the kits from mothers fed LT iodine supplementation over all iodine levels. However, kits from dams fed ST iodine diets

showed a marked increase in reverse T_3 values. These changes in reverse T_3 are most likely due to alterations in the enzymes involved in the metabolism of T_4.

Gross pathology was limited to increased size of the thyroids in kits from mothers fed diets >40 ppm iodine, and enlarged, thickened and opaque gall bladders in adults fed 320 ppm iodine supplementation. Although the adult thyroids were not significantly larger than controls, histopathology of the thyroids in both kits and adults fed >ppm iodine diets showed a hyperplasia of the follicular cells and a decreased amount of colloid. The gal bladders of the adults fed 80 ppm or more iodine showed increasing severity of hyperplasia of the pili and smooth muscle walls and increasing amounts of secretory material.

to my wife, Judy

my daughter, Sarah

and my father, Ross E. Jones Sr.

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INTRODUCTION

Iodine is a Generally Recognized as Safe (GRAS) food ingredient for man and animals (Van Gelder, 1976). Many areas, such as the midwestern U.S., are iodine deficient. Goiter in man and animals was determined to be caused by an iodine deficiency in the food and water of these areas. Because of these deficient areas, sources of iodine are added to the diet. The use of iodized salt for humans and ethylenediamine dihydriodide (EDDI) for livestock and poultry has resulted in a substantial decline in the occurrence of iodine deficiency or goiter.

The multitude of compounds containing iodine, many of which are used in therapeutic agents or medicine, have possibly reversed the iodine problem from a deficiency question to one of toxicity in certain areas (Hemken, 1979). Acute iodine toxicity has been known in cattle for years. This toxicity is generally seen when the cattle are being treated with an iodinated compound. Chronic toxicity, or iodism, has become another problem in association with feeding iodides routinely. Cattle

appear to be a major example of iodism in that excessive iodides are found in feed ingredients and in supplements. These additives are used not only for their iodine content but also as treatment or preventative measures for "foot rot" or infectious pododermatitis, "lumpy jaw" or actinomycosis, mastitis, infertility, and other disorders (McCauley et al., 1972; Feed Additive Compendium, 1975). Iodine is also present in the test dip used during milking. Hemken (1979) described the effect of dietary iodine, teat dips, udder wash and sanitizers containing iodine, and other sources of iodine on the iodine content of milk and meat. Generally the level of iodine in meat is not a problem, whereas milk's content of iodine has increased over recent years with some samples over the recommended safe limit. Excessive iodine in dairy cows has been reported in Michigan herds for which clinical signs were suggestive of pneumonia and the incidence of other infectious diseases was increased. The milk from these cows contained over 2.0 ppm iodine and the cows showed a decrease in milk production (Main, 1977; Haggard, 1978). These same clinical signs of increased susceptibility to infections and bronchopneumonia were noted in Minnesota herds on high levels of EDDI and iodine in the mineral supplement (McCauley et al., 1972). In Georgia dairy herds, Wallace

(1975) reported chronic lameness, decreased milk production, and respiratory problems associated with excessive iodine. Effects of excessive iodine have also been reported for sheep (Blaxter, 1948), lambs (McCauley et al., 1972), and mares (Drew et al., 1975).

Humans could be exposed to abnormally high levels of iodine through: iodized salt, meat or milk from cattle receiving iodine, and other foods such as seaweed which alone may contain greater than 6000 ppm iodine (Underwood, 1977). Nagataki et al. (1972) have shown that people of Japan who tend to eat iodine rich seaweed, shellfish, and marine fish have a higher T_4/T_3 (thyroxine/triidothyronine) ratio within thyroglobulin than residents of the U.S. Talbot et al. (1974) reviewed the untoward reactions to iodine in foods, and noted an increased iodine availability in foods in the diet of North Americans though no significant increase in adverse reactions to iodine was noted. Iodine sensitivity in humans was noted by Peacock and Davison (1957). They observed that 16.1 percent of asthmatic patients on iodide medication had sufficient adverse reactions to warrant discontinuation of their expectorant.

In recent years, mink ranchers, in trying to reduce the cost of feed, have decreased the amount of beef products used in feed and increased the amount of poultry

products and fishmeal included in the diet. Many ranchers have experienced abnormally high (30 percent) losses of young (kits) mink, many of which have been associated with thyroid dysfunction, anemia and "shock" (Aulerich, personal communication). The exact causes are unknown, but the rigorous, long-term selective inbreeding of dark mink is most likely one of the factors causing this low reproductive performance (Howell, 1979).

Among other possible factors, a toxic agent in the feed has been shown to be a probable cause in the past. In 1968 when mink were fed whole, raw Lake Michigan coho salmon kit mortality reaching 80 percent was noted. This mortality was directly related to the percent fish in the diet. It was shown to be caused by polychlorinated biphenyls (PCBs) in the fish (Aulerich et al., 1973). PCBs have also been implicated in causing female mink in estrus to be hypothyroid; these females also bore no young (Byrne et al., 1975).

Beef by-products, especially gullet trimmings, which contain high levels of naturally occurring thyroid-active compounds also caused reproductive problems in mink (Travis et al., 1966).

In addition research has shown mink to be more sensitive than other species to iodine and many environmental contaminants such as mercury and PCBs (Aulerich et al., 1973, 1974, 1978). As Mink are at the

top of the food chain and thus tend to accumulate pollutants, feeding high levels of fish can have adverse effects due to the concentration of contaminants these fish may have stored in their body tissues.

The experiments presented in this dissertation were conducted to determine the effects of fishmeals and iodine on mink.

LITERATURE REVIEW

Historical

In 300 B.C. the Chinese emperor Shen-nung noted the use of seaweed as a remedy against goiter. The ancient Greeks used burnt sponges as a goiter treatment. Iodine was discovered in 1820 by a Swiss physician, Coindet, and has since been used as a remedy for goiter. During the 1800's many physicians realized the relationship of the thyroid to various disease conditions: goiter, myxedema, and cretinism. Chatin, a French botanist, determined the natural occurrence of iodine in soil, water, and foods and associated the incidence of goiter with areas deficient in iodine (Werner, 1957; Pitt-Rivers and Trotter, 1964; Goodman and van Middlesworth, 1974). It has since been shown that low iodine content is the major factor responsible for goiter development and that thyroid stimulating hormone (TSH) and cyclic adenosine monophosphate (cAMP) concentration may be either normal or elevated in animals with goiter (Berthier and Lamarchand-Béraud, 1978). By the end of the 1800's iodine was determined to be concentrated in the thyroid and to reduce endemic goiter when administered.

Kendall (1915) isolated thyroxine and found it contains 65 percent iodine. Gross and Pitt-Rivers (1953) isolated triiodothyroxine, and suggested that thyroxine was the form of the secreted hormone and triiodothyronine was the activated form that stimulated tissues.

Distribution

Though every tissue of the body contains iodine, the thyroid gland contains 70 to 80 percent of total body iodine. Iodine is also concentrated in the stomach (or abomasum or proventriculus), small intestine, salivary glands, skin and hair or fur, ovaries (oocytes of birds), placenta, and lactating mammary glands (Church and Pond, 1974; Underwood, 1977). The ovarian, placental, and mammary gland iodine content is of physiological importance in that it supplies the embryo, fetus, or newborn with iodine for development (Brown-Grant, 1961).

Iodine occurs as both inorganic and organically bound forms in tissues, though normally the inorganic moiety is present in extremely low concentrations. Most extrathyroidal iodine consists of thyroxine bound to protein; also other low concentrations of compounds are found including triiodothyronine (Gross, 1962). Amniotic fluid shows increased iodine levels dependent upon maternal iodine intakes, thus demonstrating placental transfer (Etling et al., 1979). The mammary

gland produces monoiodotyrosine and some diiodotyrosine but they are of no known use to the newborn (Potter et al., 1959; Brown-Grant, 1961). Urine contains a larger portion of excreted iodine than does the feces (Stanbury and Ramalingaswami, 1964; Food and Nutrition Board, 1970; Goodman and van Middlesworth, 1974).

Iodine Metabolism

Ingested inorganic iodine is absorbed rapidly from the gastrointestinal tract by two pathways: one is in common with other halides, such as chlorine and bromine, the other is a process specific for iodine. The free iodine, or iodate (I^2), upon reaching the intestine undergoes reduction to iodide (I^-) before absorption into the blood stream. Iodinated amino acids are absorbed in this same manner, but to a lesser degree and are absorbed more slowly. Diiodosalicyclic acid (a source of added iodine) is absorbed as above, but the iodine is not split from the ring structure and is excreted more rapidly (Aschbacher et al., 1963). Iodine as EDDI is absorbed to a greater extent than iodine as a salt and is not excreted in the urine as rapidly (Miller and Swanson, 1973). This iodine-specific transport mechanism is saturated by high iodine concentrations (Gross, 1962; Barua et al., 1964; Alexander et al., 1967; Scott et al., 1969; Church and Pond, 1974;

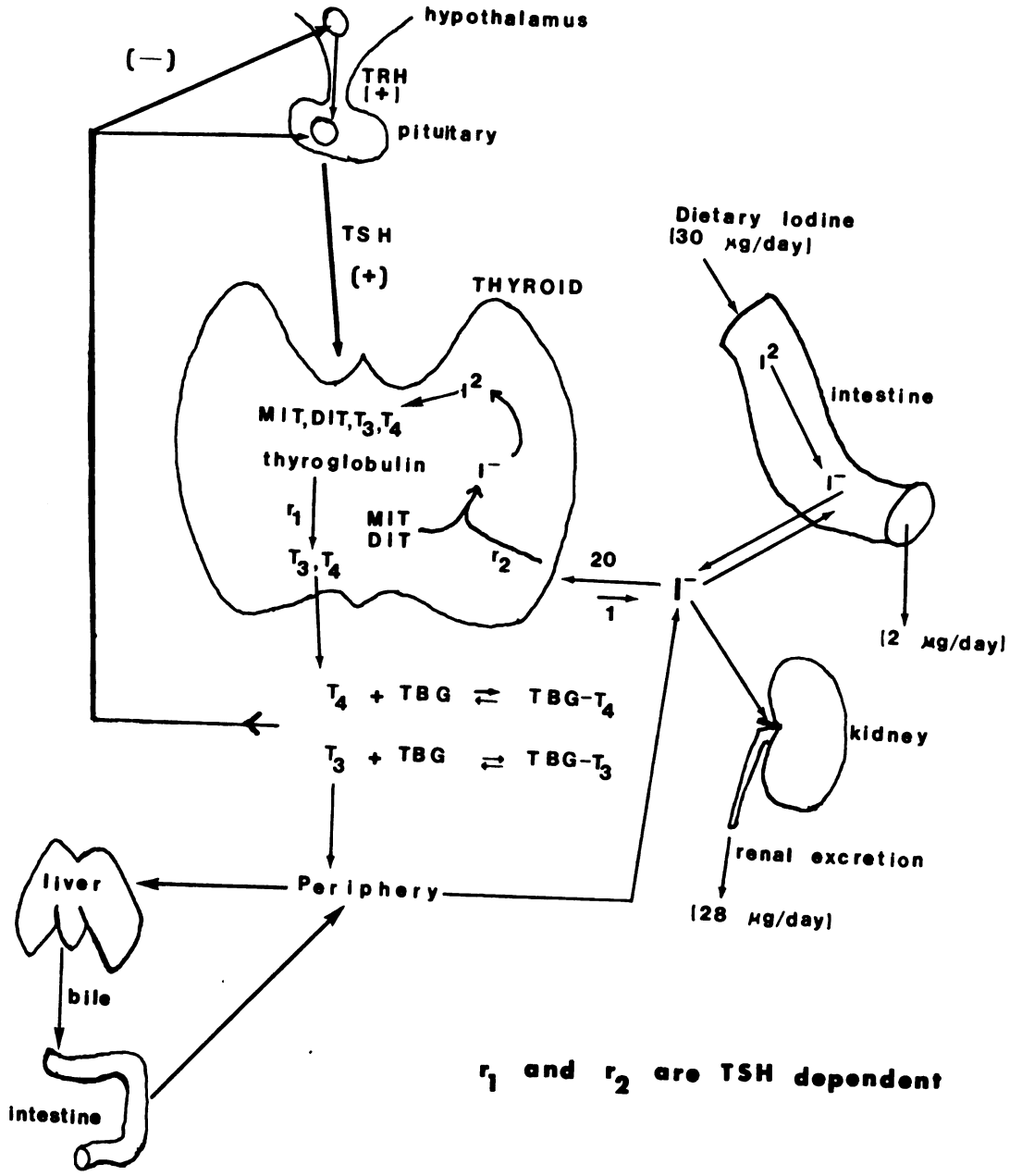
Underwood, 1977).

Nearly all absorbed iodide is trapped by the thyroid gland, a process that is energy-requiring and stimulated by TSH (see Figure 1). Antithyroid compounds of the perchlorate and thiocyanate types can inhibit the iodide transport system (Wyngaarden et al., 1952). Some iodine appears in the kidneys and a smaller amount is found in various organs previously listed (see Distribution). In the liver the thyroid hormones undergo conjugation with glucuronic acid and are then excreted as such in the bile and thus to the small intestine (Galton and Pitt-Rivers, 1959b). In the intestine these conjugates undergo hydrolysis and the free hormone or derivative is then either reabsorbed into the circulation (enterohepatic circulation) or excreted in the feces (Pitt-Rivers and Trotter, 1964). The kidneys also deiodinate hormonal iodide which either recirculates to the thyroid or is excreted in the urine (Aschbacher et al., 1963). The kidney metabolite is also the conjugate of thyroxine with glucuronic acid. However since the metabolite is absent from serum and urine its breakdown must take place in the kidney (Galton and Pitt-Rivers, 1959b). Salivary secretion of iodine is an active process in most species, with the rat being an exception (Underwood, 1977).

Figure 1. Pathway of iodine metabolism (Sodeman and Sodeman, 1974). Based on a level of iodine in the diet of 1.5 ppm, a feed consumption level of 200 g/day and an excretion rate similar to other animals the following were calculated for the mink: intake = 30 µg/day, fecal excretion = 2 µg/day and renal excretion = 28 µg/day.

TRH = thyroid releasing hormone
TSH = thyroid stimulating hormone
MIT = monoidotyrosine
DIT = diiodotyrosine
T_3 = triiodothyronine
T_4 = thyroxine
I_2^- = iodine (or iodate)
I^- = iodide
TBG = thyro-binding globulin
r_1 and r_2 = reaction rates

FIGURE 1


hypothalamus
(−)
TRH
(+)
pituitary
TSH
(+)
THYROID
Dietary Iodine
(30 µg/day)
I^2
intestine
MIT, DIT, T_3, T_4
thyroglobulin
I^-
r_1
MIT
DIT
T_3, T_4
r_2
20
1
(2 µg/day)
T_4 + TBG ⇌ TBG-T_4
T_3 + TBG ⇌ TBG-T_3
kidney
renal excretion
(28 µg/day)
liver
Periphery
bile
intestine
r_1 and r_2 are TSH dependent

The placental tissue, as well as the ovary of mammals concentrates iodine in late pregnancy, whereas the yolk of birds' eggs serves as an iodine source. In lactating females iodine is concentrated in the mammary glands where the active transfer of inorganic iodine results in a 40-fold concentration, as compared to plasma. This concentration is greatly influences by dietary iodine intake and by the stage of lactation (Salter, 1940; Flamboe and Reineke, 1959; Reineke, 1961; Lengemann, 1963, 1965; Hemken et al., 1972; Swanison, 1972; Underwood, 1977).

Of all trace elements, only iodine can pass with ease across the mammary barrier (Underwood, 1977). Even though the mammary gland during lactation has a higher capability for removal of iodine from the blood than does the thyroid, in cases of low dietary iodine intake the mammary gland reduces its intake thus keeping the thyroid supplied (Flamboe and Reineke, 1959; Swanison, 1972). The passage of both thyroxine and triiodothyronine into the milk is in very small amounts and is of no significance to the young (Potter et al., 1959).

Since the only role of iodine is in the synthesis of the thyroid hormones, thyroxine and triiodothyronine, iodine metabolism and thyroid function are inter-related. After uptake from the blood, iodide is taken into the follicular epithelial cells and stored in a colloid

protein, thyroglobulin. Certain chemicals, including thiocyanate, perchlorate, hypochlorite, iodate, biiodate, nitrate and periodate, reduce or completely inhibit the iodine concentrating ability of the thyroid gland (Wyngaarden et al., 1952). In these cells the iodine is concentrated and oxidized to a higher valence (Wolff, 1964). This more reactive iodine is transported to the luminal surface of the apical microvilli and combines with the tyrosine residues and thyroglobulin to form 3-monoiodotyrosine and 3,5-diiodotyrosine (DeGroot and Davis, 1962a; Taurog, 1970). This step can be blocked by high concentrations of iodide, anti-thyroid agents such as allylthiourea, 5-vinyl-2-thiooxazolidone, or 2-mercaptoimidazole, and sulfaguanidine, an antibacterial agent (Rosenberg et al., 1963b; Frieden and Lipner, 1971). Thyroperoxidase, which is responsible for iodination, forms iodoprotein at the luminal surface of the follicular eithelial cells. This reaction is also under the control of tyrosine iodinase (DeGroot and Davis, 1962a, 1962b). A peroxidase is again involved in the combination of two diiodotyrosine (DIT) molecules to form one thyroxine (T_4) or one monoiodotyrosine (MIT) and one DIT joining to form triiodothyronine (T_3) as follows:

1) glucose + O_2 + H_2O $\xrightarrow[\text{oxidase}]{\text{glucose}}$ H_2O_2 + gluconic acid

2) H_2O_2 + $2I^-$ + $2H^+$ $\xrightarrow[\text{peroxidase}]{\text{iodide}}$ 2 "active iodine" + $2H_2O$

3) 2 "active iodine" + L-tyrosine $\xrightarrow[\text{iodinase}]{\text{tyrosine}}$ 2MIT or DIT

4) (1MIT + 1DIT) or 2DIT $\xrightarrow[\text{peroxidase}]{\text{iodide}}$ (T_3) or T_4

(Alexander, 1959, 1961)

The hydrogen peroxide generating system (equation 1), glucose and glucose oxidase, markedly enhance utilization of iodide by the thyroid. Triiodothyronine may also be formed by the removal of one iodine from thyroxine (T_4) (Taurong and Howells, 1966).

Thyroxine and triiodothyronine are uniquely stored extra-cellular hormones in peptide linkage within the thyroglobulin molecules in the lumen of the thyroid follicle. The apical regions of the thyroid follicle cells contain two types of vesicles; one endocytotic in nature and the other exocytotic. The endocytotic vesicles represent intake of colloid from the follicle lumen which constitutes the first step in the secretion of thyroid hormone. The exocytotic vesicles transfer thyroglobulin (TG) or pre-TB, which is synthesized in the endoplasmic reticulum and golgi apparatus of the follicle cells, to the apical cell surface. TSH secretion almost completely inhibits the endocytotic vesicles, whereas the exocytotic vesicles are only slightly affected (Bjorkman et al., 1974). During

transport toward the cell base, the colloid droplets fuse with lysosomes that contain hydrolytic enzyme(s). The combination of T_4, T_3, and thyroglobulin to be released is degraded via proteolysis, by a protease system, to release both iodotyrosine and thyroactive thyronines; T_4 and T_3 are released in a ratio of 10:1 to 20:1 (Nagataki et al., 1972; Chopra et al., 1973) (see Figure 2).

The rate of thyroidal ^{131}I output in mink (19.9 percent per day with a $T_{1/2}$ = 3.48 days) is nearly identical to rats and mice, but the thyroid secretion rate in mink (0.95 γ L-thyroxine/100 gm daily) is much less than in rats (2.15 γ). By adjusting for body size, by dividing the amount of thyroxine by body weight raised to the 0.73 power, all species, from mice to goats, had thyroid secretion rates ranging from 9.1 to 16.1 γ per kilogram$^{0.73}$ and the thyroid secretion rate of the mink was 9.2 γ, thus within range of the other species (Reineke et al., 1960).

MIT and DIT cannot escape from the cell into the circulation or be directly resynthesized into thyroglobulin. Instead DIT and MIT are both deiodinated, by a deiodinase enzyme, to free tyrosine and iodide. Tyrosine is recycled into thyroglobulin and the iodide is recycled to iodinate the thyroglobulin as discussed before. This recycling of iodine is of prime importance

Figure 2. The active biosynthesis of thyroid hormones in the thyroid gland, starting with the "active molecule of iodine (I^2)" and showing the effect of TSH and blocking drugs. The active iodine selectively iodinates tyrosyl molecules of the polypeptides which then give rise to thyroglobulin. The iodinated products are MIT and DIT (Frieden and Lipner, 1971).

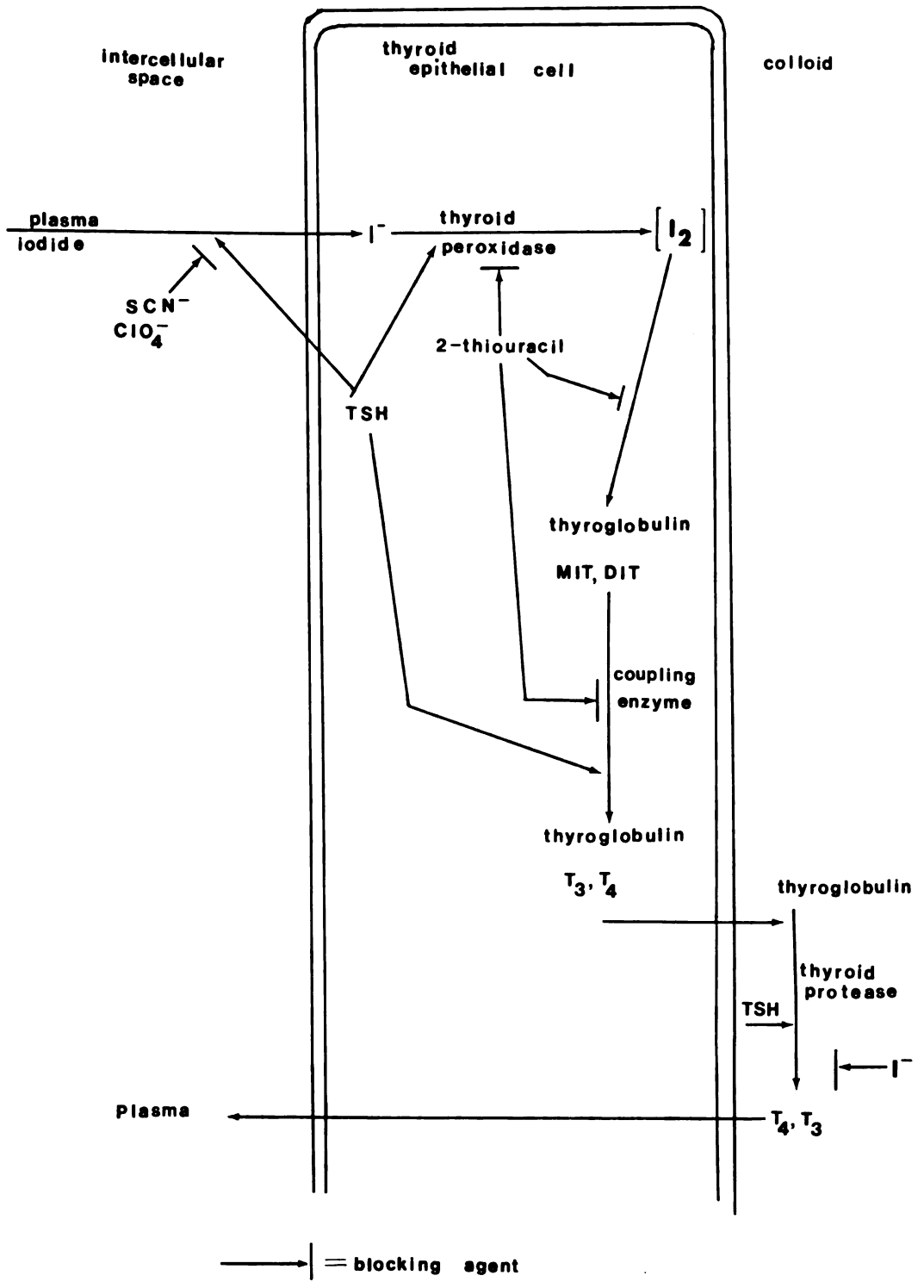

intercellular space
thyroid epithelial cell
colloid
plasma iodide
I^-
thyroid peroxidase
$[I_2]$
SCN^-
ClO_4^-
2-thiouracil
TSH
thyroglobulin
MIT, DIT
coupling enzyme
thyroglobulin
T_3, T_4
thyroglobulin
thyroid protease
TSH
I^-
Plasma
T_4, T_3
= blocking agent

FIGURE 2

in conserving iodine in normal situations, where iodine intake is low (Rosenberg et al., 1963b). In rats up to 71 percent of the iodide in the thyroglobulin is derived from deiodination of iodotyrosine (Simon, 1963). When rats are fed ten times the normal iodine level the turnover of iodine via deiodination is only 47 percent (Simon, 1963).

All activities of the thyroid gland are directly increased by hormones, specifically thyroid stimulating hormone (TSH), and indirectly decreased by hormones. These activities are modulated not only by pituitary thyrotropin but also by the iodide supply; which is a unique mechanism among the endrocine organs under pituitary control. When iodine is administered, TSH levels fall in proportion to the dose of iodine early in the treatment and iodine uptake is inversely correlated with dietary intake later in time. Thus, autoregulation by iodide occurs but at a slower rate (Sterling and Lazarus, 1977). This inhibition of iodine trapping does not interfere with the action of TSH on the other synthetic phases of thyroglobulin (Rosenberg and Bastomsky, 1965). The direct effect of TSH on the thyroid is the activation of cyclic AMP. This activated cyclic AMP: (1) enhances the resorption of colloid from the follicular lumen (endocytosis); (2) increases the release of thyroid hormones (Wilson et al., 1968);

(3) increases the number of intrathyroidal colloid droplets (Ohashi et al., 1973); (4) increases the incorporation of iodine into thyroglobulin and the synthesis of thyroglobulin (Sterling and Lazarus, 1977; Sinadinović et al., 1978). Also the possibility that 3',5' cyclic guanosine monophosphate may be involved in regulation of thyroid protein biosynthesis has been examined (Pisarev and Itoiz, 1972). Excess iodide in isolated thyroid also causes: (1) a decreased colloid droplet formation (Ohtake et al., 1973); (2) a 30 percent reduction in the stimulation of cyclic AMP production by TSH stimulation; (3) a decrease in iodide pump activity and (4) a decreased iodinating activity when thyrotropin is added (Sherwin and Tong, 1975; Hashizume et al., 1976). The degree of decrease in iodinating activity is proportional to the duration and concentration of iodide in the thyroid. In hypophysectomized rats, Yukimura et al. (1976) found that excess iodide stimulates thyroid hormone secretion. This increase in hormone was postulated to occur via an enhancement of cyclic AMP synthesis. It has also been noted that excess iodide increases glucose oxidation in the absence of TSH while blocking glucose oxidation and phospholipogenesis in the presence of TSH (Burke, 1968). More recent evidence indicates that iodine does not inhibit adenylate cyclase enzyme directly nor does it

increase activity of cAMP phosphodiesterases, but rather iodine causes a change in the transduction process by which binding of TSH to its receptor is coupled to adenylate cyclase activation (Uchimura et al., 1979).

Iodine can also impair thyroid response to cyclic GMP and probably acts at a step prior to cGMP's formation (Pisarev and Itoiz, 1972).

Yamamoto et al. (1972) have shown a decrease in intrathyroidal colloid droplets, produced by TSH, when rats and mice were fed excessive iodine.

It has been shown that administration of methimazole[1] prior to excess iodide administration relieves the inhibitory effects of iodide. Thus, iodide probably manifests its inhibitory action on thyroid hormone secretion after its conversion to some form of organic iodine (Van Sande et al., 1975).

Administration of iodide (500 mg three times a day) to normal humans for 10-12 days did not always cause a fall in serum T_3 and T_4 (Sawin et al., 1979); though others using small doses of iodine did see a decrease (Vagenakis et al., 1974; Saberi and Utiger, 1975). Ikeda and Nagataki (1976) found a poor correlation between serum thyroid hormones and TSH. Fukuda et al. (1975a, 1975b) found a marked increase in plasma T_4 and T_3,

[1]An antithyroid compound that interferes with the formation of thyroid hormone by stopping the incorporation of iodine into an organic form.

after injection of iodide to iodine-deficient rats, and a decrease in TSH. When larger doses of iodide were given no increase in T_4 or decline in TSH was noted.

Sawin et al. (1979) found an increase (> 2 times) in basal TSH after iodide administration and an increased response of TSH to thyrotropin releasing hormone.

Thyrotropin releasing hormone (TRH) is produced and stored in the hypothalamus. TRH is secreted from the hypothalamus into the hypophyseal portal vessels and acts immediately upon arrival in the adenohypophysis. Thyroid hormones inhibit the biological responses to TRH at the pituitary (Hinkle et al., 1979). TRH stimulates the beta cells of the anterior pituitary causing TSH synthesis and release. After release TSH acts upon the thyroid gland to cause release of T_4 and T_3 which are bound to thyroglobulin. The transport of T_4 and T_3 in plasma is almost exclusively by three proteins: thyroxine binding alpha-globulin, thyroxine binding prealbumin and albumin. Even though the T_4 in circulation is more than 30 times that of T_3, the protein binding is much less for T_3, and hence there is about ten times (in humans) the amount of T_3 in the unbound diffusible state. Because of the binding, thyroxine has a half-life of six to seven days in the humans while T_3 only has a half-life of one day (Nicoloff et al., 1972). In man T_3 is approximately three times as

active as T_4 while in the rat it is about five times more potent (Rosenburg and Bastomsky, 1965; Frieden and Lipner, 1971; Sterling and Lazarus, 1977).

Since T_4 undergoes peripheral deiodination to T_3 in most species (Surks et al., 1973), it may be that thyroxine is just a prohormone and the actions are all from T_3 (Schadlow et al., 1972; Ingar and Baverman, 1975; Sterling and Lazarus, 1977). Of the circulating T_3, two thirds is from T_4 conversion. Thyroxine thus loses about 33-40 percent to monoiodination, also another 15-20 percent is changed to tetraiodothyroacetic acid or conjugated and excreted, and about 50 percent is modified to 3',5',3-triiodo-L-thyronine[2], reverse T_3 or rT_3, all of which has a significant effect upon circulating T_3 levels (Rudolph et al., 1976; Bernal and Refetoff, 1977).

As plasma concentrations of T_4 and T_3 increase a negative feedback to the hypothalamus and/or pituitary occurs to inhibit the release of TRF and TSH. The rat pituitary has been shown to bind T_3 almost ten times as strongly as T_4 and, as the binding sites are saturated, the release of TSH stops (Schadlow et al., 1972). Other factors such as environmental temperatures or stressful situations regulate TSH secretion. These effects are mediated through TRH and CNS (Sterling and Lazarus, 1977).

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[2] The iodine is lacking from the A ring rather than the B ring.

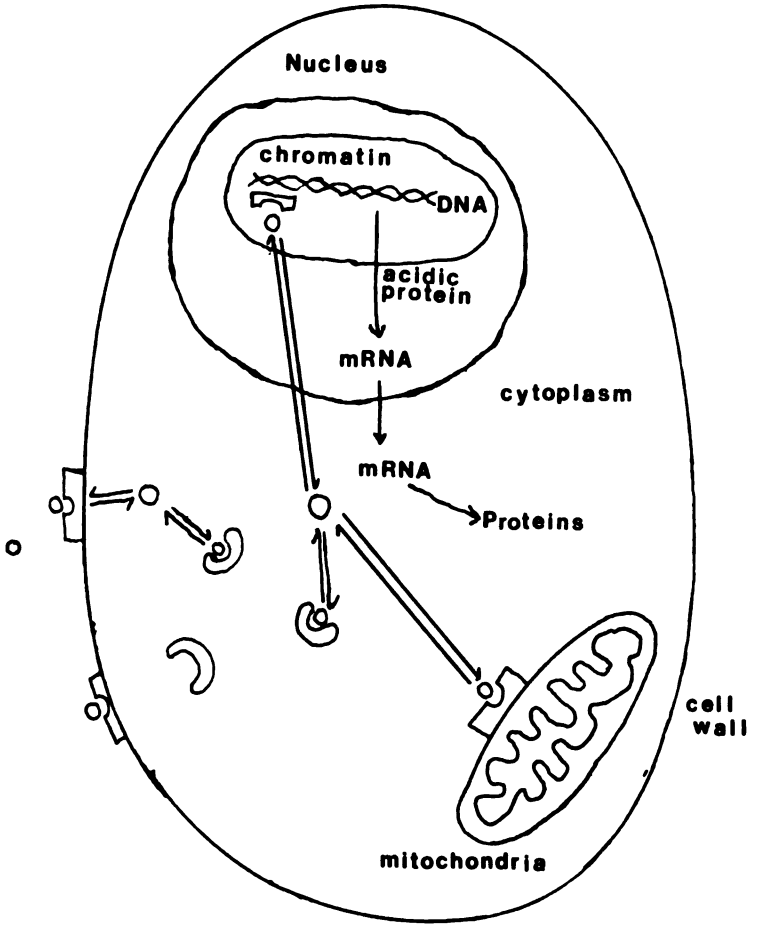
Physiologic Effects of Thyroid Hormones

The model for thyroid hormone action, through interaction with a receptor on the nuclear chromatin, is similar to the model for steroid hormones. The unbound T_3 molecule diffuses or is transported across the cell membrane and bound by a cytosol binder. This binding with the cytosol-binding protein (CPB) exist in equilibrium with free T_3. The free hormone interacts with binding proteins at recptors on the nucleus and/or mitochondria (see Figure 3). In the nucleus the thyroid hormones, as well as some of their analogues, are bound to acidic chromatin proteins. These analogues include D-T_3 isomer which is bound almost as well as L-T_3 (active form) and triiodothyroacetic acid which is bound more firmly but for a much shorter period than T_3. T_3 stimulates cellular protein synthesis via stimulation of increased transcription of DNA information which results in enhanced mRNA formation. Translation may also be stimulated by thyroid hormones which cause the release of a factor that stimulates protein synthesis by the ribosomes (Tata et al., 1963; Bernal and Refetoff, 1977; Sterline, 1979a).

In response to thyroid hormone stimulation the sodium pumps (Na^+-K^+ = ATPase, plasma membrane enzyme) increase in number and thus oxygen consumption is increased. This ATPase stimulation may be a result of

Figure 3. Model for thyroid hormones action at the cell level. The T_3 (or possibly T_4) molecule enters the cell and is bound to a cytosol-binding protein (CBP). Minute amounts of free T_3 are in equilibrium with the bound and this free T_3 interacts with the mitochondria and nucleus via receptors. T_3 then stimulates DNA which in turn stimulates mRNA and protein synthesis (Sterling, 1979a).

FIGURE 3



= CBP receptor

= T_3 receptor

= T_3 molecule unbound

stimulated nuclear transcription (Lo et al., 1976; Lo and Edelman, 1976). Thyroid hormones alos have direct and indirect effects on mitochondria. Directly the hormones bind to a lipoprotein macromolecule at the inner membrane, which is the site of oxidative phosphorylation. This binding causes an increase in the activity of oxidative phosphorylation. Indirectly, the mitochondrial enzyme α-glycero-phosphate dehydrogenase is increased, probably through action at the nucleus and mRNA from thyroid hormones. Also it has been shown that thyroid hormones indirectly increase the incorporation of amino acids into mitochondria proteins (Herd et al., 1974; Bernal and Reftoff, 1977; Sterling, 1979b).

1. Cellular Oxidation. The actions of thyroid hormones on the mitochondria may regulate the calorigenic response of the animal (Hoch, 1962; Gustafsson et al., 1965; Gross, 1971). As mentioned before, the increase in oxygen consumption may be due to the increased activity of the sodium pump; Edelman and Ismail-Beige (1974) showed an elimination of thyroid effect on oxidation by blocking the pump with ouabain. This calorigenic action of thyroid hormones may be secondary to enhanced energy-requiring protein synthesis. As noted by Stein and Gross (1962) T_4 *in vivo* failed to increase microsomal protein synthesis when mitochondria

were absent. Gustafsson et al. (1965) found an increase in size, number and metabolic activity of mitochondria in skeletal muscle after administration of thyroid hormone. These effects are concomitant with the oxidative and phosphorylative function changes seen both *in vivo* and *in vitro*, when thyroxine produces a nearly complete uncoupling of oxidative phosphorylation (Hoch, 1962). Iodine has also been shown to cause swelling of mitochondria (Greit, 1962). The rate of swelling with iodine was much more rapid than with T_4 (Rall et al., 1963), and when iodine was given to thyroidectomized rats, the animals were almost normal in their growth (Asline and Evans, 1963). Not all tissues are so affected. The brain is a notable exception showing neither a calorigenic nor a sodium pump effect, whereas liver, heart, kidney, skeletal muscle, pancreas, salivary glands, epidermis and anterior pituitary are affected. These affected tissues consumed less oxygen in a hypothyroid state [lowered basal metabolic rate (BMR) and increased oxygen consumption during hyperthyroid conditions) than normal (Bernal and Refetoff, 1977; Underwood, 1977). Other tissues not affected include the gonads and accessory sex organs, lungs, spleen, and gastric smooth muscle (Barker and Klitgaard, 1952). The measurement of BMR was used for the determination of thyroid function status (normal or abnormal) until

assessment of T_4, T_3 and other related compounds were introduced.

2. Carbohydrate and Lipid Metabolism. In hyperthyroid individuals glycogen content is decreased in liver and muscle cells, glycogen synthesis is decreased and glucose catabolism is accelerated by thyroid hormones. Some workers have reported an increase in glycogen synthesis, through this may be dose related with low doses increasing synthesis and high doses decreasing anabolism of glycogen. Hexose phosphorylation and activity of intestinal phosphokinase enzymes are increased by thyroid hormones. Overall some of the effects of thyroid hormones on carbohydrate metabolism may be indirect effects from insulin and epinephrine (Hoch, 1962; Goodman and van Middlesowrth, 1974).

Thyroid hormones potentiate insulin action and sensitive animals to the cardiovascular, glycogenolytic, and lipolytic effects of epinephrine and norepinephrine. Free fatty acid (FFA) content of fat and release of FFA is increase in hyperthyroid animals and is further increased by epinephrine. This decrease in fat storage is concurrent with reduced serum cholesterol levels from degradation to bile acids and excretion of cholesterol. Rates of synthesis for cholesterol and fatty acids are increased, however, the ability of the liver to excrete cholesterol is increased greatly (Hoch,

1962; Rosenberg and Bastomsky, 1965; Miettinen, 1968; Bernal and Refetoff, 1977).

Following thyroidectomy in rats a reduced oxidation of palmitate was noted, as well as the usual decreased oxygen consumption, but glucose, pyruvate, and acetate oxidation were not affected. The adipose tissue of these hypothyroid animals showed a decreased capacity to oxidize long-chain fatty acids and a decrease in glycerol production. This reduction in FFA oxidation partially accounts for the reduced oxygen consumption of the thyroprivic fat cells. These changes in adipose tissue function may be due to abnormalities in the function of mitochondrial membranes caused by the thyroidectomy (Bray and Goodman, 1968).

3. Protein Metabolism and Cell Growth. Induction of RNA and protein synthesis has been mentioned earlier as being induced by thyroid hormones. These hormones are essential for growth, especially during early post-natal life in all mannals, birds, and most developmental processes of amphiabian larvae including metamorphosis. A direct action of the thyroid hormones at the cellular levels is the enhanced uptake of some amino acids and carbohydrates. This effect is seen within minutes after exposure to the hormones which is opposite of the thermogenesis effects that may take days to be seen (Goldfine et al., 1975a, b). A synergism

exists between T_4 and growth hormone (GH), such that GH will stimulate normal growth without T_4, although the overall rate of growth is enhanced with T_4 (Asling and Evans, 1963; Widnell and Tata, 1966). Also, thyroid hormones, growth hormone or testosterone will stimulate the Mg^{2+}-activated RNA-polymerase reaction in liver nuclei. The combination of thyroid hormones and either GH or testosterone produces an additive effect (Widnell and Tata, 1966). In hypothyroid individuals, showing depressed protein synthesis and degradation, administration of thyroid hormones increases synthesis of RNA followed by increasing protein synthesis and decreasing nitrogen excretion (Tata et al., 1963; Tata and Widnell, 1966; Frieden and Lipner, 1971; Underwood, 1977). Another abnormality associated with hypothyroid individuals is the deposition of micro-proteins in subcutaneous and extracellular spaces. This abnormality, associated with the negative nitrogen balance, is offset by thyroid hormone administration which causes absorption of the mucoproteins and a loss of water (Goodman and van Middlesworth, 1974). Hyper-thyroid animals show net catabolism of protein rather than the normal anabolic action. This increased break-down of proteins, mainly muscle, leads to an increase in nitrogen and cretine excretion (Kekki, 1964; Bernal and Refetoff, 1977).

4. Temperature Regulation. The calorigenic action of the thyroid hormones is probably secondary to the enhanced, energy-requiring, protein synthesis. In subjects with thyrotoxicosis an increase in metabolic rate is seen, as well as tachycardia, anxiety, and increased respiration. These abnormalities are similar to beta-adrenergic stimulation in normal individuals but only tachycardia is mediated by the beta-sympathic mechanism during thyrotoxicosis (Zwillich et al., 1978). With an increase in thyroid hormones a number of enzymes increase: myocardial adenyl-cyclase (Levey et al., 1969), cytochrome aa_3,[3] cytochrome C[3], and succinate dehydrogenase.[4] This increase in cellular oxidation generates more heat and less ATP, most notably seen in cold adaptation. A cold environment elicits an increase in production of thyroid hormones, an increase in conversion of T_4 to T_3, and also other hormones. These calorigenetic effects are inhibited or reversed by inhibitors of protein and nucleic acid synthesis (Weiss and Sokoloff, 1963). Both corticotrophin (ACTH), which promotes the secretion of cortical hormone, and somatotrophin (STH), which augments the action of exogenous TSH, appear to be important factors in calorigenesis. Epinephrine is also

[3] The cytochrome enzymes function in the sequence of electon-transfer reactions in the respiratory chain from NADH [nicotinamide adenine dinucleotide (reduced)]

[4] This enzyme is found at the inner mitochondria membrane and oxidizes succinate in the citric acid cycle.

involved in that it is potentiated by thyroxine during cold acclimatization (Hoch, 1962; Albright et al., 1965; Turner and Bagnara, 1971). Norepinephrine's calorigenesis action is the immediate response to cold and produces a lipolysis and an increase in oxidation of fatty acids. This action of norepinephrine is not seen in birds where the thyroid is of prime importance (Prosser, 1973).

5. Reproduction and Interactions with the Gonads.

a. Males. Thyroid hormones are required for the normal maturation of testes in all male species. In young hypothyroid animals the gonads are reduced in size, resulting in depressed libido and spermatogenesis and underdeveloped accessory glands. Thyroxine adminsitration ahs been shown to improve the sexual performance of these animals (Magsood, 1951; Parrott et al., 1960; Underwood, 1977). In mature rams, bulls, and stallions, mild hypothyroidism is associated with decreased libido and semen quality. This decline in performance is corrected with thyroactive proteins, but not with iodine unless the animals is iodine deficient (Underwood, 1977).

Males given additional thyroid hormones (in moderate amounts) show an increase in spermatogenesis even when no hypothyroidism exists (Turner and Bagnara, 1971).

b. Females. Magsood (1951) reported the maturation of ovaries by thyroid hormones via stimulation of gonadotrophin production. As with males, thyroidectomy at an early age causes the ovaries to remain in an infantile condition. When thyroid activity is suppressed, in mature female laboratory animals, irregular and lengthened estrus cycles are seen. In farm animals fed low iodine diets, fetal development may be arrested. Thus, death of the embryo or the birth of weak, hairless young may occur. These changes are often associated with prolonged gestation and parturition (Allcroft et al., 1954; Bruce and Sloviter, 1957; Hoar et al., 1957). Parrott et al. (1960) using thyroid-deficient rats, which produced smaller litters and fewer pregnancies, showed increased conceptions and litter size by supplementing the rats with thyroid hormones.

In iodine deficient areas cattle showing suppressed or irregular estrus and infertility showed marked improvement with iodine therapy. The iodine therapy. The iodine fed 8-12 days prior to estrus improved first time conception rate and conception rate of repeat-breeders (Moberg, 1961; McDonald et al., 1961).

When thyroactive compounds, from gullet trimmings[5] or triiodothyronine and sodium-L-thyroxine pentahydrate, were fed to mink, a decrease in the number of females that whelped, kits born, kit's birth weight and livability in all treatments were seen. The intake of thyroxine from these diets was about 40 times the mink's normal secretion rate (9.5 µgm) (Travis et al., 1966).

These same results of deleterious effects upon reproduction were also shown in rats fed either dried thyroid-parathyroids (T-P) from gullet trimmings at low and high levels or gullet trimmings without the T-P. The rats on low (0.30 percent) dried T-P produced normal young but many lost weight and died (77.8 percent mortality at seven days and 95.2 percent mortality at 14 days). Gross observations of the mothers mammary glands showed a reduced growth as compared to control mothers glands. Of the high T-P fed rats only 30 percent of the mothers littered (60 percent of control mothers littered) and no pups survived to seven days (20/20 died). These mothers' mammary glands were identical to the low T-P group. Gullet trimmings minus T-P showed not adverse effects; 90 percent of the mothers whelped and all pups were alive at birth and survived for 14 days (Travis et al., 1963).

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[5]Consist of the trachea, larynx, pharynx, esophagus, and adhering muscular and glandular (thyroid-parathyroid) tissue from calves a product used in making mink feed by many commercial mink ranchers.

In a recent study Aulerich et al. (1978), feeding iodine at 10, 100 and 1000 ppm to mink during gestation and through lactation, found a decrease in the number of kits born at 100 ppm and no reproduction from females fed the 1000 ppm iodine diet. The kits, from the 100 ppm iodine-fed mothers, weighed significantly less at birth and at four weeks of age than the control kits.

Iodine Toxicity

A number of theories for the mechanism of excessive iodine effects have been given though none have been proven: (1) interference with an enzyme system(s); (2) inhibition of carbohydrate metabolism; (3) possible H_2OI^+ formation; (4) suppression of the thyroid to respond to TSH and inhibition of iodine uptake by the thyroid (Wolff, 1969), (5) metabolic acidosis (Dyck, et al., 1979).

1. Laboratory Animals. The effect of a single large (500 µg iodide) does given intraperitoneally to the rat was a depression of organic binding of iodide (protein bound) by the thyroid, but the level of binding gradually returns to normal after 16 hours. A significant increase in the MIT/DIT ratio is also seen within the first few hours when binding is decreased (Wolff and Chaikoff, 1948; Galton and Pitt-Rivers, 1959a).

Longer term treatment of rats with iodide (1 mg per day) gives an initial decrease in iodine binding followed by a return towards normal binding levels (after 3-4 days). However, the iodine binding slowly falls during the next few weeks to about 70 percent of control values (Galton and Pitt-Rivers, 1959a). Rats fed iodine at 0.119 gm per day for nine months showed no myxedema nor inhibition of organic binding of iodine, though they did develop a goiter condition (Correa and Welsh, 1960). These authors did not look at PBI during the early stage of the experiment as did Galton and Pitt-Rivers (1959a) and the experimental animals were subjected to pneumonitis and many died (36/60), thus, results of the two studies were not comparable. Acute toxicity of iodine in mice, rabbits and guinea pigs was examined by Highman et al. (1955). Severe retinal degenerative changes were seen after an i.p. injection of 100 mg/kg KIO_3, in all three species. Necrosis of renal convoluted tubules, adrenal cortical lipoid depletion, and severe fatty changes in the liver, kidney and heart were observed in mice.

Arrington and his co-workers (1965) have examined tolerance to high iodine intakes in several species. In rats fed 0 to 2500 ppm supplemental iodine up to 35 days perpartum an increase in mortality of pups after birth,

directly related to the level of iodine in the diet, was seen. Gestation time was not affected, but a prolonged parturition was noted. High levels of iodine reudced the number of pups born per litter. By 48 hours after birth there was only one surviving litter with three young in the highest level. Histological examination of the mammary glands showed normal epithelial development, however, milk secretion was diminished or absent in the treated groups. Oxytocin and prolactin administered to the mothers were ineffective in inducing or maintaining lactation. Male rats fed up to 2500 ppm iodine showed no effects; as they sired normal litters. Rabbits fed up to 1000 ppm iodine showed an increased mortality of the newborn and deaths occurred earlier than that of rats. Lactation was normal in the iodine-treated rabbits but the young died within the first few hours and did not attempt to nurse. Hamsters fed 2500 ppm iodine were not affected, except for a slight decrease in food consumption and weaning weight of the young (Arrington et al., 1965; Ammerman et al., 1964; Taylor et al., 1964). Rats fed excess iodine (20-30 mg/day) for four months showed no signs of hypothyroidism, but four of 12 died and TSH was increased in the iodine-treated group. Of the rats that died no gross or histopathologic lesions were seen (Liwendahl et al., 1972).

The LD_{50} for potassium iodate in dogs is 200-250 mg/kg. At these levels anorexia, prostration, and death occurred. Some histopathologic lesions were noted: fatty changes in the viscera, necrosis in the liver, kidney and mucosa of the intestine and urinary bladder, and retinal degeneration (Webster et al., 1966).

2. Farm Animals. Feeding of thyroid glands to White Leghorn chickens resulted in female-type feathering on male birds and hastened the normal molt of pullets, but did not have any effect on body weight or egg production (Cole and Hutt, 1927). Feeding desiccated thyroids to laying hens decreased feed consumption, body weight and egg production. Similar results by Asmundson et al. (1936) were seen when sodium iodide was fed. This study was noted that the iodine content of the egg was not related to the iodine intake of the hen.

Thyroprotein fed to broilers produced an increase in body weight in males, a decreased weight of the comb, testes, adrenals, thyroids, and pituitary of the males and an early feather growth in males and females (Wheeler et al., 1948).

More recently Arrington et al. (1967), working with pullets and laying hens, determined effects of added iodine on reproductive performance. Egg production of pullets was decreased in all treatments (625 to 2500 ppm

supplemental iodine) and varied inversely with the level of iodine. The hens fed 500 ppm iodine produced no eggs after the second week on trial. The laying hens responded about the same as the pullets, but the decrease in egg production was more severe. It was noted that the ovaries of the non-producing hens contained many ova but ovulation was not occurring. Egg weights from treated hens were decreased by the supplemental iodine. Fertility was not affected but embryonic mortality and length of hatching were increased while hatchability was decreased. Chicks that hatched from treated hens had larger thyroids and did not grow as well as control chicks (Perdomo et al., 1966; Arrington et al., 1967).

Hens given 500 mg iodine per day ceased egg production by eight days, at which time their eggs contained 7 mg of iodine. When a threshold level of iodine was accumulated in the ova, development stopped and atrophy began (Marcilese et al., 1968).

Mares fed a diet containing seaweed added at 0.89 percent, which contained iodine, produced foals with nodular goiters. This diet gave over 30 mg of iodine daily whereas the daily requirement of the horse is only 1-2 mg. The thyroids were grossly enlarged in the mares and the foals' thyroids were larger than the mares'. No other clinical signs were noted (Miyazawa et al., 1978).

Driscoll (1978) also reported goiters in foals caused by feeding a kelp supplement to mares. This kelp supplemented diet gave 35 mg of iodine daily which is in agreement with Baker and Lindsey (1968) and Drew et al. (1975) who reported that feeding 40 mg of iodine daily to a pregnant mare could result in a goitrous condition in the foal. Also, if these animals are not changed to a lower iodine containing diet death can occur in the young horses.

Pigs are much more tolerant of excessive iodine than most other species. Pregnant sows fed 1500 or 2500 ppm iodine showed no detrimental effects. Lactation, body weights of young pigs, and growth were all normal. The approximate daily intake of iodine on a per kg basis was only 41 mg which may account for the no effect (Arrington et al., 1965). Growing pigs receiving up to 400 ppm iodine showed no difference in their performance as compared to controls. However, serum iodine levels and thyroid gland weights were increased in all groups and liver iron concentration was depressed in the 400 ppm group. In pigs fed 800 or 1600 ppm iodine, along with affects mentioned above, growth rate, feed intake and hemoglobin levels were also depressed. It was noted that either oral administration or injection of iron improved the swines' performance, including hemoglobin levels (Newton and Clawson, 1974). Feeding sows 100 times

their daily iodine requirement may interfere with length of gestation, induce resorption of feti, cause abortion or reduce the number of pigs born. This level of iodine depresses hemoglobin values and hematocrits in both sows and newborn pigs (Erickson, 1977, 1978).

When thiouracil at levels of 0.175 gm to 0.544 gm per lamb per day was fed to determine the effect on daily gain, no effect on rate of growth was seen. However, at 1.148 gm/lamb/day, a significant decrease in body weight gain was noted. Feeding thiourea at 0.064 and 0.071 gm per lamb daily also depressed body weight gains. Increased thyroid weights were also reported in these groups (Andrews et al., 1947).

Lambs given iodine, ranging from 94 to 785 mg per lamb per day, showed increased body temperatures. Lambs on the higher doses had decreased feed consumption and weight gain. These animals were also lethargic. Some coughing and diarrhea were noticed in high iodine-treated lambs; a few deaths also occurred. Serum iodine concentrations were high thoughout the experiment and decreased after termination of treatments (McCauley et al., 1973). These results are in agreement with Blaxter (1948) in which lambs given iodinated casein showed: hyperthermia, anorexia, coughing, nasal discharge, increased respiratory and heart rates, and were emaciated.

In calves elevated dietary iodine depressed body weight gain and feed intake. Levels of 100 and 200 ppm iodine produced coughing and profuse nasal discharge. Serum calcium and blood hemoglobin concentrations were depressed by 200 ppm iodine. Adrenal glands were heavier as were the thyroids of the treated animals (Newton et al., 1974). Cattle fed ten times the iodine requirement showed signs of iodine toxicity including decreased milk production, lameness, nasal and lacrimal discharge, and chronic low-grade fevers (Wallace, 1975). Cows showing increased mammary and respiratory infections as reported by McCauley et al. (1972) were shown to be consuming high levels of EDDI.[6] The total iodine that these herds were getting ranged from 250 to 1700 mg per head per day. Convey et al. (1977, 1978) reported no effect on thyrotropin, thyroxine, and triiodothyronine in cows fed 1.6 and 3.3 g iodine daily for 341 days. Haggard et al. (1978) reported a shorter and milder antibody response in heifers fed 1250 mg of iodine per day. Both humoral and cell-mediated immunity were shown to be impaired, as well as reduced DNA synthesis of lymphocytes.

3. Mink. While 0.03-0.05 mg of iodine daily has been considered sufficient (Saad, 1975), others have

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[6] Ethylenediamine dihydriodide.

suggested 0.18 to 0.20 ppm (dry matter) during breeding and growth (Wood, 1962) and 0.45 ppm (dry matter) (Kiiskinen and Mäkelä, 1977). Though normal feed contains about 2.4 to 6.4 ppm iodine (Kiiskinen and Mäkelä, 1977).

Thyroprotein administered at a level to supply twice the normal level of thyroxine gave no adverse effects. A slight increase in weight gain was noted only in natural dark mink but not other color phases (Aulerich and Schaible, 1966).

Mink fed diets supplemented with iodine at 10, 100 and 1000 ppm and mink housed in cages with nest boxes sanitized with 100 ppm and 1000 ppm titratable iodine showed adverse reproductive effects at 100 and 1000 ppm added iodine levels, but increased kit survival in the treated nest box groups. The 1000 ppm treated mink whelped no kits and the 100 ppm iodine-fed mink produced fewer kits in a decreased biomass[7] (Aulerich et al., 1978).

Fish and Fishmeal

Coho salmon from the Great Lakes have shown an increased frequency of hypothyroidism in the past six

[7]Biomass - average kit body weight gain between birth and four weeks of age x the average number of kits raised per lactating female.

years (Moccia et al., 1977). It appears that environmental pollutants, either alone or together, in some manner are acting as goitrogens and are accumulating in the Great Lakes (Moccia et al., 1977). Fish from the North Atlantic, especially near the mouth of the St. Lawrence River, have had the same problems with environmental pollutants. The effects of these pollutants on fish do not appear to affect the thyroid hormone production in all cases. Salmon from Lake Ontario had eight times the frequency of goiter as Lake Michigan fish but also had four times the T_3 and T_4 content (Moccia et al., 1977). Another fish, the lake trout, from Lake Michigan, was shown by Hunn (1964) to be iodine deficient. Mink have been shown to be hypersensitive to contaminants in the past and thus may be more sensitive than animals lower on the food chain, such as fish. Fish fed raw to mink have been shown in the past to induce anemia in mink (Skrede, 1970a). Reproductive failure has been shown in mink to be caused by the feeding of Great Lake fish. These fish were contaminated not with a goitrogenous substance, but by polychlorinated biphenyls[8] (Ringer et al., 1972).

The nutrient composition of various fishmeals has been determined, iodine content was 0.51 to 0.54 ppm for

[8]At high levels PCBs do cause hypothyroidism during estrus and the reproductive season (Byrne, 1974).

British Columbia whole herring meal (March et al., 1963) and 1.3 to 126 ppm (29.7 ppm average) for Atlantic Coast herring fish meal (Power et al., 1969).

Feeding mink a diet containing 40 percent of mackerel with added fat (3 percent) produced an increased kit mortality (30.3 percent) compared to 16.4 percent (Jarosz and Berteczko, 1976). However, Taranov (1976) fed a diet containing 50 percent fishmeal (wet weight) without affecting breeding results, though it was specified that the fish meal should be of good quality and a vitamin supplement added.

Breeding

The mink's reproductive cycle begins in July with anestrus and low plasma estradiol levels and undeveloped ovaries, oviducts and uteri. During the second half of anestrus, October to December, there is a slight increase in the plasma estradiol concentrations which continues into proestrus, January to February, and reaches a maximum at this time. Oviducts and uterine weights increase during this time, prior to breeding. Estrus starts during late February and continues through March, during which ovarian weights are maximal while estradiol concentrations are reduced. After mating, ovarian weights are decreased, uterine

weights increased and plasma estradiol levels are decreased further (Møller, 1973; Pilbeam et al., 1979).

Females exhibit a delay of implantation of the ova and both rebreeding and fertilization may occur during this period. Generally growth of the follicle starts 18-20 hours after mating, followed by rupture and release of the egg. Ovulation occurs by approximately 48 hours after copulation. Females are ready for re-mating 7-10 days after the first mating since the corpora lutea do not secrete any progesterone. The embryos implant about 28-30 days before parturition. The mean length of gestation is approximately 51 days (Venge, 1959; Enders and Enders, 1963; Duby, 1969).

When mating takes place a second time (at an interval of six days or more), ovulation takes place again. Because of this, superfetation may take place; the development of embryos at different ages taking place in the same mother.

Superfecundation may also take place. This refers to the more or less simultaneous fertilization of two ova by spermatozoa of different males. This occurs generally when a female is mated on successive days by different males (Hansson, 1947; Enders, 1952).

Rationale

The thyroid gland which contains 70-80 percent of the body's total iodine is involved in the metabolism of thyroxine (T_4) and triiodothyronine (T_3). These hormones are necessary for biological functions such as metabolic rate, body and fur growth, reproduction and lactation. Substances that interfere with the formation rate of thyroid hormones, will interfere with the animals' biological functions controlled by these hormones. Some chemical goitrogens can produce severe hypothyroidism associated with thyroid hyperplasia, especially if the animals are on a marginally iodine deficient diet (Capan, 1978). Belzile et al. (1974) produced thyroid hypertrophy in mink by feeding a diet containing 20 percent rapeseed flour. The hypertrophy was due to the presence of a goitrogen in the flour (glucosinolate). Reproductive failure is seen in many domestic animals on iodine deficient diets.

Conversely, excessive iodine intake can also cause thyroid hyperplasia while interferring with normal thyroid functions, as it reduces thyroid uptake of iodine. Thyroactive compounds at sufficiently high levels to cause thyroidal stimulation and reproductive failure (Travis et al., 1966), have been found in mink feed. A

more recent study showed that iodine levels as low as 10 ppm, fed for only 69 days, decreased the gestation period, whereas a level of 100 ppm decreased the gestation period and decreased the number of kits born (Aulerich et al., 1978). This decrease in kits may be due to the fact that iodine crosses from the mother's blood through the placenta and concentrates in the fetus; thus causing death of the embryo via iodine toxicity (Salter, 1940).

Coho salmon from the Great Lakes have shown an increased frequency of hypothyroidism during the past six years. It appears that environmental pollutants, either alone or together, are acting as goitrogens and are accumulating in the Great Lakes (Moccia et al., 1977). Fish from the North Atlantic, especially near the mouth of the St. Lawrence River, have had similar thyroid disorders attributed to environmental pollutants. The effects of these pollutants on fish do not appear to affect hormone production in all cases. Salmon from Lake Ontario had eight times the frequency of goiters as Lake Michigan fish but also had four times the plasma T_4 and T_3 content (Moccia et al., 1977). Another fish, the trout, from Lake Michigan was shown by Hunn (1964) to be iodine deficient. In the past mink have been shown to be hypersensitive to certain environmental contaminants and, thus may be more sensitive than animals

lower on the food chain, such as fish (Aulerich et al., 1971).

OBJECTIVES

1. Determine the effects on reproduction and growth of feeding high levels of fishmeals to adults and young mink and rats.
2. Determine the levels of iodine that produce a decrease in reproductive performance of the female mink and/or effects on the kits.
3. Investigate the transport of iodine in mink via determinations of the amount of iodine in the lactating mother's milk and urine.
4. Determine the effects of iodine on adult and kit mink thyroid parameters, including thyroid weight, T_4, T_3, rT_3, and T_4-binding index.
5. Determine the gross and histopathologic lesions in the mink caused by excess iodine.

EXPERIMENTAL PROCEDURES

A. Introduction

A long-term and a short-term feeding trial were conducted to determine the effects of excess levels of dietary iodine on various nutritional, physiological, and pathological parameters of adult mink and their offspring.

Various fishmeals were fed at high levels to screen for their effect on reproduction in Sprague-Dawley rats. These trials were conducted to determine if any fishmeal may have contained an ingredient in sufficient quantity to affect the thyroid or reproduction of the animals. Any fishmeal diet having an adverse effect on the rats was tested further using mink.

All mink in these studies were housed and cared for at the Michigan State University Fur Animal Project Ranch located at the Poultry Science Research and Teaching Center. The rats were cared for in accordance with current good laboratory practices. For the mink, standard ranch procedures were followed.

B. Experiment

1. Animals and diets

Rats: The animals were Sprague-Dawley and were obtained from Spartan Research Animals, Inc.[1], or Harlan Industries[2].

Upon arrival all animals were weighed and placed in separate cages, 23.7 cm (l) x 17.8 cm (w) x 17.5 cm (h). They were given "clean" feed and water daily for a two week acclimation period. The rats were reweighed after two weeks to determine if any abnormal weight changes took place. If any abnormal weight changes were noted (loss of > ten percent of body weight) the animal was not used. The animals were randomly divided into groups of nine females and three males per treatment. Feed and water were provided ad libitum throughout the experiment. For trial 1, one group served as controls and the other five groups were placed on a control diet (see Appendix A) supplemented with various fishmeals at 40 percent (see Table 1). For trial 2, one group served as control and one as a repeat for BFS Canadian herring. Another group was fed Great Lakes fishmeal. Trial 3 consisted of a control group and a Menhaden fishmeal treated group.

During trial 1, rats were weighed weekly for four weeks prior to breeding, For trial 2 and 3, rats were weighed on day 1, 14, and 28 before being bred.

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[1] 5735 N. Shoeman Road, Haslett, MI 48840.

[2] P.O. Box 29176, Cumberland, IN.

Table 1. Types of fishmeals[a] fed to Sprague-Dawley rats for a minimum of three weeks prior to mating through weaning. Trial number in which each fishmeal was used and number of females per group per respective trial is given.

Name	Trial Number	Number of Females
Control	1, 2, 3	9, 6, 9
White Fishmeal	1	9
Boston Fishmeal	1	9
BFS Ocean Maid	1	9
BFS Canadian Herring	1, 2	9, 9
British Columbia	1	9
Great Lakes Fishmeal	2	9
Menhaden Fish	3	9

[a]See Appendix B for complete address of suppliers of fishmeals.

The newborn rats (pups) were weighed at birth and again at weaning. Feed consumption was determined at bi-weekly intervals.

Mink: All animals used were standard dark mink (Mustela vison) from the MSU breeding stock, born during May of 1978. Twelve females and three males were placed on each treatment. The control group and five iodine treatment groups were started August 8, 1978 for determination of long-term effects on iodine. The animals were immature and approximately three months old, they were on treatment to maturity through one reproductive season (11 months total duration).

The iodine diets were prepared by adding iodine, as potassium iodide (see Appendix D) mixed in a one liter solution, to 100 kg of regular feed. The feed was mixed in a large commercial mixer for a minimum of five minutes. For the diets started August 8, 1978 the levels of added iodine were 10, 20, 40, 80 and 160 ppm. Five additional diets were started February 18, 1979 and consisted of four iodine supplemented diets (40, 80, 160 and 320 ppm) and one fishmeal diet: BFS Canadian herring. The fishmeal was added to the control diet at 30 percent (for composition of diets see Appendix C). All diets were fed ad libitum and water was available at all times. All animals were housed individually in wire cages, out-of-doors in a commercial-type, open-sided

mink sheds. The cages were single-tier, 30.5 cm (w) x 76.2 (l) x 47.7 cm (h) or 30.5 cm (w) x 61.0 cm (l) x 38.1 cm (h) with an attached nest box and water cup. Three subgroups of each treatment, which consisted of four females and one male, were randomly placed throughout the shed. Each mink identification card, placed above the cage, was color coded with the color code on the respective feed containers used to store the various diets. This procedure aided in the prevention of feed being fed to the wrong mink.

Body weight measurements were made at monthly intervals, except during the reproductive season when handling the bred females might have an adverse effect on reproduction.

2. Breeding

During the reproductive season, females were housed, individually, in breeder cages, [61 cm (w) x 76.2 cm (l) x 45.7 cm (h)] to which a nest box, 30.5 cm (w) x 25.4 cm (l) x 25.4 (h), was attached on the outside of the cage.

Bedding, used in the nest box, consisted of shredded wood and this provided for insulation in the winter and for nesting during the reproductive season. The mink season began on March 1, 1979, and lasted until the end of the month.

International Microcapillary Centrifuge[3]. After centrifugation, the packed red cell volume in each tube was measured using a microcapillary reader.

Blood smears for differential counts were prepared using fresh flowing blood, containing no anticoagulants, and a clean glass slide. The blood was allowed to air dry before straining. The smears were then stained with Camco Quik Stain[4] (buffered idfferential Wright's Stain) for five to ten seconds, followed by dipping in distilled water for 15-20 seconds and rinsed by dipping in clean distilled water for a few seconds, then allowed to dry.

Serum calcium was determined by spinning whole coagulated blood at 3000 rpm for ten minutes and removing the serum. The serum was analyzed on a Corning 940 calcium analyzer[5] (see Appendix D for theory of operation).

Plasma and serum were used in the determination of chloride. The plasmas was collected from whole blood with added heparin and was spun for ten minutes at 3000 rpm. The chloride determinations were performed on a digital Chloridometer[6] Model 4-2500 (see Appendix F for theory of operation).

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[3]International Equipment Company, Needham Heights, MA 02194.

[4]Cambridge Chemical Products Inc., Ft. Lauderdale, FL 33309.

[5]Corning Scientific Instruments, Medfield, MA 02052.

[6]Buckler Instruments, Fort Lee, NJ 07024.

Most females were mated to a male in their respective dietary group. Some females were mated to males in other groups for two reasons: one, not all the males in their group were as sexually active as they should have been; and two, so as to help determine if the iodine was having an effect on the males fertilization capacity. Attempts were made to mate the females every fourth day until a positive mating was secured. A positive mating was confirmed by checking post-coital vaginal aspirations for motile sperm. This check also helped determine if the treatment was affecting the male's spermatism. Positive matings were followed by a second mating attempt eight days later.

During the whelping period (April 20 to May 15) the female's nest box was checked daily for evidence of new-born kits. At one day of age and at four weeks, the kits were counted, sexed and weighed. Whelping females were also weighed on the day of whelping and at one month afterwards. Length of gestation, litter size, kit mortality, sex ratio, kit biomass during lactation, and lactating female weight changes were recorded.

3. Packed cell volume and milk sampling

Packed cell volumes were determined by collecting blood by nail clip into a heparinized capillary tube. After sealing one end of the capillary tube, it was centrifuged at 4500 rpm for 7.5 minutes in an

Erythrocyte and leukocyte counts were determined on whole blood collected either via a nail clip or heart puncture with an anticoagulant, heparin. Gounts were performed on a Coulter Counter Model Z[7] (See Appendix G for theory of operation). Hemoglobin determinations were performed on Hemoglobinometer[7] (see Appendix H for theory of operation).

Milk samples were collected from female mink after an intramuscular injection of 0.06 ml of anesthetic[8] (at 50 mg/ml) and 0.5 ml (10 units) of oxytocin[9] to induce milk let-down. Milking consisted of placing a plastic needle cup over the teat to which the vacuum pump[10] had been attached and the mammary gland was gently squeezed with the fingers. Regular pulsations were produced via a finger over a T-tube in the line between the collecting vial and the pump. The pump developed a maximum of 322 mm Hg vacuum.

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[7]Coulter Electronics Inc., Hialeah, FL 33010.

[8]Telazol[R] manufactured by Warner-Lambert Co., Pharmaceutical Research Division, Ann Arbor, MI.

[9]United States Biochemical Corp., P.O. Box 22400, Cleveland, OH.

[10]Duoseal vacuum pump, The Welch Scientific Co., 730 N. Linden Ave., Skokie, IL.

4. Pathology

Necropsy was performed on all rats and mink that died during the course of the experiments, most kits and pups born dead, randomly selected kits at one week of age, and randomly selected adult mink and rats at termination of the experiment. All internal organs were examined with particular close attention to the thyroid. Histopathology was performed on randomly selected kits born dead, kits sacrificied at one week, and selected adult female mink necropsied at termination. Sections were taken from the thyroid, liver, adrenals, gonads (adults only), spleen, stomach (kits) or intestine (adults), kidneys, heart (kits only), and brain (adult only). Organs from the adult mink were weighed before samples were taken.

All tissues were fixed in 10% buffered formalin and strained with hematoxylin and eosin.

Total Iodine Determination

Previously frozen milk and urine samples from adult female mink were thawed and prepared in duplicate. Total iodine content of samples was determined using a Polarographic Analyzer-174A[11] with a Houston XY 2000 recorder[12].

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[11]Princeton Applied Research, Princeton, NJ.

[12]Brinkman Instrument, Westbury, NY.

Thyroid

To determine if added iodine was having an effect on the basal metabolic rate of mink, body temperatures were taken on adult male and female mink the second through the fifth months of iodine treatment. At weaning time adult female mink and kits had blood samples taken for T_4, T_3, T_4-binding index and reverse T_3 determinations.

T_3 and T_4 were determined by radiommunassay (RIA) in which free T_3 and T_4 were measured in the serum. The basic principle of this technique is discussed in Appendix H. The T_4-binding index test was not a RIA but rather was a comparison of the equine which tends to be normally higher than other species; for discussion of method see Appendix H. The test for rT_3 determines the amount of 3, 3', 5'-triiodothyronine in the serum and basically follows the method of Chopra (1974), for explanation of the method see Appendix H.

Animals and Fishmeal Diets

Mink: Thirty kits approximately nine weeks old were randomly divided into three groups of ten kits each. The kits, one male and one female per cage, were placed in cages the same size as the adults were kept in. Group one was placed on the control ration (Appendix C), group two was fed the control diet plus British Columbia fishmeal (supplemented at 30%) and group three was fed

the control diet plus Canadian herring (supplemented at 30%) (see Appendix B for supplier). The kits were weighed at the start and weekly thereafter for five weeks at which time feeding of the fishmeal diets stopped and all kits were placed on the control diet. Feed and water were both provided ad libitum throughout the experiment.

Rats: Twenty rats approximately four weeks old were randomly divided into two groups of ten pups each. The rats were placed two per cage, the cages were the same size as those used for the adults. One group was placed on the control ration (Appendix A) and the second group was fed control diet plus Great Lakes fishmeal (alewife) supplemented at 40% (see Appendix B for supplier). The pups were weighed at the start and weekly thereafter for three weeks, at which time the experiment was terminated. Feed and water were both provided ad libitum throughout the experiment.

Statistical Analyses

The data were subjected to a one-way analysis of variance. Level of significance of difference between means was determined by the Chi-Square, F-test or Dunnett-t test.

RESULTS

Rats

Pre-treatment body weight, weight at initiation and termination of treatment are presented in Table 2. No significant changes took place during these periods.

Body weight changes for the period prior to mating are presented in Table 3 and feed consumption in Table 4. No significant differences were noted in either body weights or feed consumption for any treatment group as compared to their respective control group. Reproduction in the rats is shown in Table 5 for trial 1 and Table 6 for trials 2 and 3. The three control groups were not significantly different from each other. The rats fed the Boston whitefish supplemented diet produced more pups born dead and greater pup mortality to three weeks of age. However, the pup's body weight and weight gain, during days 14 to 21, were not affected. The rats fed the Boston mixed fishmeal diet performed similar to the rats that received the Boston whitefish diet, though a significant decrease in pups born alive was noted (2.8), as well as an increase in pups born dead and a decreased survival to three weeks as compared to the controls. Rats

Table 2. Mean body weights of rats at the time of arrival to the laboratory, at the beginning of treatment and at the end of treatment

Dietary Treatment	Mean Body Weight[a] at time of arrival (week -2)	at start of treatment (week -0)	at termination of treatment (week +5)
Control[b]	231	274	348
40% Boston whitefish fishmeal	232	277	361
40% Boston mixed fishmeal	228	261	344
40% Ocean Maid fishmeal	231	264	346
40% Canadian herring fishmeal	230	258	345
40% British Columbia fishmeal	227	258	333

[a]Each group consisted of nine females and three males.

[b]Standard rat diet.

[c]All fishmeals were added on a dry weight basis.

Table 3. Body weight change of adult rats (n =♂3 ,♀9 per treatment) fed various fishmeals, supplementing the control diet at 40%[a].

Dietary Treatment	Sex	Days:	Body Weight Change (g/r/d) 0-7	8-14	14-21	22-28
Control[b]	♂		1.58	1.36	1.86	0.66
	♀		4.60	1.11	5.13	0.53
40% Boston whitefish fishmeal	♂		1.60	2.47	2.20	0.68
	♀		4.82	0.43	5.85	0.51
40% Boston mixed fishmeal	♂		2.28	2.69	1.42	1.51
	♀		4.00	1.90	4.49	-1.25
40% Ocean Maid fishmeal	♂		2.09	1.17	3.08	0.74
	♀		4.13	-2.43	4.29	1.07
40% Canadian herring fishmeal	♂		0.45	2.39	3.19	1.32
	♀		2.87	6.14	2.63	0.93
40% British Columbia fishmeal	♂		1.56	0.61	2.43	1.00
	♀		2.91	3.81	3.07	-0.33
		Days:	0-14		15-28	
40% Great Lakes fishmeal (alewife)	♂		1.57		2.78	
	♀		0.59		0.88	
Control	♂		2.48		2.33	
	♀		1.42		1.56	
40% Menhaden fishmeal	♂		5.36		-4.00	
	♀		2.64		6.50	
Control	♂		4.85		-2.09	
	♀		3.29		5.50	

[a]Added on a dry weight basis.

[b]Standard rat diet.

Table 4. Feed consumption of adult rats (n = 3♂, 9♀ per treatment) fed various fishmeals supplementing the control diet at 40%[a].

Dietary Treatment	Days:	Feed Consumption (g/r/d) 0-7	8-14	15-21	22-28
Control[b]		24.11	19.73	21.33	27.57
40% Boston whitefish fishmeal		22.69	19.00	22.61	23.52
40% Boston mixed fishmeal		24.81	18.62	19.78	25.33
40% Ocean Maid fishmeal		23.43	19.92	22.15	24.86
40% Canadian herring fishmeal		21.31	19.17	20.43	24.99
40% British Columbia fishmeal		18.77	18.48	18.81	23.15

	Days:	0-14	15-28
40% Great Lakes fishmeal (alewife)		20.16	21.50
Control		18.90	21.93
40% Menhaden fishmeal		26.01	26.52
Control		25.25	23.80

[a]Added on a dry weight basis.

[b]Standard rat diet.

Table 5. Reproductive success, weaning weight, and survival of rats fed various fishmeals fed at 40%[a] of the diet.

Diet	No. females whelped/no. mated	Mean No. of Pups per litter	Mean No. of Pups at birth alive	Mean No. of Pups at birth dead	Mean No. of Pups alive at 3 weeks/ mated	Mean No. of Pups alive at 3 weeks/ littered	Mean Weight of Pups at 3 weeks (g)	Mean Weight of Pups gain/pup (g/day)
Control	7/9	10.6	10.3	0.3	6.89	8.86	36.6 ± 0.95[c]	2.0
40% Boston whitefish fishmeal	5/9	10.8	9.4	1.4[c]	3.44[d]	6.20	32.7 ± 1.94	2.7
40% Boston mixed fishmeal	6/9	10.2	7.5[d]	2.7[e]	3.11[d]	4.67[d]	30.0 ± 1.67	2.5
40% Ocean Maid fishmeal	3/9	12.0	10.3	1.7[e]	2.78[d]	8.33	34.8 ± 0.33	2.2
40% Canadian herring fishmeal	3/9	12.7	9.0	3.7[e]	1.44[d]	4.33[d]	32.0 ± 0.85	1.1[d]
Repeat of 40% Canadian herring fishmeal	5/9	9.4	7.2[d]	2.2[e]	0.55[d]	1.00[d]	27.5 ± 0.81	1.1[d]
40% British Columbia fishmeal	6/9	10.2	8.2	2.0[e]	2.33[d]	3.50[d]	27.1 ± 0.36	1.2[d]

[a]Added on a dry weight basis.

[b]From 14 to 21 days of age.

[c]Standard error.

[d]Significantly less than control ($P<0.05$).

[e]Significantly greater than control ($P<0.05$).

Table 6. Reproductive success, weaning weight, and survival of rats fed Great Lakes alewife and Menhaden fishmeals fed at 40%[a] of the diet.

Diet	No. females whelped/no. mated	Mean No. of Pups whelped	at birth alive	at birth dead	alive at 3 weeks/ mated	alive at 3 weeks/ littered	Mean Weight of Pups at 3 weeks (g)	Mean Weight of Pups gain/pup (g/day)
Control[e]	5/6	10.0	9.0	1.0	6.00	7.20	31.1 ± 0.61[c]	1.8
40% Great Lakes fishmeal (alewife)	4/9	8.7	6.7[d]	2.0	2.11[c]	4.75[d]	29.8 ± 0.72	1.2
Control	7/9	10.7	10.7	0.0	7.67	9.86	33.1 ± 0.95	2.02
40% Menhaden fishmeal	5/9	10.3	10.0	0.3	3.44[d]	6.20[d]	29.0 ± 0.79	1.57

[a]Added on a dry weight basis.

[b]From 14 to 21 days of age.

that consumed the diet containing Ocean Maid fishmeal had only three litters, although an average 12 pups were born per litter, of those 1.7 pups were dead at birth. However, about the same number of pups lived to three weeks as the control pups and the pups from the Ocean Maid fishmeal fed group gained about the same as the control pups, 36.6 and 34.8 grams, respectively. Animals fed Canadian herring fishmeal also produced only three litters, and more pups were born dead than in the controls. However, they also showed a decrease livability through weaning. The repeat group of rats on Canadian herring fishmeal had fewer live pups but also fewer born dead than in the first trial. However, there was a significant increase in pup mortality during the first three weeks and a decrease in body weight of surviving pups. British Columbia fishmeal supplemented diets when fed to rats produced an increase in the number of pups born dead, a decrease in the number of pups at three weeks of age and the weaning weights were depressed by 26.0 percent from that of controls. Reproduction results in the rats fed Great Lakes fishmeal and Menhaden fishmeal are shown in Table 6. Rats fed the Great Lakes (alewife) fishmeal supplemented diet had fewer female litter (83 percent for control and 44 percent for Great Lakes alewife fishmeal) which produced fewer live pups and the survival of pups to weaning was

decreased, however, weaning weight was not significantly affected.

Rats fed Menhaden fishmeal supplemented diets were not as adversely affected as those fed other fishmeals. The number of pups surviving to weaning was reduced to 6.20 pups per female from control value of 9.86 pups per female.

Mink

Body weight changes for mink during the first six months on the long term iodine treatment are shown in Table 7. There were no significant differences between the animals in the treated groups and the controls during any period. By the fifth month (January) all groups lost weight, a time of year when weight loss is considered normal. The initial mean body weight of control amounts was less than any treatment group. However, the control group did gain more weight over all as compared to the treated groups. The final mean body weights are 1105, 982, 1034, 1077, 1010 and 994 for 0, 10, 20, 40, 80 and 160 ppm iodine treated groups, respectively. Packed cell valumes during the first six months of treatment are shown in Table 8. There were no significant differences in packed cell volumes between any treatment group and the control over any period. The gradual rise in packed cell volumes to values above 55

Table 7. Body weight changes (in grams) of male and female mink fed various levels of supplemental iodine during a six month period (August 1978 to February 1979).

Iodine Treatment Level (ppm)	Initial Weight (g)	Weight Change (g)[a]					
	August	September	October	November	December	January	February
0	672 ± 32.3[b] (15)[c]	58 ± 31.6 (15)	244 ± 31.6 (15)	33 ± 18.5 (15)	22 ± 10.5 (15)	-42 ± 26.3 (15)	28 ± 23.2 (15)
10	773 ± 53.0 (15)	172 ± 2019 (15)	65 ± 22.8 (15)	36 ± 8.5 (15)	14 ± 12.8 (14)	-102 ± 22.2 (12)	-7 ± 22.3 (12)
20	801 ± 30.1 (15)	160 ± 19.1 (14)	51 ± 15.5 (14)	13 ± 10.1 (14)	26 ± 12.2 (14)	-82 ± 19.8 (14)	35 ± 17.8 (13)
40	824 ± 36.1 (15)	159 ± 21.7 (15)	92 ± 32.7 (15)	30 ± 16.3 (15)	42 ± 15.5 (15)	-82 ± 14.8 (15)	19 ± 24.7 (15)
80	778 ± 25.9 (15)	99 ± 31.4 (15)	168 ± 45.1 (15)	19 ± 10.9 (15)	6 ± 16.0 (15)	-66 ± 23.7 (15)	7 ± 12.5 (15)
160	804 ± 40.5 (15)	85 ± 33.3 (15)	164 ± 36.4 (15)	54 ± 12.6 (15)	10 ± 11.5 (15)	-83 ± 19.8 (15)	-27 ± 16.7 (14)

[a] Data reported as group mean ± standard error.

[b] No significant differences in any treatment group as compared to control during any period ($P > 0.05$).

[c] Number in parenthesis = number of animals.

Table 8. Hematocrit values of mink fed various levels of supplemental iodine over a six month period (August 1978 to February 1979).

Iodine Treatment Levels (ppm)	Hematocrit[a] (%)						
	August	September	October	November	December	January	February
0	51.2 ± 0.59[b] (15)[c]	53.2 ± 0.35 (15)	55.1 ± 0.52 (15)	55.6 ± 0.32 (15)	55.6 ± 0.46 (15)	53.3 ± 0.64 (15)	56.7 ± 0.49 (15)
10	50.5 ± 0.87 (15)	51.4 ± 0.57 (15)	51.2 ± 0.55 (15)	54.6 ± 0.47 (15)	54.6 ± 0.48 (14)	54.4 ± 0.40 (12)	56.0 ± 0.75 (12)
20	50.4 ± 0.62 (15)	53.7 ± 0.47 (15)	54.1 ± 0.62 (15)	57.4 ± 0.57 (14)	55.9 ± 0.53 (14)	54.7 ± 0.61 (14)	57.1 ± 0.57 (13)
40	52.0 ± 0.62 (15)	53.7 ± 0.44 (15)	53.5 ± 0.45 (15)	56.4 ± 0.39 (15)	55.8 ± 0.37 (15)	53.6 ± 0.55 (15)	56.6 ± 0.58 (15)
80	51.4 ± 0.66 (15)	51.9 ± 0.51 (15)	51.8 ± 0.74 (15)	55.1 ± 0.56 (15)	55.5 ± 0.50 (15)	53.0 ± 0.51 (15)	56.0 ± 0.73 (15)
160	50.1 ± 0.74 (15)	51.7 ± 0.47 (15)	52.1 ± 0.58 (15)	53.9 ± 0.46 (15)	54.0 ± 0.38 (15)	54.2 ± 0.48 (15)	57.6 ± 0.54 (14)

[a] Data reported as group mean ± standard error.

[b] No significant differences in any treatment group as compared to control during any period ($P > 0.05$).

[c] Number in parenthesis = number of animals.

percent by month six (January) is a normal physiological function in mink during cold weather (Asher et al., 1976).

Serum calcium, hemoglobin, red blood cell and white blood cell counts measured during months five and six of iodine treatment are shown in Table 9. Only the hemoglobin of mink fed 40 ppm iodine was significantly decreased (-23.3 percent) at the fifth month but not at the sixth month. At five months the number of red blood cells in mink fed 40 ppm was significantly less than the control group by 22.4 percent.

Body temperatures, for mink fed the iodine-treated diets, during the first four months are shown in Table 10. No significant differences in body temperature were noted between the control and any treatment group during any month of the test.

Urinary iodine levels of mink fed 0, 10, 20, 40 or 80 ppm iodine supplemented diet, for five months, are presented in Table 11. As the level of iodine in the diet increased the level of iodine in the urine increased also. The correlation between dietary iodine and iodine extreted via the kidneys was +0.985.

Reproduction

The number of females that whelped per number mated, total number of kits whelped and percent dead, the average number of kits (alive and dead) per female that whelped

Table 9. Mean blood parameters of mink fed various levels of supplemental dietary iodine for five and six months.

Iodine Treatment Level (ppm)	n	Serum Calcium at 5 mo. on Treatment (mg%)	Hemoglovin (g/dl)		RBC (x 10^6)		WBC at 6 mo. on Treatment
			5 mo.	6 mo.	5 mo.	6 mo.	
0	5	10.8 + 0.22[a]	21.5 ± 0.47	22.4 ± 0.51	10.01 ± 0.29	10.27 ± 0.20	8100 ± 1346
10	5	10.4 ± 0.17	18.5 ± 0.97	20.8 ± 0.72	9.01 ± 0.40	9.88 ± 0.46	9400 ± 967
20	5	10.7 ± 0.25	18.8 ± 0.92	22.1 ± 0.49	8.34 ± 0.44	10.26 ± 0.31	10700 ± 1951
40	5	10.7 ± 0.18	16.5[b] ± 1.12	20.9 ± 0.75	7.77[b] ± 0.58	10.19 ± 0.29	9700 ± 911
80	5	10.1 ± 0.14	18.6 ± 0.87	21.9 ± 0.40	8.23 ± 0.44	9.64 ± 0.21	8400 ± 1046
160	5	10.6 ± 0.11	21.3 ± 0.34	22.5 ± 0.72	9.89 ± 0.35	10.13 ± 0.29	9000 ± 2117

[a]Standard error.

[b]Significantly different from control ($P < 0.05$).

Table 10. Mean rectal body temperature of adult mink fed various levels of supplemental iodine.

Iodine Treatment Level (ppm)	n	Body Temperature (°C)			
		Initial	1 month	3 months	4 months
0	8	38.1 ± 0.20[a, b]	38.5 ± 0.18	39.8 ± 0.12	39.8 ± 0.16
10	8	38.2 ± 0.10	38.4 ± 0.25	39.7 ± 0.12	39.9 ± 0.07
20	8	38.1 ± 0.13	38.4 ± 0.14	39.3 ± 0.17	39.5 ± 0.18
40	8	38.3 ± 0.14	38.3 ± 0.17	39.5 ± 0.24	39.5 ± 0.23
80	8	38.0 ± 0.13	38.4 ± 0.16	39.6 ± 0.16	39.5 ± 0.11
160	8	37.9 ± 0.24	38.7 ± 0.11	39.5 ± 0.20	39.9 ± 0.08

[a]Standard error.

[b]No significant differences in any treatment group compared to the control during any time period ($P > 0.05$).

Table 11. Urinary iodine levels of adult female mink fed various levels of supplemental iodine for five months.

Iodine Treatment Level (ppm)	n	Urinary Iodine Level (ppm)[a]
0	1	3.6
10	1	20.0
20	1	29.0
40	1	68.0
80	1	100.00

[a]Urine volumes did not vary significantly from each other.

and per female mated are shown in Table 12. The number of females that whelped decreased slightly in groups fed 10 ppm iodine (LT) and 20 ppm (LT) diets, 67 and 70 percent, respectively, versus 92 percent for control. However, the groups fed the 160 ST and 320 ST diets were greatly affected as to the number of females whelping. Only 55 percent of the females whelped that were fed the 160 ST diet while only 27 percent of the females fed the 160 LT diets whelped and only eight percent of the females fed 320 ST whelped. The females fed the 320 ST, 160 LT and 160 ST had a high percentage of kits born dead, 100, 67 and 39 percent respectively, while all other groups were less than the control. The average number of kits per female that whelped was decreased in the animals fed the 160 ST and 320 ST diets, all other groups were about equal to the control's 5.18 per female.

Length of gestation, mean birth weight of live kits and average number of live kits per female that whelped are shown in Table 13. Gestation was significantly shortened from the control (53.0 days) in three groups; 40 LT, 40 ST and 80 ST, all by about five days. The one female in the 320 ST iodine-treated group that whelped had a gestation period 11 days longer than that of the control animals. Birth weights were significantly decreased in the 160 ST treatment group and the fishmeal group by 24.6 and 16.8 percent respectively, no other

Table 12. Effect of feeding various levels of supplemental iodine or fishmeal on the number of females whelped, kits born alive and dead, and average total kits per female.

Treatment		No. females	No. kits whelped		Avg. No. kits/female	
iodine (ppm)	fishmeal	whelped/mated	total	% dead	whelped	mated
0	(control)	11/12	57	19.3	5.18	4.75
10	(LT)[a]	6/9	41	4.9	6.83	4.56
20	(LT)	7/10)	37	10.8	5.29	3.70
40	(LT)	11/12	58	13.8	5.27	4.83
40	(ST)[b]	9/10	45	6.7	5.00	4.50
80	(LT)	9/12	45	13.3	5.00	3.75
80	(ST)	9/11	46	8.7	5.11	4.18
160	(LT)	3/11	15	66.7	5.00	1.36
160	(ST)	6/11	23	39.1	3.83	2.27
320	(ST)	1/12	2	100.0	2.00	0.17
40%	Canadian herring	9/12	59	16.9	6.55	4.92

[a]Long term supplementation (Aug. 8, 1978 to July 1, 1979).

[b]Short term supplementation (Feb. 17, 1979 to July 1, 1979)

Table 13. Effect of feeding various levels of supplemental iodine or fishmeal on the number of live kits whelped per female, birth weight and length of gestation.

Treatment		No. live kits/ female whelped	Mean birth wt. of live kits (g)	Mean gestation (days)
iodine (ppm)	fishmeal			
0		4.18	9.36 ± 0.24[a]	53.0 ± 1.20[a]
10	(LT)[b]	6.50	9.29 ± 0.21	50.0 ± 1.81
20	(LT)	4.71	10.27 ± 0.34	51.1 ± 2.82
40	(LT)	4.54	8.48 ± 0.27	48.5 ± 1.63[e]
40	(ST)[c]	4.67	8.39 ± 0.19	48.4 ± 1.38[e]
80	(LT)	4.33	9.26 ± 0.24	52.0 ± 2.42
80	(ST)	4.67	8.80 ± 0.24	48.0 ± 1.09[e]
160	(LT)	1.67	8.34 ± 0.98	55.3 ± 1.76
160	(ST)	2.33	7.06 ± 0.30[d]	51.7 ± 2.56
320	(ST)	0	---	64[f]
40% Canadian herring		5.44	7.79 ± 0.20[d]	50.2 ± 2.56

[a]Standard error.

[b]Long term supplementation (Aug. 8, 1978 to July 1, 1979).

[c]Short term (Feb. 17, 1979 to July 1, 1979).

[d]Significantly less than control ($P < 0.001$)

[e]Significantly less than control ($P < 0.05$).

[f]One female in this group had two kits born dead.

group was significantly affected. The number of live kits per female that whelped was decreased in the 160 LT and 160 ST dietary groups by 2.51 and 1.85 kits per female, respectively, from the control (4.18 kits per female).

Mean kit weight at weaning, average number of kits raised to four weeks per lactating female, percent survival from birth to four weeks and "biomass" are shown in Table 14. Weaning weight was significantly increased in three groups: 20 LT, 40 LT and 160 LT. Kits in the 20 LT group were 14.4 percent heavier and kits in the 40 LT group were 10.4 percent greater, while kits in the 160 LT group were 44.8 percent heavier than control kits. However, there were only three kits in the latter group with one mother. Two groups had significantly depressed weaning weights as compared to the control kits: 160 ST and fishmeal. The kits in the 160 ST group weighed 26.4 percent less than control kits, while the kits in the fishmeal group weighed 10.4 percent less than control kits. Although the percent survival of kits to four weeks was increased from the control in the 10 LT and 20 LT groups from 57 to 72 and 91 percent, respectively, this control group is considered below normal values obtained from this mink ranch. The kits in the 80 ST group were the only other group above 70 percent survival. While the survival of most

Table 14. Effect of feeding various levels of supplemental iodine or fishmeal on the number of kits raised to four weeks of age, percent kit survival, weaning weight, and biomass.

Treatment		n	Avg. No. kits raised to 4 wks/ lactating ♀	% kit survival to 4 wks.	Kit wt. 4 wks. (g)[a]	Biomass[b]
iodine (ppm)	fishmeal					
0 (control)		26	3.7	57	125 ± 5.4	428
10 (LT)[c]		28	5.6	72	137 ± 4.5	715
20 (LT)		30	5.0	91	143 ± 4.0[e]	661
40 (LT)		34	3.8	68	138 ± 5.5[e]	490
40 (ST)[d]		28	4.0	67	133 ± 4.2	498
80 (LT)		27	3.4	69	134 ± 5.5	498
80 (ST)		38	4.8	91	133 ± 3.9	591
160 (LT)		3	3.0	60	181 ± 5.4[e]	517
160 (ST)		3	1.5	36	92 ±22.9[f]	127
320 (ST)		0	---	--	----------	---
Canadian herring	40%	26	3.8	47	112 ± 5.5[f]	398

[a] Data reported as group mean ± standard error.

[b] Biomass - average kit body weight gain between birth and 4 weeks of age x the average number of kits per lactating female.

[c] Long term supplementation - (Aug. 8, 1978 to July 1, 1979)

[d] Short term supplementation - (Feb. 17, 1979 to July 1, 1979)

[e] Significantly greater than control ($P < 0.01$).

[f] Significantly less than control ($P < 0.01$).

kits from treated groups was above the control kit survival, the kits in the 160 ST and fishmeal groups had survival below the control, being 36 and 47 percent, respectively. The average number of kits raised per female generally followed the percent survival (correlation coefficient = +0.780). The 160 ST had only 1.5 kits raised per female while all other groups were equal to or greater than 3.0 kits per female.

Biomass is a measure of growth of kits during lactation which takes into account the number of young being raised per female, thus not giving more significance to one kit raised by one female than to four kits raised by one female (the four kits individually would normally be lighter than body weight than the single kit). The biomass was greater in the 10 and 20 LT groups than the other groups. The 160 ST group biomass was only 29 percent of the control value.

Adult female body weight at whelping and weaning is shown in Table 15. At whelping the three females fed the 160 LT diet weighed significantly less than the control group by 7.8 percent. All other groups were not significantly different from the control. At weaning no group was significantly less than the control, however, the one nursing female fed the 160 LT treatment only weighed 770 grams which was 129 grams or 14.3 percent less than the control mean body weight.

Table 15. Body weight of female mink at whelping and weaning fed various levels of supplemental iodine or fishmeal

Treatment iodine (ppm)	fishmeal	n	at whelping	n	at weaning	Mean change (g)
0		11	1001 ± 27.1[a]	7	899 ± 28.0	-102
10 LT[b]		6	985 ± 11.8	5	884 ± 31.7	-101
20 LT		7	1013 ± 42.7	6	863 ± 35.5	-150
40 LT		11	1015 ± 29.7	9	914 ± 26.2	-101
40 ST[c]		9	1028 ± 16.2	7	880 ± 27.8	-148
80 LT		10	1010 ± 54.1	8	964 ± 48.3	- 46
80 ST		9	997 ± 36.9	8	924 ± 36.5	- 73
160 LT		3	923 ± 13.3[d]	1	770	-153
160 LT		6	1087 ± 53.8	2	1072 ± 42.5	- 15
320 ST		1	980	0	----	---
	40% Canadian herring	9	1043 ± 60.1	6	933± 65.0	-110

[a] Standard error

[b] LT = Long Term supplementation (Aug. 8, 1978 to July 1, 1979).

[c] ST = Short Term supplementation (Feb. 17, 1979 to July 1, 1979).

[d] Significantly less than control ($0.10 > P > 0.05$).

Iodine did not affect the males fertilization capacity, as males fed high iodine levels and bred to females on low iodine levels fertilized the females as shown below:

Males' treatment level (ppm iodine)	n	Whelping females' treatment level (ppm iodine)
160	1	20
160	2	40
160	1	80
320	1	160

No increased numbers of abnormal or dead sperm were noticed in any treatment group when post-coital vaginal aspirations were examined.

Thyroid Function

Total thyroxine levels (nanograms per ml) for adult female mink and for kits approximately four weeks of age are shown in Table 16. A significant decrease in T_4 levels in groups from 40 LT to 320 ST occurred; however, groups 10 LT to 40 ST did not decline significantly in their levels compared to control levels. The T_4 level in the 40 LT group was significantly decreased by 64.7 percent, however the 80 ST and 80 LT groups T_4 levels were only decreased by about 28 percent. The T_4 level in mink on 160 ST were decreased by 38 percent while the animals in the 160 LT group showed a 67.9 percent decline in their T_4 levels. The females fed 320 ST also were

Table 16. Effect of feeding various levels of supplemental iodine to adult female mink on total serum thyroxine (T_4) levels in these mink and their kits.

Iodine treatment level (ppm)	T_4 nanograms/ml serum[a]			
	n	adults	n	kits[b]
0	7	18.4 ± 1.35	5	30.6 ± 3.49
10 LT[c]	6	14.9 ± 2.07	5	28.6 ± 2.02
20 LT	5	19.2 ± 1.38	6	26.5 ± 1.07
40 ST[d]	6	15.9 ± 1.20	6	26.5 ± 2.54
40 LT	6	6.5 ± 2.29[e]	7	24.8 ± 1.76
80 ST	6	13.0 ± 2.87[e]	6	25.8 ± 5.22
80 LT	5	13.3 ± 2.83[f]	6	22.1 ± 4.64
160 ST	6	11.4 ± 3.48[g]	2	14.6 ± 1.10[h]
160 LT	6	5.9 ± 3.14[h]	3	19.8 ± 8.42
320 ST	6	5.2 ± 2.09[f]	-	---

[a] Data given as goup mean ± standard error.

[b] Approximately four weeks of age.

[c] ST = Short Term supplementation (Feb. 17, 1979 to July 1, 1979).

[d] LT = Long Term supplementation (Aug. 8, 1978 to July 1, 1979).

[e] Significantly less than control ($P < 0.005$).

[f] Significantly less than control ($0.25 > P > 0.10$).

[g] Significantly less than control ($P < 0.05$).

[h] Significantly less than control ($P < 0.025$).

significantly decreased in their T_4 levels to 5.2 ng/ml or 71.7 percent of the control's T_4 level. The T_4 level in kits was significantly decreased in only the 160 ST treatment group. The T_4 values are plotted in Figure 4 for both adults and kits. The adults fed the LT diets had variable T_4 levels. The adults fed the ST diets had a gradual decline in T_4 levels with increasing iodine levels from 15.9 ng/ml (40 ST) to 5.2 ng/ml (320 ST). Kits from mothers fed the LT diets had a gradual decline in their T_4 levels. Kits T_4 levels were not related to the T_4 levels of their mothers. When kits T_4 levels were plotted on semi-log paper versus the iodine dosage of the mother's diets a straight line was obtained with a correlation of -0.997 between dose and T_4 level. The overall decline from the control to the 160 LT group was 35.3 percent. The kits from mothers fed ST diets had a gradual decline in their T_4 levels at 40 and 80 ppm, but dropped markedly at the 160 ppm iodine treated group to 14.6 ng/ml which was a 52.3 percent decline from the control.

Triiodothyronine (T_3) levels of adult female mink and kits approximately four weeks of age are shown in Table 17. In adults only on 40 LT and 160 LT treatment was a significant decrease in T_3 noted, being 47.6 and 44.4 percent of the control value, respectively. The T_3 levels in kits were also significantly depressed at

Figure 4. Effect of feeding various levels of iodine for 1) ten months (long term) or 2) four months (short term) on thyroxine (T_4) levels of adult and kit (four weeks old) mink.

FIGURE 4

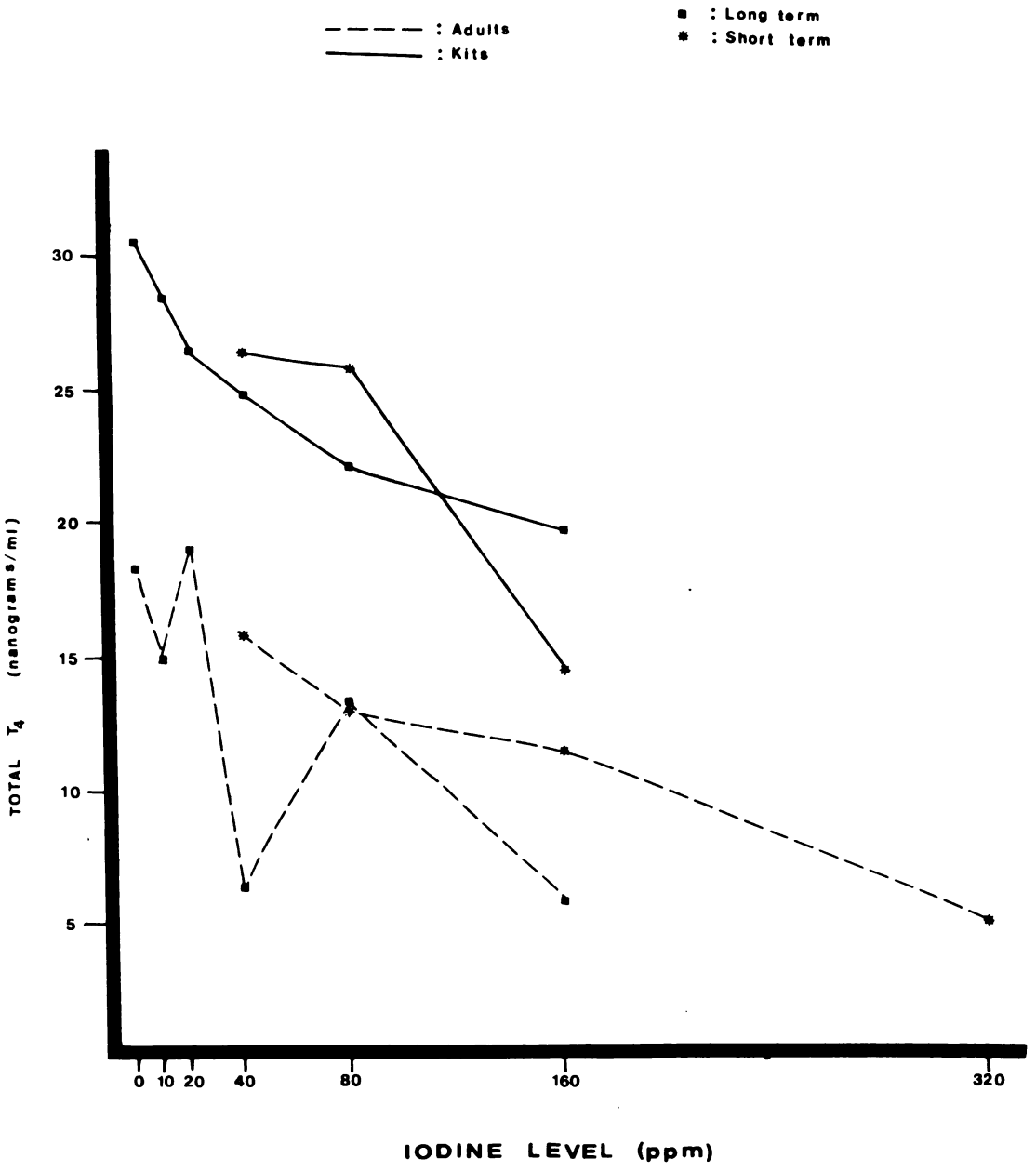

: Adults
: Kits
: Long term
: Short term
TOTAL T_4 (nanograms/ml)
30
25
20
15
10
5
0 10 20 40 80 160 320
IODINE LEVEL (ppm)

Table 17. Effect of feeding various levels of supplemental iodine to adult female mink on triiodothronine (T_3) levels of these mink and their kits.

Iodine treatment level (ppm)	T_3 nanograms/ml serum[a]			
	n	adults	n	kits[b]
0	7	0.63 ± 0.109	5	0.72 ± 0.134
10 LT[c]	6	0.55 ± 0.132	5	0.84 ± 0.056
20 LT	5	0.64 ± 0.104	6	0.80 ± 0.097
40 ST[d]	6	0.57 ± 0.072	6	0.82 ± 0.116
40 LT	6	0.33 ± 0.048[e]	7	0.59 ± 0.037[e]
80 ST	6	0.42 ± 0.075	6	0.61 ± 0.115
80 LT	5	0.41 ± 0.084	6	0.75 ± 0.074
160 ST	6	0.50 ± 0.114	2	0.19 ± 0.085[f]
160 LT	6	0.35 ± 0.098[f]	3	0.63 ± 0.115
320 ST	6	0.49 ± 0.063	-	---

[a]Data given as group mean ± standard error.

[b]Approximately four weeks of age.

[c]ST = Short Term supplementation (Feb. 17, 1979 to July 1, 1979).

[d]LT = Long Term supplementation (Aug. 8, 1978 to July 1, 1979).

[e]Significantly less than control ($P < 0.05$).

[f]Signigicantly less than control ($0.10 > P > 0.05$).

only two levels: 40 LT being 18.1 percent lower and 160 ST was 73.6 percent below control T_3 levels. T_3 levels of both adults and kits are plotted in Figure 5. Adult T_3 levels generally paralleled the T_4 levels, having the same rises and declines. However, the kit's T_3 levels were not similar to their T_4 levels, but the kits from dams fed the LT diets did tend to follow the adults T_3 increases and decreases. The kits from mothers fed the ST diets had a marked decline in their T_3 levels over the range of iodine levels, of -76.8 percent falling from 0.82 ng/ml (40 ST) to 0.19 ng/ml at 160 ST.

T_4-binding indices for adult females and kits are shown in Table 18. The T_4-binding indexes of the adults fed the 20 LT and 40 ST diets were the only ones significantly lower than the control, by 52.6 and 59.2 percent, respectively. The T_4-binding index of kits was significantly reduced in four of the treatment groups: 10 LT, 20 LT, 40 ST and 160 LT, and significantly increased in the 160 ST group which was 52.4 percent greater than the control group. The T_4-binding index of the 10 LT, 20 LT, 40 ST and 160 LT groups was reduced from the control by 57.6, 44.6, 45.0 and 48.8 percent, respectively. The T_4-binding index of the adults is plotted in Figure 6. Except for the animals fed 10 LT and 320 ST diets, a general trend was seen in which the T_4-binding was opposite of T_4 values (when T_4 increased,

Figure 5. Effect of feeding iodine at various levels for 1) ten months (long term) or 2) four months (short term) on triiodothyronine (T_3) levels of adult and kit (four weeks old) mink.

FIGURE 5

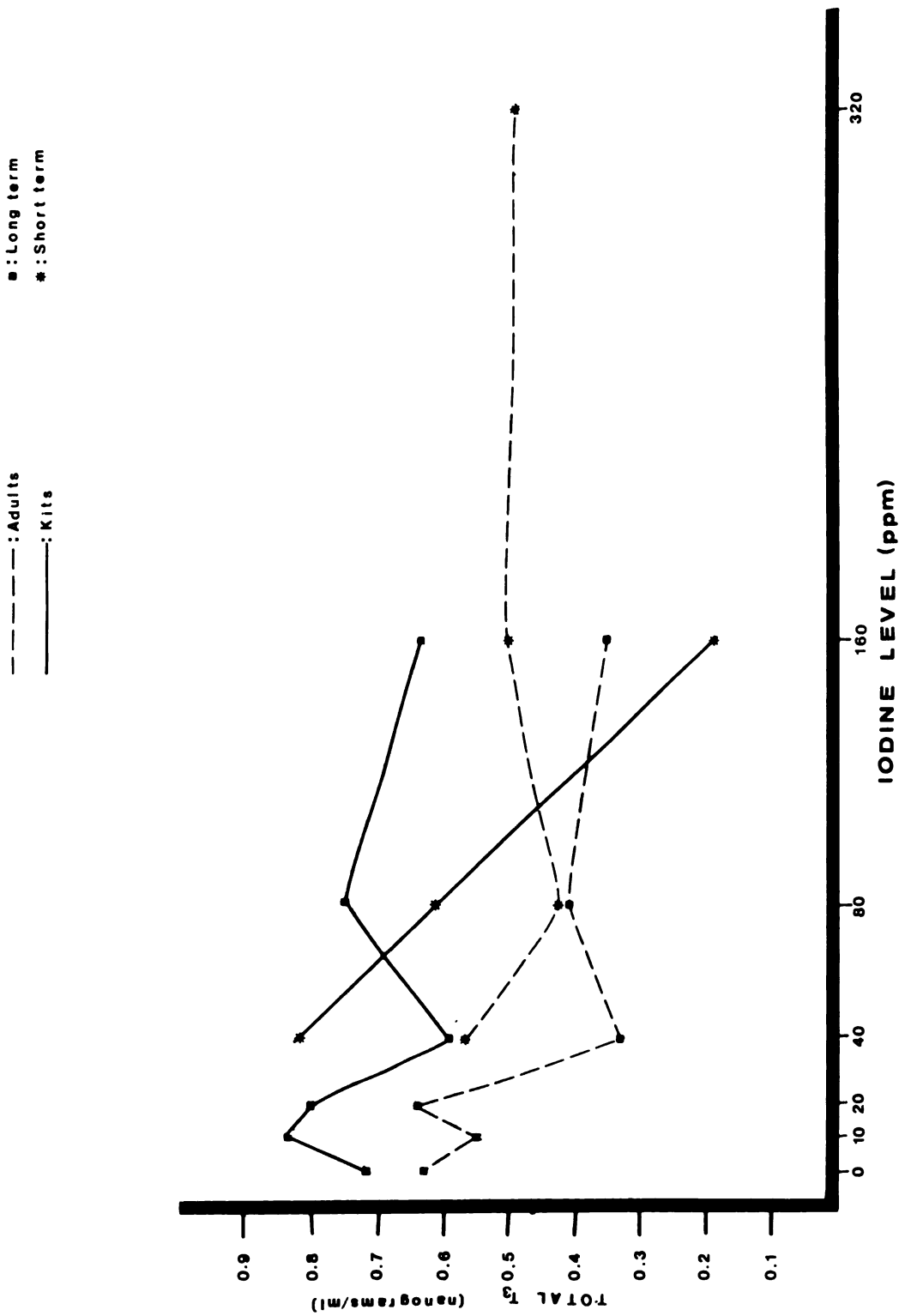

■: Long term
✱: Short term
——: Adults
——: Kits
TOTAL T3 (nanograms/ml)
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
0
10
20
40
80
160
320
IODINE LEVEL (ppm)

Table 18. Effect of feeding various levels of supplemental iodine to adult female mink on T_4-binding index of these mink and their kits.

Iodine treatment level (ppm)	T_4-binding index[a]			
	n	adults	n	kits[b]
0	7	7.6 ± 2.06	5	86.6 ± 21.59
10 LT[c]	6	4.5 ± 0.62	5	36.7 ± 12.70[e]
20 LT	5	3.6 ± 0.46[f]	6	48.0 ± 11.62[f]
40 ST[d]	6	3.1 ± 0.76[e]	6	47.6 ± 15.69[f]
40 LT	6	6.4 ± 1.00	7	64.6 ± 9.56
80 ST	6	5.5 ± 1.71	6	70.7 ± 12.02
80 LT	5	4.5 ± 1.16	6	60.8 ± 17.26
160 ST	6	5.9 ± 1.49	2	132.0 ± 2.00[g]
160 LT	6	5.4 ± 1.69	3	44.3 ± 4.48[f]
320 ST	6	5.8 ± 0.65	-	---

[a]Data given as group mean ± standard error.

[b]Approximately four weeks of age.

[c]LT = long term supplementation (Aug. 8, 1978 to July 1, 1979).

[d]ST = short term supplementation (Feb. 17, 1979 to July 1, 1979).

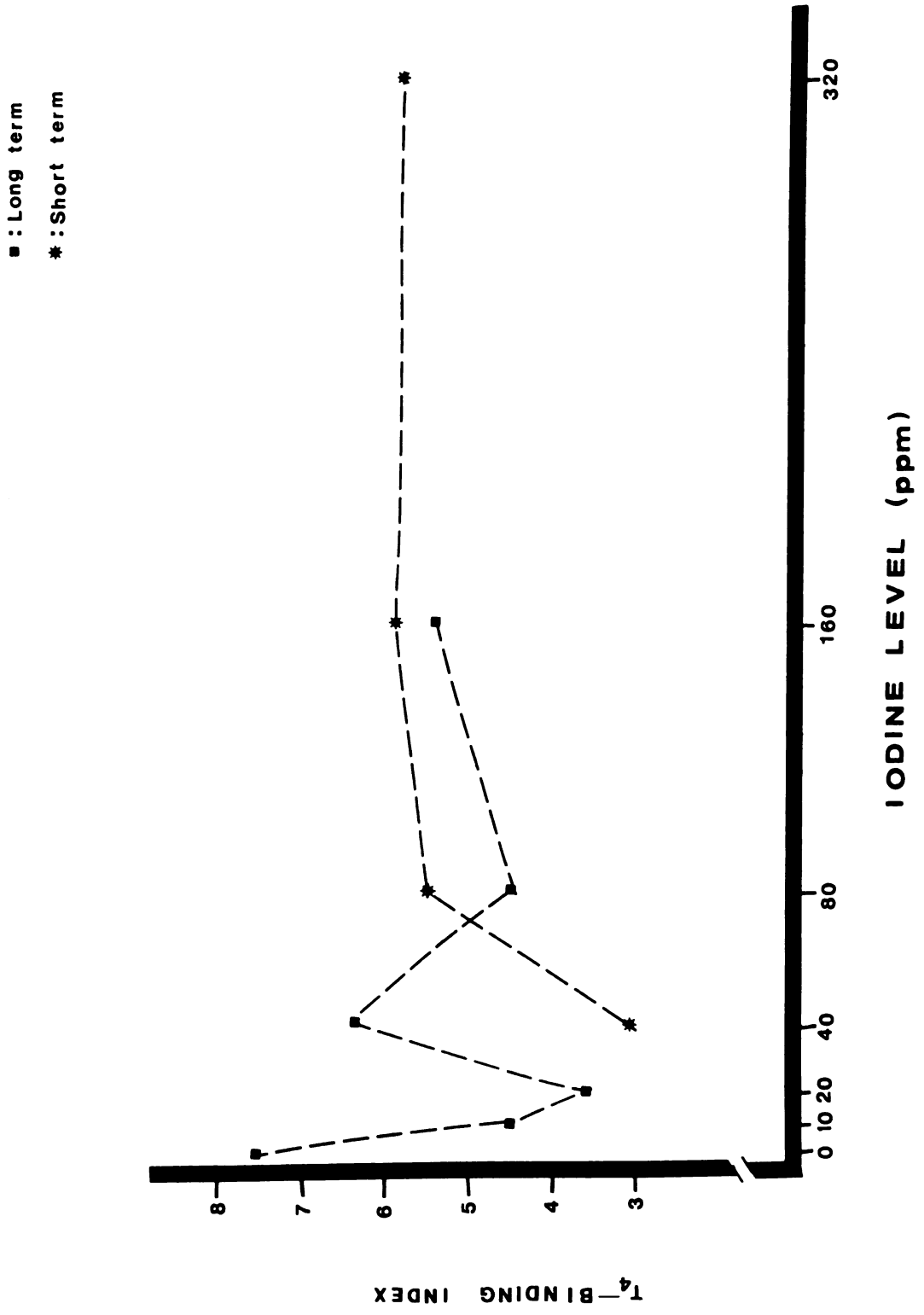
[e]Significantly less than control ($0.10 > P > 0.05$).

[f]Significantly less than control ($P < 0.25$).

[g]Significantly greater than control ($P < 0.25$).

Figure 6. The T_4-binding index of adult mink fed iodine at various levels for 1) ten months (long term) or 2) four months (short term).

FIGURE 6


■ : Long term
✱ : Short term
T_4—BINDING INDEX
8
7
6
5
4
3
0 10 20 40 80 160 320
IODINE LEVEL (ppm)

T_4-binding decreased). The T_4-binding indexes of the kits is plotted in Figure 7. Kits from mothers fed LT diets had a rise from 36.7 (10 ppm) to 64.6 ng/ml (40 ppm) then a dropping off to 44.3 (160 ppm). The kits fromdams on St diets had a marked increase from 48.0 (40 ppm) to 132.0 ng/ml (160 ppm). This increase was opposite the effect noted for both T_4 and T_3.

Reverse-T_3 (rT_3) for the adult female mink and kits is shown in Table 19. The rT_3 levels of adults were significantly decreased at 40 LT, 160 LT and 320 ST by 47.2, 47.2 and 49.1 percent, respectively, compared to the control animals. The rT_3 levels of kits in the 160 ST treated group were significantly increased over controls by 83.1 percent while rT_3 levels of kits from the 160 LT treated mothers were significantly decreased by 27.1 percent. Reverse-T_3 levels for adults and kits are plotted in Figure 8. A decline in rT_3 of adults fed diets greater than 10 ppm supplemented iodine, with a peak in the animals fed LT diets at 80 ppm which was similar to T_4 and T_3. The rT_3 of adults fed the ST diets had similar levels, from 40 to 160 ppm, and a slight decline in animals fed 320 ppm (the four groups averaged 0.73 $\pm$ 0.036 ng/ml). The rT_3 values of kits from the dams fed LT diets had a steady decline at all iodine levels, this was similar to the T_4 levels of kits mentioned earlier. The rT_3 levels of kits from the

Figure 7. The T_4-binding index of kits from feeding iodine at various levels for 1) ten months (long term) or 2) four months (short term) to their parents.

FIGURE 7

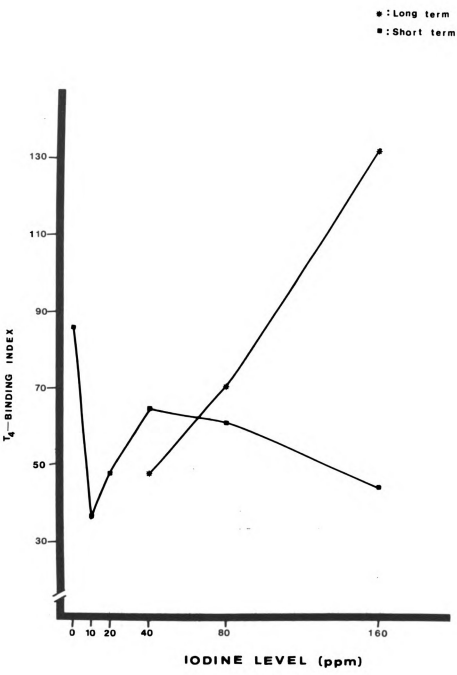

●: Long term
■: Short term
130
110
90
70
50
30
T_4—BINDING INDEX
0
10
20
40
80
160
IODINE LEVEL (ppm)

Table 19. Effect of feeding various levels of supplemental iodine to adult mink on reverse-T_3 (rT_3) values of these mink and their kits.

Iodine treatment level (ppm)	rT_3 nanograms/ml serum[a]			
	n	adults	n	kits[b]
0	7	0.53 ± 0.054	5	0.59 ± 0.072
10 LT[c]	6	0.55 ± 0.039	5	0.54 ± 0.037
20 LT	5	0.44 ± 0.068	6	0.54 ± 0.048
40 ST[d]	6	0.36 ± 0.061	6	0.46 ± 0.034
40 LT	6	0.28 ± 0.038[e]	7	0.51 ± 0.047
80 ST	6	0.41 ± 0.046	6	0.49 ± 0.063
80 LT	5	0.39 ± 0.081	6	0.48 ± 0.078
160 ST	6	0.43 ± 0.076	2	1.08 ± 0.020[f]
160 LT	6	0.28 ± 0.069[g]	3	0.43 ± 0.057[h]
320 ST	6	0.27 ± 0.044[g]	-	---

[a]Data given as mean ± standard error.

[b]Approximately four weeks of age.

[c]LT = long term supplementation (Aug. 8, 1978 to July 1, 1979).

[d]ST = short term supplementation (Feb. 17, 1979 to July 1, 1979).

[e]Significantly less than control ($P < 0.005$).

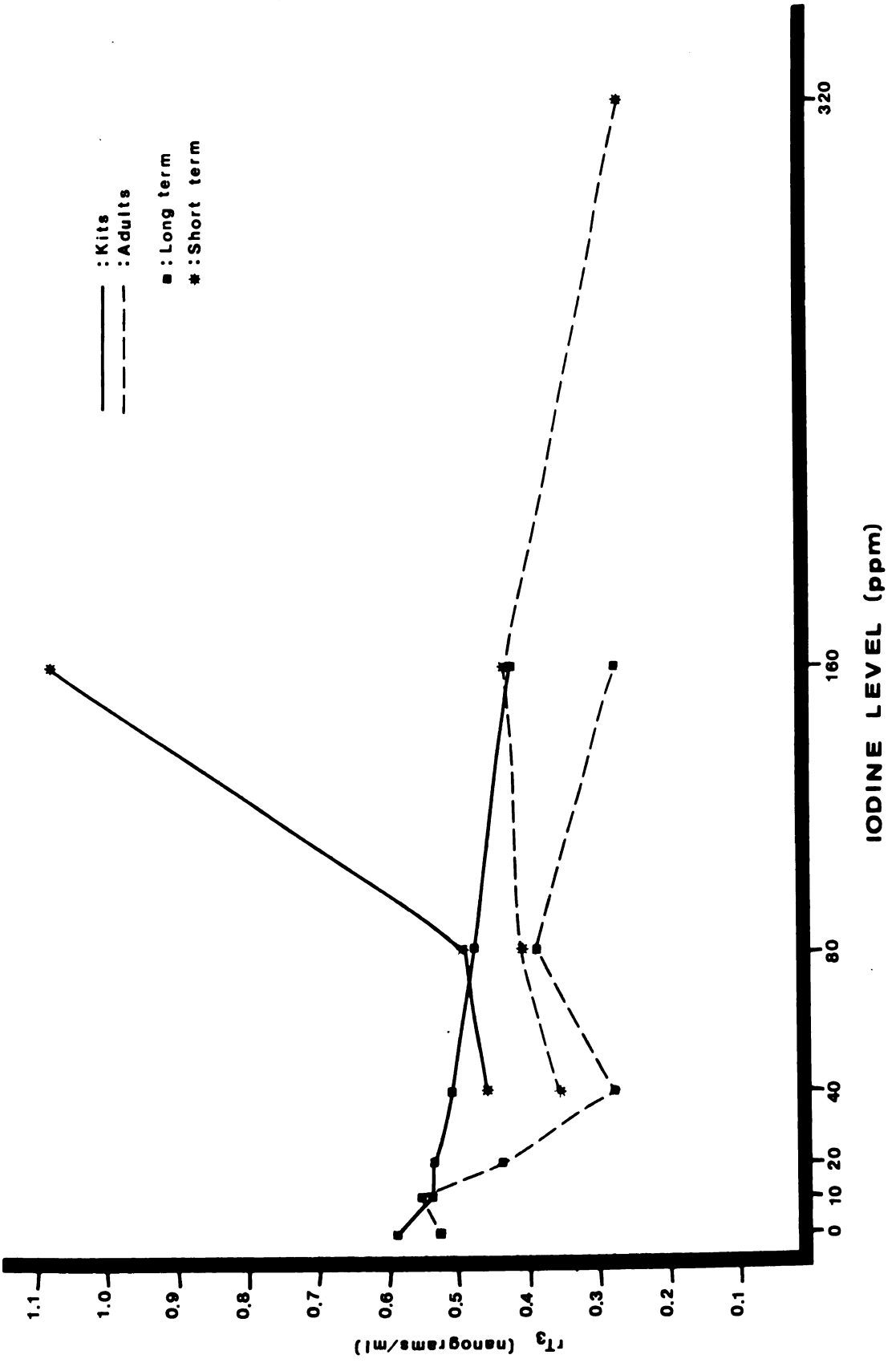
[f]Significantly greater than control ($P < 0.10$).

[g]Significantly less than control ($P < 0.01$).

[h]Significantly less than control ($0.10 > P > 0.05$).

Figure 8. Effect of feeding various levels of iodine for 1) ten months (long term) or 2) four months (short term) on 3, 3',5'-triiodothyronine (rT_3) of adult and kit (four weeks old) mink.

FIGURE 8

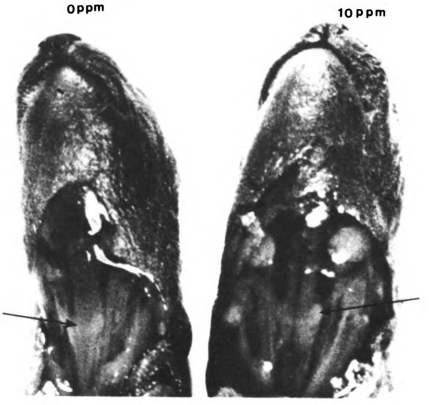

: Kits
: Adults
■ : Long term
✱ : Short term
rT3 (nanograms/ml)
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
10
20
40
80
160
320
IODINE LEVEL (ppm)

mothers fed the ST diets rose from the 40 ppm group to the 80 ppm group by 6.5 percent and increased markedly from the 80 ST to 160 ST group, by 120.4 percent.

Pathology

Thyroids of kits examined at birth had an increase in size as the level of iodine increased above 20 ppm iodine in the diet fed to the mothers. Figure 9 shows the normal thyroid glands of control kits and kits from mothers fed 10 and 20 ppm iodine. Grossly, no differences were noted, the size of the glands were similar. Figure 10 shows the thyroid glands of kits from the 40 and 80 ppm-treated groups. The thyroids of the kits from the 40 ppm-treated groups were sightly enclarged, while the thyroids of kits from the dams fed 80 ppm iodine were much larger than the controls' thyroids. Kits at birth and one week of age from the 160 ppm iodine treated group are shown in Figure 11. At birth the thyroids were enlarged in comparison to the controls' thyroids. At one week of age the thyroids of kits from the 160 ppm-treated group were greatly enlarged compared to the controls' thyroids. Figure 12 shows the thyroids of two kits that were born dead (the only kits whelped from the 320 ppm iodine treatment). The upper kit's thyroid was not much larger than the controls' thyroids, however, the lower one's thyroid was markedly

Figure 9. Thyroid glands (arrows) of day old kits whelped by females fed 0, 10 and 20 ppm iodine supplemented diets. No gross enlargements are noticeable in these animals.


0ppm
10ppm

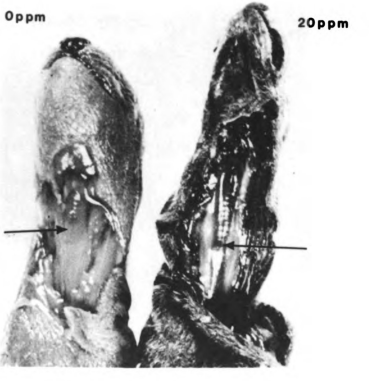
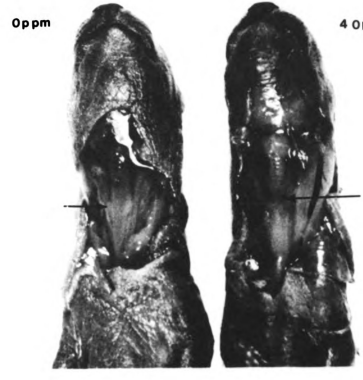

0ppm
20ppm

Figure 10. Thyroid glands (arrows) of day old kits whelped by females fed 0, 40 and 80 ppm iodine supplemented diets. There is a slight enlargement in the thyroid in the kit from the 40 ppm iodine-treated group, and the thyroid from the 80 ppm iodine-treated groups is much larger than the control thyroid.


0ppm
40ppm


0ppm
80ppm

Figure 11. Thyroid glands (arrows) of day old and one week old kits whelped by females fed 0 and 160 ppm iodine-supplemented diets. A noticeable enlargement in the thyroid gland is present both at one day of age and at one week of age in the kits from the 160 ppm iodine-treated group.

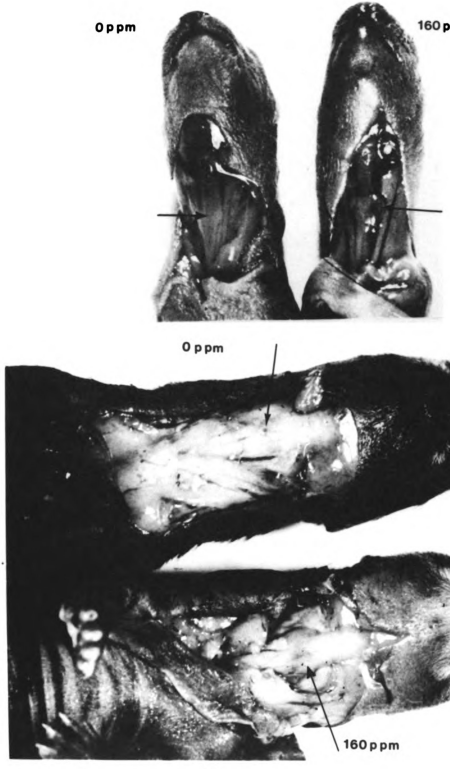
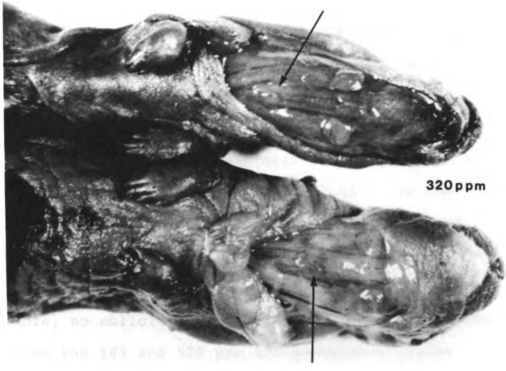

0 ppm
160 ppm
0 ppm
one
week
old
160 ppm

Figure 12. Thyroid glands (arrows) of day old kits whelped by females fed 320 ppm iodine supplemented diets. The thyroid gland of the kit in upper picture does not appear larger than the control thyroid, however, the thyroid gland of the kit in the lower picture is grossly enlarged.

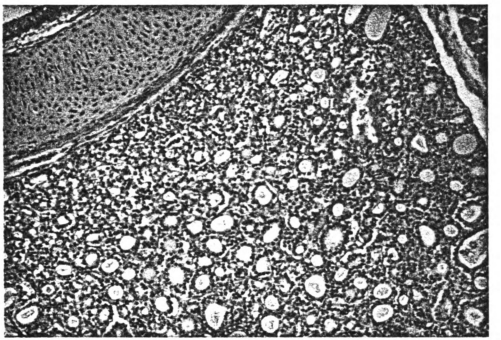

320ppm

increased in size.

Histologically the controls' thyroids were normal (see Figure 13) with rounded follicles containing colloid. The follicles varied in shape from the 10 and 20 ppm-treated groups (see Figures 13 and 14) had thyroids similar in appearance to the controls' thyroid glands. However, the kits from the 40 ppm iodine-treated group showed a slight hyperplasia with an increased number of follicles (see Figure 14). Each individual follicle was atypical in size and shape being smaller and more irregular than the controls' thyroids. There was less colloid present in these thyroid follicles than the controls'. In the thyroids of kits from the 80 ppm group the extent of hyperplasia was more than in those from the 40 ppm group and individual follicles were not ealisy distinguishable, no colloid was present (see Figure 15). The kits from the 160 and 320 ppm iodine-treated groups (see Figures 15 and 16) had extensive hyperplasia, complete loss of follicular architecture and an absence of colloid. Kits from parents fed the fishmeal-supplemented diet showed no histologic changes in their thyroids (see Figure 17).

Adults on all levels of iodine, except 320 ppm, showed no gross abnormalities. All animals had normal sized thyroid glands. The adults fed 320 ppm iodine had

Figure 13. Photomicrograph of the thyroids of four-week-old kits whelped by females consuming 0 (upper photograph) or 10 ppm (lower photograph) iodine supplemented diets. The structure of each thyroid is normal. (580 X)



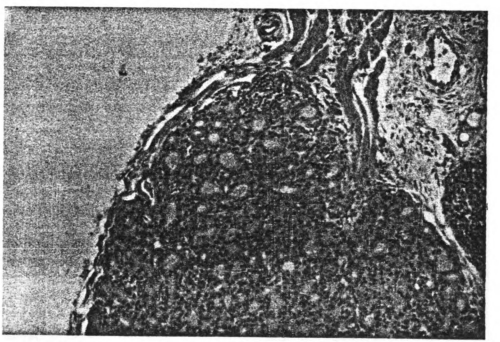
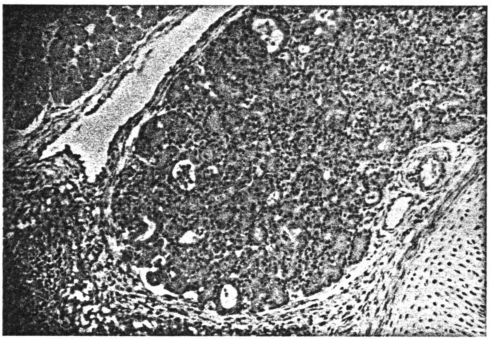


Figure 14. Photomicrograph of the thyroids of four-week-old kits whelped by females consuming 20 (upper photograph) or 40 ppm (lower photograph) iodine-supplemented diets. The thyroid gland of the kit from the 20 ppm treated group appears normal. However, the thyroid gland of the kit from the 40 ppm group is slightly hyperplastic (A) and an increased number of follicles with a decrease in architecture is noticeable. (580 X)



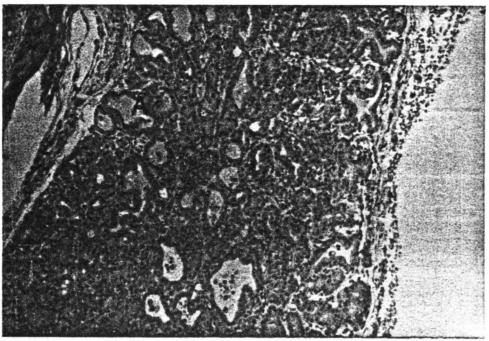
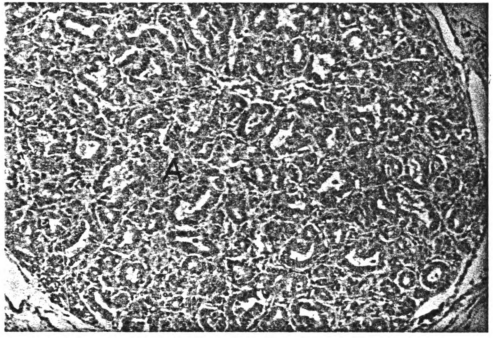


Figure 15. Photomicrograph of the thyroids of four-week-old kits whelped by females consuming 80 (upper photograph) or 160 ppm (lower photograph) iodine-supplemented diets. The thyroid gland of the kit from the 80 ppm group has a greater degree of hyperplasia than the kit from the 40 ppm group. Also, the individual follicles are not as easily distinguishable. The thyroid of the kit from the 160 ppm treated group has extensive hyperplasia (A) as well as a complete loss of architecture and an absence of colloid. (580 X)


A

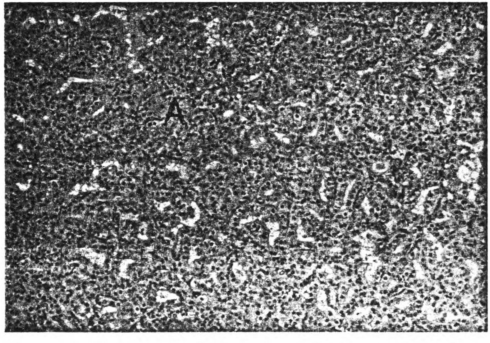

A

Figure 16. Photomicrograph of the thyroid of a four-week-old kit whelped by females consuming 320 ppm iodine supplemented diet. The Thyroid of the kit from the 320 ppm group has extensive hyperplasia (A), a complete loss of architecture and an absence of colloid.

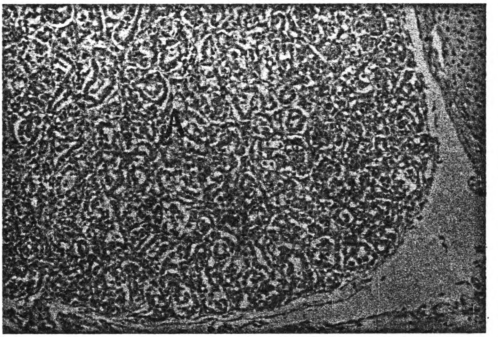
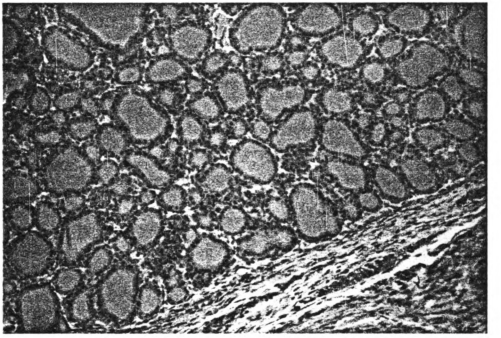


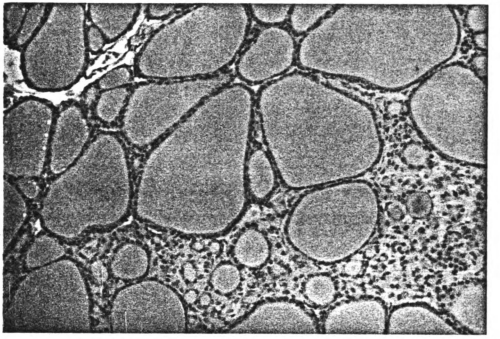
Figure 17. Photomicrograph of the thyroid of a four-week-old kit whelped by females consuming a diet supplemented with 30 percent fishmeal. No abnormal changes are noticeable in the structure of the thyroid gland.



gall bladders that were firm, slightly lighter in color than the controls, thickened and containing a copious amount of bile. Two of the four animals on the 320 ppm treatment had enlarged hemorrhagic ovaries. No other gross abnormalities were noted.

Adults fed 40 ppm or less iodine supplementation showed no histopathologic lesions (see Figures 18 and 19). Females in the 80 ppm-treated group (see Figure 20) displayed a mild hyperplasia of the follicular cell, with some overall decrease in the lumen size and colloid content, some vacoulation was also present. The thyroid glands of animals fed 160 ppm iodine diets (see Figure 20) were similar to those of the 80 ppm treated group in that the follicles were slightly hyperplastic with an increase in interfollular cellular material and the lumens were decreased in size and vacuolated. Thyroids of females fed 320 ppm supplemental iodine diet (see Figure 21) were very hyperplastic with a decreased number of follicles, abnormal follicular architecture and a decreased amount of colloid. The new follicles that are present were enlarged, very irregular, vacoulated and there were numerous cells within the colloid. Adults fed the diet supplemented with fishmeal showed no histopathologic changes in their thyroid glands (see Figure 22).

Figure 18. Photomicrograph of the thyroids of adult female mink fed 0 (upper photograph) or 10 ppm (lower photograph) iodine-supplemented diets. The cellular structure of the thyroid gland is normal in both cases. (232 X).



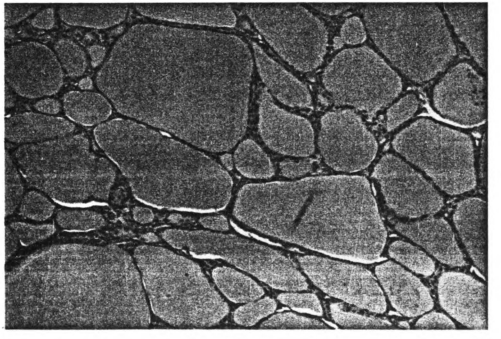
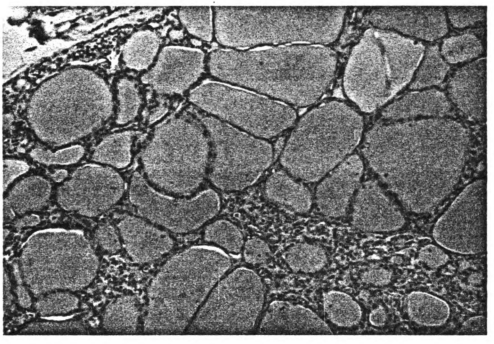


Figure 19. Photomicrographs of the thyroids of adult female mink fed 20 (upper photograph) or 40 ppm (lower photograph) iodine-supplemented diets. The cellular structure of the thyroid glands is normal in both cases. (232 X).



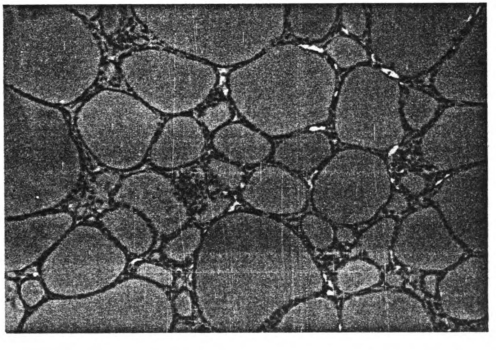
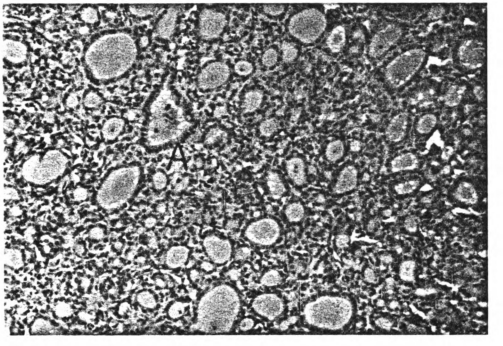


Figure 20. Photomicrograph of the thyroids of adult female mink fed 80 (upper photograph) or 160 ppm (lower photograph) iodine-supplemented diets. Thyroid gland from females in both the 80 ppm and 160 ppm treated groups have a mild hyperplasia (A) of the follicular cells, a slight decrease in lumen size and colloid content, and some vacoulation. (232 X).


A

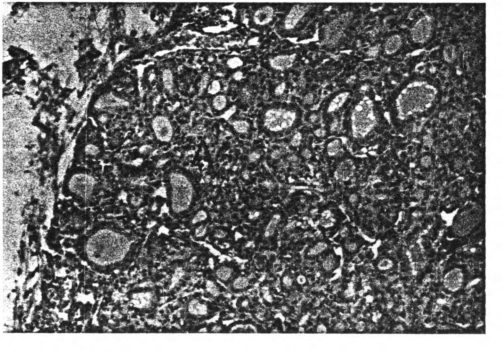


Figure 21. Photomicrograph of the thyroid of an adult female mink fed 320 ppm iodine-supplemented diet. The thyroid glands of the 320 ppm-treated group have extensive hyperplasia (A), a decreased number of follicles, abnormal follicular architecture and a decreased amount of colloid. The few follicles present show enlargement, irregular shape, vacoulization and numerous cells within the colloid. (232 X).

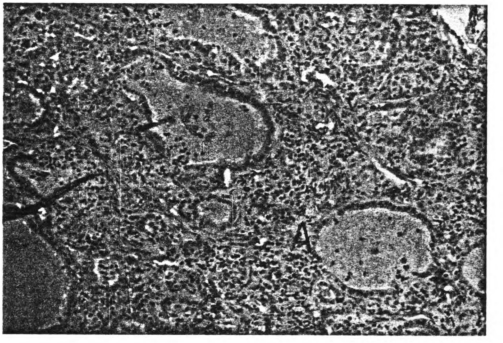
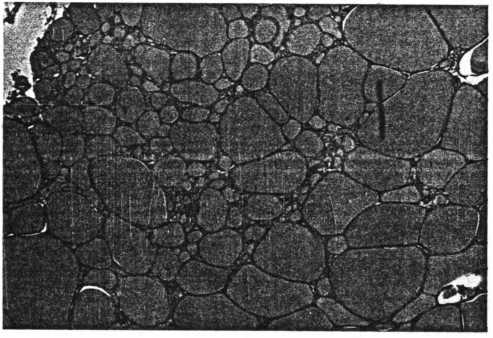
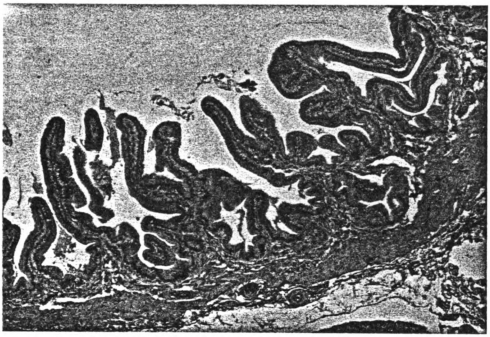

A

Figure 22. Photomicrograph of the thyroid of an adult female mink fed a diet supplemented with 30 percent fishmeal. The thyroid gland is normal in appearance. (232 X).



Gall bladders from adult females fed 40 ppm or less iodine supplementation showed no pathologic changes (see Figures 23 and 24). These gall bladders showed only small amounts of secretory material, normal pili and thin walls. However, adults fed 80 ppm or greater iodine-supplemented diets showed increasing lesions as the level of dietary iodine increased. A slight hyperplasia of the pili and a slight thickening of the smooth muscle in the wall were seen in gall bladders from adults fed 80 ppm iodine (see Figure 25). Animals fed 160 ppm iodine treated diets (see Figure 25) had gall bladders that were hyperplastic and the muscular walls were thickened. The amount of secretory material was greatly increased and there were foci of lymphocytes scattered throughout the pili. Gall bladders of mink fed 320 ppm iodine supplementation (see Figure 26) were extremely hyperplastic, the pili were more numerous and greatly enlarged. The walls were greatly thickened with increased connective tissue. The amount of secretory material was also greatly increased and foci of lymphocytes were also present and more numerous than the sites from the gall bladders of the animals fed 160 ppm iodine. Adults fed the diet supplemented with fishmeal showed no pathologic changes in their gall bladders (see Figure 27).

Figure 23. Photomicrograph of the gall bladders of adult female mink fed 0 (upper photograph) or 10 ppm (lower photograph) iodine-supplemented diets. Both gall bladder are normal in appearance. (232 X).



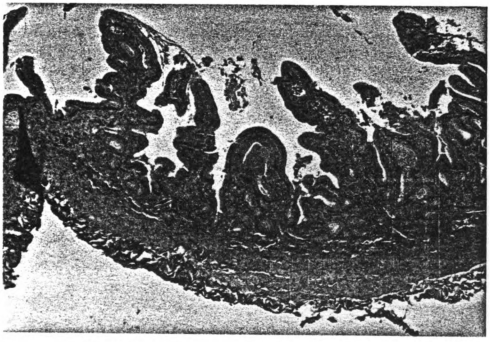
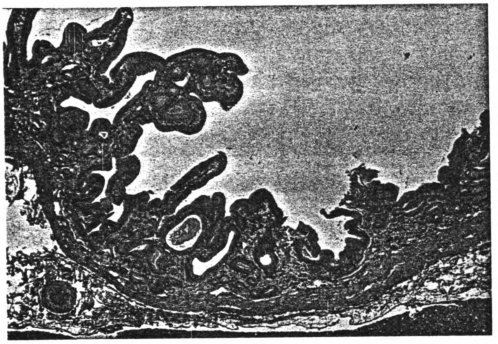


Figure 24. Photomicrograph of the gall bladders of adult female mink fed 20 (upper photograph) or 40 ppm (lower photograph iodine-supplemented diets. Both gall bladders are normal in appearance. (232 X).



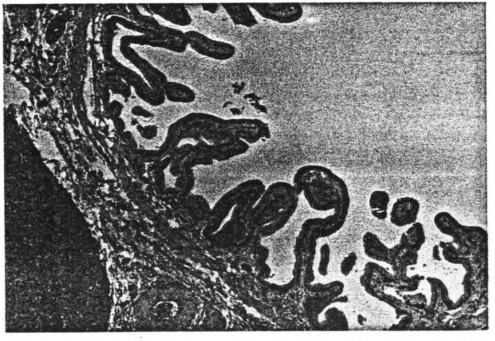
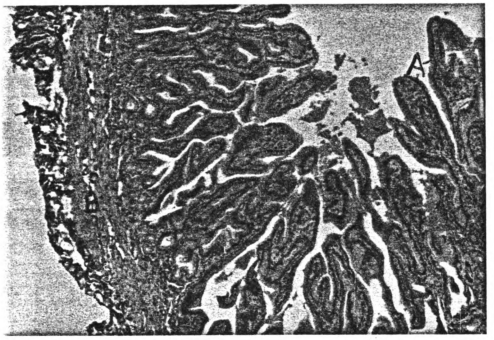


Figure 25. Photomicrograph of the gall bladders of adult female mink fed 80 (upper photograph) or 160 ppm (lower photograph) iodine-supplemented diets. The gall bladder of the adults from the 80 ppm group has a slight hyperplasia (A) of the pili and a thickening of the smooth muscle wall (B). The gall bladders of the animals from the 160 ppm group has extensive hyperplasia (A) as well as thickening of the walls (B). (232 X).


A

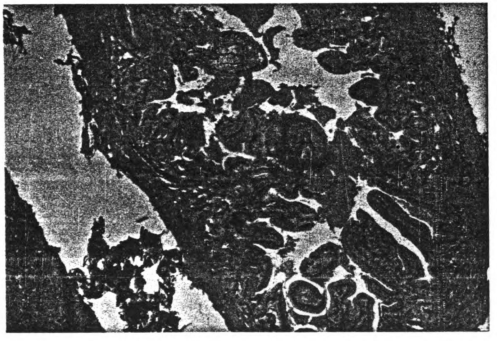


Figure 26. Photomicrograph of the gall bladder of an adult female mink fed 320 ppm iodine-supplemented diet. The gall bladder of the females on 320 ppm treatment are extremely hyperplastic in both pili cells (A) and smooth muscle cells (B) of the wall. The foci of lymphocytes (C) present are larger and more numerous than in lower treatment groups. (232 X).

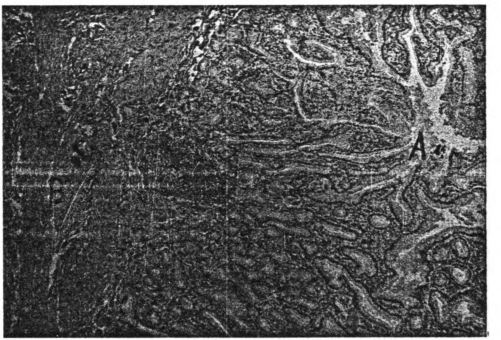
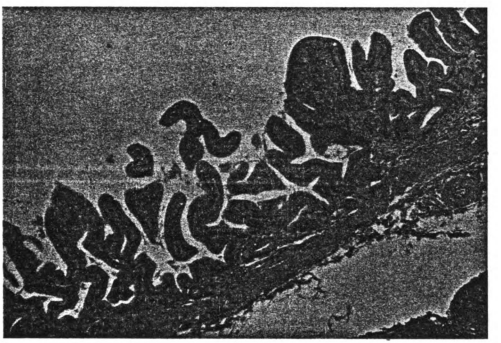

A

Figure 27. Photomicrograph of the gall bladder of an adult female mink fed a diet supplemented with 30 percent fishmeal. The gall bladder of the adults from the fishmeal treatment are normal in their architecture. (232 X).



In both the 160 and 320 ppm iodine-treated groups a couple of animals had a small amount of amyloid within the cortex of the adrenal gland.

Milk Iodine, Blood Parameters and Organ Weights at Termination

Iodine levels in milk from mothers fed various levels of supplemental iodine are presented in Table 20. At the lower treatment levels, 10 and 20 ppm, the iodine levels in the milk samples tended to increase as iodine in the diet increased. However, at higher iodine treatment levels the amount of iodine in the milk tended to level off.

Differential counts of leukocytes from adult females killed at the end of the experiment are shown in Table 21. Animals fed diets of 80 ppm iodine supplementation and less showed no changes in white cell counts. Mink on 160 ppm iodine showed a significant decrease (38.2 percent) in neutrophils and a significant increase (39.1 percent) in lymphocytes. However, the females in the 320 ppm group did not show this effect, but did have a significant decrease (57.5 percent) in eosinophils. Overall, there tended to be a slight increase in lymphocytes in groups fed 20 ppm iodine or more. The animals fed 30 percent fishmeal showed no effects from the diet on their differential leukocyte

Table 20. Iodine levels in milk of adult female lactating mink fed various levels of supplemental iodine for approximately ten months.

Treatment		n	Iodine in milk (ppm)
Iodine (ppm)	Fishmeal		
0		1	0.7
10		1	9.3
20		1	36.0
40		1	17.6
80		2	33.5[a]
160		1	45.0
	40% Canadian herring	1	1.8

[a] Group mean

Table 21. Leukocyte counts of adult female mink fed various levels of iodine or added fishmeal.

Treatment			%					
Iodine (ppm)	Fishmeal	n	Basophils	Eosinophils	Bands	Neutrophils	Lymphocytes	Monocytes
0		5	1.6	4.0	0.4	46.6	44.7	2.7
10		2	2.5	4.5	0.0	49.0	40.0	4.0
20		4	1.5	4.2	0.5	39.3	52.0	2.5
40		2	2.5	4.0	2.0	40.0	47.5	4.0
80		2	0.0	4.0	0.5	47.5	47.0	1.0
160		4	1.3	5.2	0.2	28.8[a]	62.2[a]	2.3
320		4	0.8	1.7[b]	3.0	43.0	49.5	2.0
40%	Canadian herring	2	2.0	5.0	0.0	46.5	43.5	3.0

[a] Significantly different from control ($P < 0.005$).

[b] Significantly different from control ($P < 0.05$).

counts.

Numbers of red blood cells (RBC), white blood cells (WBC), hemoglobin (Hb) and packed cell volumes (PCV) for adult females at the end of the experiment are shown in Table 22. There was no significant difference in RBC counts between any treated group and the control, although, the RBC counts of all treated groups were greater than the control. There tended to be an increase in RBC with increasing iodine levels in the diet (correlation coefficient = +0.865). White blood cell counts (WBC) from any treated group were not significantly different from control WBC values. Hemoglobin levels were also not significantly affected by any treatment, although, the 320 ppm-treated group was decreased by 10.7 percent. Packed cell volume (PCV) were significantly decreased by 10.3 percent in adult females fed 320 ppm iodine supplementation and overall there was a trend toward lower PCV values with increasing iodine levels (correlation coefficient = -.0836). The PCV of the animals on the fishmeal treatment was not affected.

Levels of chloride in the plasma or serum and levels of calcium in the serum are shown in Table 23. Chloride was measured since an increase in blood levels of chloride ions will cause an increased chloride excretion in the urine which affects renal clearance of iodide (Stanbury and Ramalingaswami, 1964). In the control animals,

Table 22. Red blood cell count (RBC), white blood cell count (WBC), hemoglobin (Hb), and packed cell column (PVC) of female mink fed various levels of iodine or added fishmeal.

Treatment						
Iodine (ppm)	Fishmeal	RBC[a] (x 10^6)/mm^3	WBC[a]/mm^3	Hb[a] (g/dl)	n	PVC[a] (%)
0	5	7.68 ± 0.68	8820 ± 668.9	20.5 ± 0.41	10	54.5 ± 0.64
10	2	7.89 ± 0.13	8900 ± 600.0	19.9 ± 1.05	4	53.0 ± 0.91
20	4	7.81 ± 0.39	8250 ± 295.8	19.1 ± 0.38	8	53.6 ± 0.71
40	2	7.91 ± 0.11	8650 ± 450.0	19.2 ± 1.55	4	50.9 ± 2.68
80	2	8.03 ± 1.23	9100 ± 900.0	20.4 ± 0.20	4	53.3 ± 0.25
160	4	8.50 ± 0.31	8550 ± 232.8	19.6 ± 0.22	8	51.8 ± 0.35
320	4	8.41 ± 0.22	9775 ± 756.5	18.3 ± 0.95	8	48.9 ± 1.64[b]
	40% Canadian herring 2	8.10 ± 0.30	8850 ± 650.0	18.6 ± 0.65	4	55.0 ± 1.47

[a] Data given as group mean ± standard error.

[b] Significantly less than control ($P < 0.001$).

Table 23. Plasma and serum chloride and serum calcium of female mink.

Treatment		n	Chloride[a] mEq/L		Serum calcium[a] mg/100 ml
Iodine (ppm)	Fishmeal		Plasma	Serum	
0		10	122.7 ± 1.97[b]	117.3 ± 1.30	10.62 ± 0.138
10		4	126.2 ± 2.70	112.4 ± 2.41	10.68 ± 0.112
20		8	128.8 ± 3.39	114.9 ± 1.58	11.23 ± 0.226
40		4	126.5 ± 5.76	119.5 ± 0.42	10.45 ± 0.230
80		4	121.7 ± 1.97	112.5 ± 2.86	10.82 ± 0.364
160		8	124.9 ± 3.26	120.9 ± 2.31	10.59 ± 0.160
320		8	124.3 ± 1.60	116.3 ± 2.20	10.48 ± 0.218
40%	Canadian herring	4	129.1 ± 2.04	111.3 ± 5.57	11.08 ± 0.491

[a] Data given as group mean ± standard error.

[b] No significant differences in any treatment group as compared to control ($P > 0.05$).

plasma chloride levels were slightly higher than serum chloride levels, 125.5 versus 115.6 mEq/L, respectively. There were no significant differences between any treatment group and the control for either plasma or serum chloride. Calcium was measured since 1) high levels of serum calcium increase the renal clearance of iodide and 2) calcium forms an unabsorbable complex with iodine in the gut (Simpson, 1947; Stanbury and Ramalingaswami, 1964). Serum calcium was not affected by any level of iodine nor from feeding 40 percent fishmeal. The overall mean of serum calcium was about equal to the level of serum calcium determined at five months in the experiment (see Table 9), 10.74 versus 10.54 mg/dl, respectively.

Organ weights of mink killed at the termination of the experiment are shown in Tables 24, 25 and 26. There was no significant difference in brain weights for any treatment group as compared to the control. Thyroid weights were also not significantly different from control values, but all thyroids of iodine treated animals were heavier than controls; 70 mg versus 58 mg, respectively for mean weights. Animals fed the fishmeal diet showed no effect on their thyroid or brain weight. Liver, spleen and kidney weights are shown in Table 25. Weights of livers from females fed 10 and 160 ppm supplemental iodine were significantly increased over controls by 33.4 and 31.2 percent, respectively. Although no other group's

Table 24. Brain and thyroid weights of adult female mink fed various levels of iodine or added fishmeal.

Organ	Treatment: Iodine (ppm)	Treatment: Fishmeal	n	Mean organ weight	Mean organ weight as percent of: Body weight	Mean organ weight as percent of: Brain weight
				(g)	(g/100g)	
Brain	0		5	7.34 ± 0.198[a,b]	0.95 ± 0.059[a]	---
	10		2	7.53 ± 0.365	0.85 ± 0.085	---
	20		4	7.99 ± 0.229	0.93 ± 0.040	---
	40		2	7.36 ± 0.325	0.97 ± 0.165	---
	80		2	7.75 ± 0.000	0.85 ± 0.080	---
	160		4	7.61 ± 0.325	0.96 ± 0.040	---
	320		4	7.57 ± 0.090	0.89 ± 0.009	---
		40% Canadian herring	2	7.32 ± 0.410	0.96 ± 0.055	---
				(mg)	(mg/100g)	(g/100g)
Thyroid	0		5	58 ± 3.7	7.4 ± 0.32	0.79 ± 0.066
	10		2	75 ± 5.0	8.4 ± 0.70	0.97 ± 0.040
	20		4	68 ± 2.5	7.9 ± 0.46	0.85 ± 0.031
	40		2	75 ± 5.0	10.2 ± 2.85	1.03 ± 0.115
	80		2	65 ± 5.0	7.2 ± 1.20	0.84 ± 0.065
	160		4	63 ± 7.5	7.8 ± 0.71	0.82 ± 0.082
	320		4	75 ± 6.5	8.9 ± 0.09	0.99 ± 0.080
		40% Canadian herring	2	50 ± 10.0	6.4 ± 0.60	0.68 ± 0.100

[a]Standard error

[b]No significant differences in any treatment group as compared to control ($P \leq 0.05$)

Table 25. Effect of feeding various levels of iodine or added fishmeal on liver, spleen, and kidney weights in adult female mink.

Organ	Treatment		n	Mean organ weight (g)	Mean organ weight as percent of:	
	Iodine (ppm)	Fishmeal			Body weight	Brain weight
Liver	0		5	23.33 ± 0.94[a]	2.73 ± 0.042[a]	292.9 ± 20.52[a]
	10		2	28.45 ± 1.35	2.93 ± 0.050	369.5 ± 8.20[b]
	20		4	23.96 ± 0.78	2.79 ± 0.109	300.0 ± 4.74
	40		2	23.68 ± 4.53	3.02 ± 0.070	319.8 ± 47.40
	80		2	25.58 ± 3.31	2.77 ± 0.100	330.0 ± 42.60
	160		4	27.98 ± 1.48	3.52 ± 0.153	370.8 ± 28.78[b]
	320		4	24.01 ± 1.98	2.83 ± 0.188	316.7 ± 22.33
		40% Canadian herring	2	24.68 ± 2.82	3.21 ± 0.015	336.1 ± 19.70
Spleen	0		5	2.73 ± 0.38	0.35 ± 0.045	37.22 ± 5.45
	10		2	3.44 ± 0.84	0.38 ± 0.035	45.28 ± 8.97
	20		4	3.17 ± 0.22	0.37 ± 0.016	39.94 ± 3.51
	40		2	2.97 ± 1.20	0.37 ± 0.075	39.74 ± 14.56
	80		2	3.29 ± 1.08	0.35 ± 0.085	42.45 ± 13.94
	160		4	2.92 ± 0.15	0.37 ± 0.025	38.89 ± 3.74
	320		4	3.68 ± 0.93	0.43 ± 0.099	48.35 ± 11.51
		40% Canadian herring	2	4.65 ± 0.35	0.61 ± 0.025	63.47 ± 1.24[c]
Kidney	0		5	4.24 ± 0.210	0.54 ± 0.020	58.22 ± 4.23
	10		2	4.20 ± 0.020	0.48 ± 0.045	55.29 ± 0.52
	20		4	4.40 ± 0.375	0.51 ± 0.039	55.00 ± 4.07
	40		2	4.20 ± 0.185	0.56 ± 0.095	57.04 ± 0.01
	80		2	4.46 ± 0.105	0.49 ± 0.035	57.49 ± 1.36
	160		4	4.58 ± 0.125	0.58 ± 0.022	60.62 ± 3.22
	320		4	4.06 ± 0.174	0.48 ± 0.012	53.55 ± 1.70
		40% Canadian herring	2	4.27 ± 0.045	0.56 ± 0.060	58.42 ± 2.66

[a]Standard error.
[b]Significantly greater than control ($P \leq 0.10$).
[c]Significantly greater than control ($P \leq 0.05$).

Table 26. The effect of feeding various levels of iodine or added fishmeal on heart, adrenal and ovarian weights of adult female mink.

Organ	Treatment: Iodine (ppm)	Treatment: Fishmeal	n	Mean organ weight (g)	Mean organ weight as percent of: Body weight (g/100g)	Mean organ weight as percent of: Brain weight (g/100g)
Heart	0		5	5.29 ± 0.21[a,b]	0.68 ± 0.034[a]	72.36 ± 3.67[a]
	10		2	6.54 ± 1.04	0.37 ± 0.005	86.46 ± 9.23
	20		4	5.67 ± 0.36	0.66 ± 0.038	70.91 ± 3.37
	40		2	6.20 ± 1.44	0.79 ± 0.015	83.60 ± 15.89
	80		2	6.79 ± 0.96	0.74 ± 0.035	87.61 ± 12.39
	160		4	5.76 ± 0.35	0.73 ± 0.027	76.17 ± 5.83
	320		4	6.23 ± 0.74	0.73 ± 0.074	82.28 ± 9.37
		40% Canadian herring	2	6.32 ± 1.18	0.32 ± 0.065	85.70 ± 11.32
				(mg)	(mg/100g)	(g/100g)
Adrenal	0		5	78 ± 2.0	9.9 ± 0.35	1.06 ± 0.036
	10		2	95 ± 5.0	9.3 ± 0.45	1.17 ± 0.100
	20		4	83 ± 9.5	10.5 ± 1.16	1.12 ± 0.085
	40		2	70 ± 10.2	9.1 ± 0.65	0.95 ± 0.095
	80		2	95 ± 15.1	10.5 ± 0.25	1.23 ± 0.195
	160		4	78 ± 9.0	9.7 ± 1.16	1.01 ± 0.091
	320		4	78 ± 8.2	9.2 ± 0.94	1.03 ± 0.099
		40% Canadian herring	2	85 ± 15.0	11.6 ± 3.40	1.18 ± 0.270
				(mg)	(mg/100g)	(g/100g)
Ovary	0		5	144 ± 15.0	18.6 ± 2.31	1.95 ± 0.177
	10		2	150 ± 30.0	16.5 ± 0.50	2.28 ± 0.005
	20		4	178 ± 29.0	22.0 ± 4.30	2.38 ± 0.468
	40		2	165 ± 5.0	22.0 ± 4.00	2.25 ± 0.035
	80		2	150 ± 30.0	16.0 ± 2.00	1.94 ± 0.385
	160		4	103 ± 11.1	13.2 ± 1.79	1.35 ± 0.130
	320		4	160 ± 21.2	19.0 ± 2.62	2.11 ± 0.269
		40% Canadian herring	2	135 ± 35.0	17.5 ± 2.51	1.83 ± 0.375

[a]Standard error.
[b]No significant differences in any treatment group as compared to control ($P \leq 0.05$).

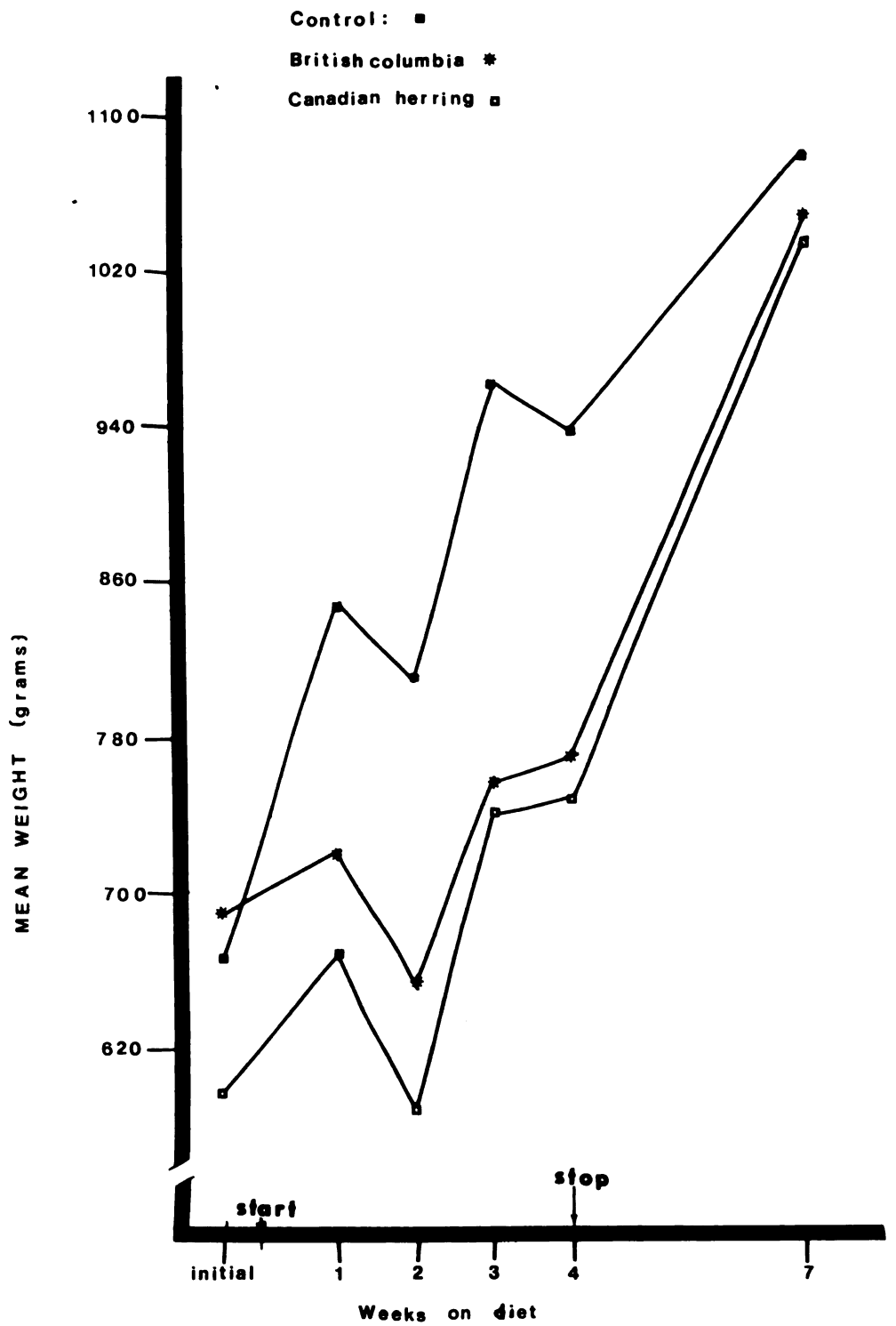
liver weights were significantly greater than the control. Spleen weights of treatment groups were all greater than the control (average 3.4 grams greater) but not significantly, except for the animals fed 40 percent added fishmeal. The spleens of females fed the fishmeal diet were 70.3 percent greater in weight than control animals' spleens. There were no significant differences in kidney weights in any group as compared to the control. Table 26 shows the heart, adrenal and ovarian weights as mean weight and as percent of body and brain weight. There were no significant differences between control and any treatment group for these organ weights.

Fishmeal Feeding to Young Animals

Body weights of juvenile mink fed the control and supplemental fishmeal diets are shown in Figure 28. Generally the control animals showed an increase in body weight over the entire period. However, the young mink fed the fishmeal supplemented diets showed only a slight increase in body weight while on the diets followed by a marked increase to near control's weight after the fishmeal diets were removed and control diet was fed. The average gain in body weight through the third week for mink fed the control diet, British Columbia fishmeal and Canadian herring fishmeal-supplemented diets was

Figure 28. The effect of feeding two types of fishmeal supplemented diet (40 percent fishmeal) for four weeks on body weight of juvenile mink. Initial = nine weeks old; feeding supplemented diet started at week 0 (arrow) and stopped after four weeks (arrow) and regular feed began.

FIGURE 28


Control: ■
British columbia ✱
Canadian herring ▫
1100
1020
940
860
780
700
620
MEAN WEIGHT (grams)
start
stop
initial
1
2
3
4
7
Weeks on diet

8.18, 2.45 and 4.73 grams per day per animal, respectively. Thus, the control's gained about twice as much body weight as those fed the fishmeal diets.

Young rats showed about the same effect as mink though not as dramatic. The body weight changes for young rats fed the control and Great Lakes fishmeal-supplemented diets are shown in Table 27. Initial body weights were about the same, however, the rats on fishmeal supplementation did not gain as well as the control animals.

Table 27. Effect of feeding Great Lakes (GL) alewife fishmeal at 40% of the diet on body weight gain of young growing rats over a three week period.

Diet	Initial weight (g)	Weight gain[a] (g/r/d) days 1-7	days 8-14	days 15-21
Control	57.2	6.65 ± 0.31	4.20 ± 1.42	6.30 ± 0.54
GL fishmeal	59.3	5.29 ± 0.24[b]	3.67 ± 0.59	4.24 ± 0.43[b]

[a] Data given as group mean ± standard error.

[b] Significantly different from control ($P < 0.05$).

DISCUSSION

Fishmeal

The purpose of this experiment was to determine if adding fishmeal at high levels (> 30 percent) to a normal diet would adversely affect the adults and/or young rats and mink. No effects were noted from feeding added fishmeal on body weight changes before or after breeding and gestation (see Table 3 for rats and Table 7 for mink), nor was nay effect noted in feed consumption by rats (see Table 4). However, reproduction parameters were affected by added fishmeal. The rats on fishmeal diets had more pups born dead and generally had greater mortality from birth to weaning time than control rats. Thus, high levels of added fishmeal were affecting late gestation and postpartum development, possibly from the excess protein the diet was supplying. Percent survival of mink kits from dams on fishmeal supplemented diets was ten percent less than control kit survival (see Table 14). Therefore, the additional fishmeal diets were also affecting growth and development in a negative manner. Weaning weights were also affected in a like manner with decreased body weight of pups from

mothers on fishmeal diets (see Tables 5 and 6). As with rats, the additional fishmeal affected the mink kits after birth, which may point to 1) either the fishmeal has a residual effect after paruration or 2) the effect of the fishmeal is being transmitted via the milk or causing a decrease in milk production.

No other noticeable effects were seen in any fishmeal treated group, except the adult female mink had larger spleens than control mink; the reason for this is unknown.

The results of decreased reproductive performance and postpartum mortality are in agreement with those of Black et al. (1954; 1957) on feeding fishmeal to chickens. These workers reported a progressive decline in the hatchability of eggs laid by hens fed increasing quantities of whitefish fishmeal supplemented up to 11.1 percent of the control diet. No attempt was made to equalize the energy or mineral content of the diets of the hens by Black et al. (1954; 1957) or of the diets for mink and rats in this experiment. As in this research on mink and rats, the research on the adult hens fed the supplemented fishmeal diets showed no adverse effects on adult body weight.

Storage of fishmeals tends not to cause any significant changes in the nutritional value of the meal (Ousterhout et al. 1959; Lea et al. 1960; Sullivan et al.

1960). However, if the processed fishmeal is overheated or scorched during or after processing, then a decrease in amino acids (especially lysine) occurs (Ousterhout et al.,1959; Lea et al., 1960). Contamination by toxic substances is of concern in fishmeals as these contaminants have been found in the past to cause problems in mink. These substances include dimethylnitrosamine causing hepatotoxicosis (Stout et al., 1968; Stout and Adair, 1970), polychlorinated biphenyls causing reproductive failure and deaths (Ringer et al., 1972), rancidity, mercury and hydrocarbon pesticide contamination also causing reproductive and livability problems (Hartsough, 1965; Aulerich and Ringer, 1970; Aulerich et al., 1971). Thus, fishmeals added to the diet, especially at high levels, could pose serious problems and unless the quality can be guaranteed, it is not recommended to supplement diets with high percentages of fishmeal.

A more recent report by Sonstegard and Leatherland (1979), concerning the problems of fish, noted that when Great Lakes coho salmon (Oncorhynchus kisutch) were fed to rats, as the only source of food, thyroid hyperplasia and hypothyroidism resulted. Serum T_4 levels were reduced and thyroid glands weighed significantly more than controls, but serum T_3 levels were not affected. These observations were not attributed to the level of

iodine in the fish (2.2 ppm) but rather to the presence of goitrogenic materials especially orgnochlorines which are goitrogenic in mammals and birds (Sonstegard and Leatherland, 1979).

The depressed weight gain in the kits fed the fishmeal supplemented diets may have been caused by the excess amount of protein in the diet and/or the decreased amount of fat, as no residual effects from the fishmeal were noted after its removal from the diet. Young rats showed about the same results as mink, that is, not growing as well when fed a diet supplemented with fishmeal (see Table 27). However, the effect was not as pronounced as in the young mink. Again, the lack of fat and increased protein probably played a major role in the lack of body weight gain. These results are in agreement with the results of Sugahara et al. (1969) who fed crude protein levels of up to 48 percent to pigs and found decreasing body weight gain, as protein in the diet increased. Also, when pigs were fed 32 and 48 percent protein, a depressed lipogenesis was noted, as well as decreased activity of several adipose-tissue enzymes associated with fatty-acid synthesis (O'Hea and Leveille, 1969). Excess protein also produces a stress on animals, which along with decreased growth could be the main causes for the postpartum mortality seen in both the rats and mink.

The level of iodine in some fishmeals varies greatly with some as high as 126 ppm of iodine (Power et al., 1969). Thus these high levels found in ocean fish could cause probelms if fishmeal were fed to mink at a high percent of the diet as shown by this experiment. These high levels of iodine in fish may be due to their eating habits or their migration routes.

Iodine

Iodine fed at up to 160 ppm to mink for six months prior to mating appeared to have some effect on body weight gain as the 160 ppm group started at a higher mean weight than control animals, but was less than control body weight after six months. Though this effect is not significant the minor differences may have been due to a slight decrease in basal metabolic rate from the excess iodine.

The normal rise in packed cell volumes (see Table 8) seen in mink as cold weather approachs was noted in all groups on this experiment and the values are comparable to others found previously (Rotenberg and Jørgensen, 1971; Ringer et al., 1974; Asher et al., 1976).

The variability observed in the mink body temperatures recorded was not unexpected since measurements of the body temperature of mink are 1) difficult to take,

2) inconclusive because of the nervous temperament of the animal thus, causing variations and 3) that an excited mink's body temperature may be as high as 42°C (Kennedy, 1951). "Normal" body temperatures of 39.5 to 40.5°C reported for mink by Berestov (1971) are quite close to the temperatures determined in this experiment. The difference in body temperature between the first two months and the third and fourth months (about 1°C greater) may have been due to the animals metabolic rate increased as environmental temperature decreased.

Blood indices of mink (see Table 9) at five and six months of treatment were comparable to values reported by other researchers. A serum calcium level of 10.55 mg/dl over all the groups tested is in agreement with Rotenberg and Jørgensen (1971) who reported a value of 10.72 mg/dl and with 10.66 mg/dl reported by Asher et al. (1976). The packed cell volumes ranged from 16.5 to 22.5 g/dl and are in agreement with reported figures of 24.5 g/dl (Rotenberg and Jørgensen, 1971), 17.2 g/dl (Narasimhalu et al., 1978), 14.5 to 21.5 g/dl (Asher et al., 1976), 19-21 g/dl (Skrede, 1970b) and 13.5 to 17.5 g/dl (Kubin and Mason, 1948). Although red blood cell counts in this study varied greatly (7.77 to 10.27 million/mm^3 they are in agreement with reported values (all values in million/mm^3) of 5.4 to 10.2 (Fletch and Karstad, 1972), 7.62 (Rotenberg and Jørgensen,

1971), 6.86 to 9.7 (Asher et al., 1976) and 5.73 to 9.35 (Kubin and Mason, 1948). White blood cells were also variable (8,100 to 10,700/mm^3) but were in agreement with others, such as 3,650 to 11,150/mm^3 (Kubin and Mason, 1948) and 5, 574 to 6,203/mm^3 for males and females, respectively (Rotenberg and Jørgensen, 1971). These highly variable results obtained in "normal" mink tend to obscure any small changes that may be taking place in these parameters caused by the treatments.

The urinary excretion of iodine (see Table 11) for mink fed higher iodine diets is in agreement with the fact that animals tend to balance their body burden of iodine through urinary loss of iodine (Stanbury and Ramalingaswami, 1964; Sodeman and Sodeman, 1974).

Iodine appears to have affected reproduction (see Tables 12, 13 and 14) in mink in a manner similar to the effect in other species fed high levels of supplemental iodine. Rabbits fed iodine in the diet, at 250 ppm starting two days before parturition, produced a normal litter. However, survival of the young, to four weeks was only 30 percent, and a decreased weight at weaning was noted. The mother's mammary glands were devoid of milk (Arrington et al., 1965). However, rats tend to tolerate higher levels of iodine than rabbits and mink. When iodine was fed at 500 ppm for 35 days pre-partum only slightly fewer young were born and the

survival of pups was reduced from 93 percent (control) to 84 percent (Ammerman et al., 1964). Other species are much less affected by iodine in the diet. Hamsters fed a 2500 ppm iodine-supplemented diet showed no effect on percent survivability of the young to weaning time, however, weaning weights were significantly decreased. Reproduction in ewes fed supplemental iodine at 0.007 percent, 109 to 133 days before parturition, was not affected (Aschbacher, 1968). Swine fed 1500 and 2500 ppm showed no adverse effects on reproduction parameters (Arrington et al., 1965). When gullet trimmings with attached thyroid-parathyroid gland tissue were fed to both mink and rats, adverse effects on reproduction were seen. A decraase in the number of females whelping, young born and survival of young to weaning was noted. This product is high in iodine and thyroid hormone content (Travis et al., 1963, 1966). The mink kit's survival to 14 days was only 22.7 percent (control = 88.8 percent) (Travis et al., 1966). Whereas, the survival of the control kits to four weeks of age in this experiment (see Table 14) was only 56.5 percent and is considered too low for "normal" mink. However, the survival of the kits from the 10 and 20 ppm iodine-treated groups was increased over the control animals by 15.3 and 34.4 percent, respectively. Thus the iodine being excreted in the milk and affecting the kit's

thyroids may have had a positive effect on the survival of the kits. The kits survival from the 160 ST group was 20.8 percent lower than the control animals, showing a possible negative effect of iodine. However, kits survival in the 160 LT dietary group was equal to control kits survival. Since 1) a major route of excretion of iodine is the enterohepatic circulation and 2) the hepatic excretory system is not fully developed in the newborn, the deaths of the kits may be due, in part, to the increased iodine and the inability of the kit to handle the excretion of the iodine. It has recently been reported that iodide inhibits the cholinergic stimulation of prostaglandins, PGE_2 and $PGF_{2\alpha}$, synthesis in dogs (Boeynaems et al., 1979). This effect, as well as the ability of iodine to depress the responsiveness of the thyroid to TSH (Rapoport et al., 1977; Van Sande et al., 1975) and the increased conversion of T_4 to rT_3 seen in the kits from the ST groups, may also be involved in the increased mortality seen in kits from mothers treated with high levels of iodine.

Body weight of kits at weaning was reduced in the 160 ST group. This is consistent with the findings of Aulerich et al. (1978) where 100 ppm supplemental iodine fed from March 25 to June 1 decreased weaning weight by 19.6 percent from that of the control. When gullet trimmings, which contain high levels of thyroid tissue, were fed at 15 percent of the diet impaired kit weight

gain, during the first 14 days after birth, was noted (Travis et al., 1966). The decreased gestation period of adult females on three of the levels (40 LT, 40 ST and 80 ST) of iodine treatment is in agreement with that of Aulerich et al. (1978), who reported a decrease in gestation period in all mink fed iodine-supplemented diets. The decreased number of kits born alive per female in the 160 ST and 160 LT groups is in agreement with the findings of Aulerich et al. (1978) where a decrease in live kits at birth was noted with females fed 100 ppm added iodine diets. Birth weights of control kits (9.36 grams) and all treatment levels birth weights (8.34 to 10.27 grams), except 160 ST, showed no effect from iodine treatment. These results and the depressed weight of the kits from the 160 ST diet (7.06 grams) are all in agreement with the results of Travis et al. (1966) where control kits birth weight was 8.3 grams and kits from mothers fed thyroid compounds weighed 6.5 grams. However, Aulerich et al. (1978) did not find a difference between the 100 ppm iodine-supplemented group (8.3 grams) and the control kits birth weight (9.5 grams).

The kits "biomass" was affected similarly to that reported by Aulerich et al. (1978) where a decrease from control (421.2) was seen in animals on 100 ppm supplemental iodine (279.7). The "biomass" of animals from

this study (see Table 14) decreased from the control value (428.4) by 70.4 percent in the 160 ST group. Also in agreement is the increased biomass noted in mink fed low levels of iodine. Aulerich et al. (1978) reported a 43.6 percent increase in biomass in animals fed 10 ppm added iodine and this study (see Table 14) also showed an increase in both 10 and 20 ppm iodine-treated groups of 66.8 and 54.3 percent, respectively. Of the mink fed the high level (320 ST) of iodine only one of the 12 females whelped and that one produced only two dead kits. This is in agreement with the lack of any kits whelped on the 1000 ppm iodine diet fed to mink in the study by Aulerich et al. (1978). These results tend to point ot the facts that 1) a low level, of iodine (10 and 20 ppm) added to the diet may improve the number of kits raised to weaning per lactating female and their biomass, 2) a high level, approximately 100 to 200 ppm iodine may have an adverse effect tending to decrease the number of kits raised to weaning, as well as the biomass (three to five months of excess iodine before breeding is worse than ten months of excess iodine before breeding), and 3) very high levels, 320 ppm and above, completely inhibit the reproductive process after mating.

Body weights of adult females at whelping and weaning time (see Table 15) for controls and for females on

low level iodine-supplementation are in agreement with Travis and Pilbeam (1978) who reported a weight of 1049 g at whelping and a loss of weight (body weight = 901 g) at weaning. The low body weight (923 g) of dams, on 160 LT iodine treatment, at whelping is in agreement with Travis and Pilbeam (1978) where a low reproductive performance female mink was tested and weighed 118 g less than control mink at whelping time. However, the body weight (770 g) at weaning of the females fed the 160 LT diet was severely affected.

Thyroid

T_4 levels of 18.4 ng/ml see Table 16) for adult female mink fed control diet are comparable to those reported by Byrne (1974) of 13.5 to 32.3 ng/ml with a mean of about 19 ng/ml. The decrease in adult T_4 and T_3 levels (see Figures 4 and 5) at higher (>20 ppm) iodine treatment levels is in agreement with work done with rats (Yamada and Lewis, 1968), where it was shown that excess iodide blocks thyroid hormone release. Also in white leghorn cockerels, fed diets supplemented with iodine (2 µg/g of diet), a reduced thyroidal activity and altered distribution of iodide in the iodoamino acids of the thyroglobulin were shown (Rosenberg et al., 1963a). No work has been done

previously in mink on thyroid response to iodine treatment. Ohtake et al. (1973) noted a decrease in thyroid hormone secretions in rats with administration of iodine via injections. Wolff and Chaifoff (1948) determined that a decrease in hormone formation took place in rats when excess levels of iodine blocked uptake of iodine by the thyroid and thus synthesis of the hormones. Iodine does not appear to inhibit the secretion of thyroid hormone, however excess iodine has been reported to inhibit or block the effect of TSH on thyroidal secretions (Onaya et al., 1966) but others report it may not (Onaya and Halmi, 1967; Sawin et al., 1979). T_4 and T_3 levels in kits, from mothers on long term (LT) diets, generally responded to the level of iodine in the diets in a manner similar to the parents, except at a higher level than adults. The kits, from mothers on short term (ST) diets, showed a more marked decrease in T_4 and a more dramatic decline in T_3 than the long-term diet groups. In relation to the time of mating it appears from these data that the short-term diets caused a greater effect upon thyroid hormones in the young than did the long-term diets. These results may have been due to the fact that the mothers on the ST diets had less time to adapt to the excess levels before mating and implantation than did their counterparts on the LT diets. This theory is based on the fact that the degree

of iodinating activity of the thyroid hormone is proportional to the duration and concentration of iodide in the thyroid (Sherwin and Tong, 1974). Also, these high levels of iodine may be affecting the embryos during the delay period before implantation or the implanted fetuses are absorbed early in gestation since no aborted fetuses were noted.

T_4-bindng index (see Table 18 and Figures 6 and 7) is a measure of the plasma globulins (TG) capacity to bind additional T_4. Generally an inverse relationship exists between total T_4 and T_4-binding index. When total T_4 is high then most of T_4 in circulation is bound to TG, thus the ability of TG to bind more is decreased as compared to when T_4 is low and not as much would be bound (Etta, 1971). In the adult mink on 10 ppm iodine, when T_4 levels decreased from the control values, the expected increase in T_4-binding was not seen, but rather a drop from control of 40.8 percent. This decline may infer that the levels of globulin have decreased; thus even though there is less T_4, the capacity to bind T_4 molecules is also decreased. All other groups follow the relationships stated above in that for every increase or decrease of T_4 there is a corresponding decrease or increase in T_4-binding index. Kits T_4-binding indexes did not follow the relationship as well as adults. The kits from mothers on 10 ppm were similar to the dams in

that they showed a significant decline in T-4-binding indexes with decreasing T_4 values. However, the 80 LT and 160 LT groups of kits showed a decline in both T_4 and T_4-binding index as did the 10 ppm group. Thus, these groups may have had a decrease in TG. Kits from dams on short-term diets showed a marked increase in their T_4-binding index, more so than the decrease seen in T_4 levels. There may have been a slight increase, therefore, in the thyroglobulin of these animals, especially the animals in the 80 and 160 ppm iodine-treated group.

Reverse-T_3 (rT_3), a normal conversion product of T_4 in most animals, levels (see Table 19 and Figure 8) in the adults on long-term diets were similar to T_4 levels. The 40 LT group of mink showed a decrease in rT_3, but the rT_3 level of the 80 LT group rose slightly compared to the 40 LT group. However, the rT_3 of the animals fed 160 LT iodine-treatment was at about the same level as the mink fed 40 LT iodine-treated feed. The reason for the rise and fall in these three treatment groups in T4, T3 and rT3 values (and the opposite effect in T_4-binding index) is unknown. This relationship of T_4 by rT_3 is in agreement with the report by Bernal and Refetoff (1977) which stated that the deiodination of T_4 to T_3 was finely regulated except in some abnormal conditions. The adult rT_3 levels on ST

diets did not parallel the levels of T4 as did the levels of rT_3 in adult animals on the LT diets. While adult T_4 levels were falling in animals fed ST diets and T_3 showed a mild depression only in animals fed the 80 ppm ST iodine-treatment, rT_3 showed a gradual rise and fall over the four ST diets. It may be that the animals on the ST diets had not adapted as well (not enough time) and the amount of conversion to rT_3 had remained the same (or increased slightly) while T_4 was decreasing.

Kits from mothers fed LT diets had rT_3 levels that paralleled T_4 levels, as each hormone showed a gradual decline for each level of iodine in the diet. Thus, probably no effect of iodine on the conversion of T_4 to rT_3 was demonstrated. However, the rT_3 of kits from mothers fed ST diets was markedly altered from expected values. Instead of a marked decline, as in T_4 and T_3 determinations, a marked increase in rT_3 was noted, especially in the 160 ppm iodine-treated group. In some clinical states, including malnutrition, hepatic and renal failure, and chronic illness, a low serum T_3 level is associated with reciprocal elevations in serum rT_3 (Chopra et al., 1975). Sterlin and Chodos (1956) noted that the rate of degradation of T_4 was increased in thyrotoxicosis and decreased in hypothyroidism.

Conversion of T_4 to T_3 is catalyzed by iodothyroine-5'-deiodinase (i-5'-d) while conversion of T_4 to rT_3 is catalyzed by iodothyronine-5-deiodinase (i-5-d). Thus, a rise (or fall) in T_3 with a comcomitant decline (or increase) in rT_3 is possible via an affect on the particular enzyme involved in the conversion. Also, iodothyronine-5'-deiodinase is more sensitive to diminished availability of glutathione than i-5-d. Thus, when carbohydrate metabolism is decreased, a decrease in glutathione is produced and, therefore, a reduction in i-5'-d (Henneman, 1979). It has been reported that rT_3 interfers with thyroid hormone uptake by target cells, or binding by the thyrotrophs and may also increase the T_3 clearance rate thus reducing T_3 in the circulation (Lewis et al., 1979).

Pathology

Generally, gross observations of the thyroid glands (see Figures 9, 10, 11 and 12) of kits were in agreement with previous studies with other animals, in that added levels of iodine caused an enlargement of the thyroid gland (Wolff, 1969; Baker and Lindsey, 1968; Correa and Welsh, 1960; Newton and Clawson, 1974). This increased size of the thyroid glands was probably due to the transfer of iodine across the placenta. If the thyroid enlarges too much, a tracheal obstruction can

occur and cause death of the fetus. The continued enlargement of the thyroid gland after birth is due to the iodine that is readily secreted into the milk.

Histologically the thyroid glands tended to follow the gross observations. Those glands that were enlarged showed hyperplasia of the follicular cells and a decrease in colloid. These changes are in agreement with other investigators (Werner, 1957; Cheville, 1976), who reported that in both man and animals that excess iodine causes the thyroid gland to become hypertrophied. This hypertrophy is an epithelial hyperplasia, showing an increase in size or number of the follicular cells resulting in a decreased follicular lumen and a decrease in colloid content. This hyperplasia may lead to an exhaustion atrophy where the follicles become smaller and nonproductive (Werner, 1957). Thyroids from adult mink on the higher levels (greater than 40 ppm supplemental iodine) displayed the same changes histologically but the changes were not as marked.

It has been shown in rats that a diet containing excess iodine and contaminated with polyborminated biphenyls (PBB) causes more severe pathologic changes than either iodine or PBB would cause alone (Sleight et al., 1978). Thus, iodine apparently has an additive effect, at least in rats, causing increased liver weights, disruption of serum proteins, lipoproteins and

lactic dehydrogenase isozymes (Sleight et al., 1978). Since mink are sensitive to both PBB and iodine, the mink may show adverse effects without being exposed to a toxic level of either chemical but a combination of the two chemicals. When polychlorinated biphenyls (PCB) were fed to mink at 0.5, 2.0 and 5.0 ppm T_4 levels generally increased and peripheral degradation of thyroxine also increased except during estrus and pregnancy. However, at the 0.5 ppm level PCB consistently caused increased T_4 levels even during estrus and pregnancy (Byrne, 1974).

The changes seen in the gall bladder of mink on excess iodine may be due in part to the normal enterohepatic circulation of iodine. Iodine is in the class B compounds of biliary excretion types as its bile/plasma ratio is greater than 1. As excessive iodine passes through the gall bladder some pathologic changes may occur. Thus, as the increased concentration of iodine continues to pass through the gall bladder the changes become more pronounced. These same changes in the gall bladder of cystic mucosal hyperplasia were seen in the beagle dog fed another halogen, chlorine as sodium dichloroacetate (Katz et al., 1980). However, the changes were not as severe as those found with the iodine treated mink and when another halogen of even lower molecular weight, fluorine, was tested no changes

were noted in the gall bladder (Katz, personal communication). It may be that the halogen, especially those of higher molecular weight, cause hyperplastic lesions in the gall bladder through, as yet, an unknown mechanism.

The small amount of amyloid noted in a couple of animals is probably a normal occurrence. One year old and older mink have been noted to be affected with spontaneous amyloidosis in a variety of organs including the adrenal glands. However, it is much more prevalent in older mink (greater than three years old) and is correlated with somatic deterioration (Schwartz et al., 1971).

Milk, Blood and Organ Weights

Milk samples analyzed for iodine (see Table 20) showed that at the higher dietary levels, 80 and 160 ppm iodine, the amount of iodine excreted into the milk is not proportional to the iodine content of the diet. It appears that a maximum excretion of iodine into the milk is reached at about 20 to 40 ppm iodine in the diet.

This secretion of iodine, increasing as dietary iodine increases, by mammary tissue is in agreement with results found in other species (Flamboe and Reineke, 1959; Reineke, 1961; Hemken et al., 1972; Swanison, 1972). Generally iodine is passed into the milk as

iodide, unlike the mechanism in the thyroid gland where the iodide is in organic combination with thyroglobulin (Reineke, 1961).

Differential counts (see Table 21) for control and nonaffected groups are in agreement with previous reported results as shown below:

	Cell Type[1] (percent)				
Reference	Lymph	Neut	Mono	Eosin	Baso
This study (control)	44.7	47.0	2.7	4.0	1.6
Kennedy (1935)	46.7	44.6	2.0	6.4	0.3
Asher et al. (1976)	36.0	50.5	8.2	2.4	2.9
Rotenberg and Jørgensen (1971)	52.5	38.4	7.0	0.6	1.5

[1]Lymph = lymphocyte, Neut = neutrophil, Mono = monocyte, Eosin = eosinophil, Baso = basophil.

The effect seen in the females fed 160 ppm iodine-supplemented diet, where neutrophils decreased and lymphocytes increased, is opposite to the effect seen in dairy cattle on excess iodine where increased neutrophils (33 to 43 percent) and decreased lymphocytes (55 to 46 percent) were noted (Hillman, personal communication). However, as in the mink fed 320 ppm iodine, where eosinophils were decreased, a trend in decreased eosinophils was also noted in cattle fed excess iodine (Hillman, personal communication).

Red blood cell counts (RBC) at the termination of this study (overall mean equaled 8.03 million/mm^3, see

Table 22) are comparable to other workers' findings previously mentioned. However, the counts generally were less than the determinations done at five and six months of this experiment, 8.88 and 10.06 million/mm^3, respectively (see Table 10). These trends of increasing RBC and decreasing hematocrits points to the probability that the size of the red blood cells was decreasing as the iodine level increased. Hemoglobin levels (overall for the iodine treated animals and control was 19.5 g/dl) are in agreement with previous reports of 19.4 g/dl by Skrede (1970b). White blood cell counts were similar to the earlier results (9,220/mm^3 overall mean) at 8,860/mm^3 and to other reported results previously mentioned in this section.

Serum calcium levels in the mink (see Table 23) did not change from those taken earlier in this experiment with overall means being 10.54 mg/dl at five months (eight months of age), and 10.74 mg/dl at 11 months of treatment (14 months old), thus, in agreement with research results previously mentioned.

Serum chloride levels of mink have not been previously reported, but levels in foxes were reported to be about 140 mEq/L (Berestov, 1971) and in other animals (all mEq/L): rats = 110, sheep = 116, chicken = 112 and dog = 106 (Spector, 1956). Thus, the overall average for mink in this study, of 115.6 mEq/L chloride for

serum, is slightly higher than most other animals but lower than the serum chloride value of fox. The slightly higher levels seen in plasma points to the fact that a small amount of chloride is bound to the coagulating proteins.

Organ weights (see Tables 24, 25 and 26) tended to be in agreement with those reported previously.

	Organ Weight as Percent of Brain Weight			
Reference	Heart	Liver	Spleen	Kidney
This study (control)	72.4	292.9	37.2	58.2
Aulerich et al. (1974)	75.8	399.5	35.5	65.3
Aulerich and Ringer (1979)	79.8	377.6	23.3	71.3

The control livers weighed less than the previously reported animals' livers, however, the females on the 10 and 160 ppm iodine supplement had liver weights that were significantly greater than controls but were close to the reported values of Aulerich and Ringer (1979). Thus, a true increase in the liver weight of these animals may not have occurred.

Thyroid weight of treated mink, though not significantly different from control, tend to show an increase in size, which would follow the kits thyroid responses and the histopathology of the adults involved.

CONCLUSIONS

1. Feeding diets containing 40% supplemental fishmeal caused no adverse effects on adult body weights, in either mink or rats, or of blood parameters in mink. However, reproduction was severely affected and growth of young was retarded in both mink and rats, probably due to the excessive protein in the diet.

2. Levels of approximately 100 ppm iodine tend to have detrimental effects on reproduction in mink, including decreased young born, weaning weight, survivability, and biomass. These changes are probably due to the placental transfer of iodine to the developing kit.

3. Excess iodine is excreted via the milk and urine. Increased mortality and enlarged thyroid glands in kits were associated with the excessive iodine in the milk.

4. Triiodothyronine (T_3) values were slightly decreased in all adults and kits from mothers fed iodine-treated diets, for a long term (LT) over all levels.

The T_3 levels of kits from adults fed iodine supplementation for a short term (ST) were markedly decreased over all levels. Thyroxine (T_4) levels in both adults and kits were decreased as iodine content in the diet increased. T_4-binding index in adults was generally inversely related to the adults' T_4 values as was expected. However, T_4-binding index in the kits was directly related to the kits' T_4 levels, especially in the kits from dams fed ST diets. Reverse T_3 levels generally declined in all adults and the kits from mothers fed LT iodine supplementation over all iodine levels. However, kits from dams fed ST diets showed a marked increase in rT_3 levels. These changes in rT_3 are most likely due to alterations in the enzymes involved in the metabolism of T_4.

5. Gross pathology was limited to increased size of thyroids in the kits from mothers fed diets >40 ppm iodine, and enlarged, thickened and opaque gall bladders in adults fed 320 ppm iodine supplementation. Although the adults' thyroids were not significantly larger than controls, histopathology of the thyroids in both kits and adults fed >40 ppm iodine diets showed a hyperplasia of the follicular cells and a decreased amount of colloid. The gall bladders

of the adults fed 80 ppm or more iodine showed increasing severity of hyperplasia of pili and increasing amounts of secretory material and connective tissue.

APPENDICES

APPENDIX A

Rat Mash Diet

Ingredient	Amount (pounds)	%
Corn	111	55.5
Soybean Meal	45	22.5
Fishmeal	20	10.0
Alfalfa Meal	10	5.0
Dried Whey	5	2.5
Corn Oil	6	3.0
Salt	1	0.5
Vitamin, Mineral Mix	2	1.0
	200	100.0

APPENDIX B

Fishmeal

Fishmeal	% Crude Protein[1]	Supplier
Boston Whitefish	61.2	Ashery Products Marystown Ltd., Canada
Boston fish mix	61.2	The Lake Group Ltd., Gaultons, NFLD, Canada
BFS Ocean Maid	58.1	BFS Corporation, Glencoe, MN
BFS Canadian Herring	66.9	BFS Corporation, Glencoe, MN
British Columbia	68.3	B.C. Packers, Quebec Ltd., Gaspe, Quebec, Canada
Great Lakes (alewife)	65.6	Wisconsin Trawlers, Inc., Rt. 2, Oconto, WI 54153
Menhaden	59.9	Zapata Haynie Corp., Houston, TX 77001

[1]Determinations courtesy of Dr. R. Emery, Dept. Dairy Science, M.S.U.

APPENDIX C

Composition of Control Mink Diet

Ingredient		Amount	
		Pounds	%
Cereal[1]		200	16.7
Fish		150	12.5
Chicken		240	20.0
Beef Trimmings		40	3.3
Beef Lungs		40	3.3
Beef Tripe		80	6.7
Beef Liver		40	3.3
Eggs		75	6.3
Powdered Milk		13	1.1
Water		322	26.8
		1200	100.0
Fishmeal Diet			
	Control Diet	154	53.8
	Fishmeal	66	23.1
	Water	66	23.1
		286	100.0

[1]XK-40 Mink cereal, XK Mink Food Inc., Thiensville, WI 53092

APPENDIX D

Potassium Iodide Neutral KI F.W. 166.00
Mallinckrodt Inc., St. Louis, Missouri 63147
Lot No. ERY A
Meets ACS Specifications

Maximum Limits of Impurities	%
Alkalinity (as K_2CO_3)	0.002
Barium (βa)	0.002
Calcium Magnesium & R_2O_3 Ppt.	0.005
Chloride, Bromide (as Cl)	0.01
Heavy metals (as Pb)	0.0005
Insoluble matter	0.005
Iodate (IO_3)	To pass test (approx. 0.0003)
Iron (Fe)	0.0003
Loss on Drying at 150°C	*0.1
Nitrogen Compounds (as N)	0.001
Phosphate (PO_4)	0.001
Sodium (Na)	0.005
Sulfate (SO_4)	0.005
* Not in total	0.0371→ 99.9629%
pH of a 5% solution (25°C)	6.0-9.2 pure

APPENDIX E

Calcium Analyzer Model 940
Theory of Operation

This analyzer determines calcium concentration via fluorometric determination. The fluorometer measures the emitted fluorescence of excited molecules. The light source is in the range of 300-500 mm and acts to excite the molecules. A primary filter isolates the wavelength of the exciting energy and a secondary filder (at right angles to the exciting beam of light) selects the wave-length to be measured. The intensity of the fluorescent light which strikes the detector is a function of the concentration of fluorescent compound in the cuvette.

When the dye calcein, a fluroescein derivative, is added to the sample an intensely fluorescent non-dissociated complex is formed with the calcium ions in the alkaline medium. The analytical procedure is based upon the quenching of this fluorescence by chelating the calcium ions with the titrant EGTA (Ethylene glycol bis [β-aminoethyl ether] n, n'-tetra-acetic acid). The

titration stops when all the calcium has been chelated by the EGTA and a predetermined level of fluorescence is attained. This minimal level of fluorescence is an inherent property of calcein.

APPENDIX F

Digital Chloridometer
Theory of Operation

The chloridometer is designed for the rapid determination of chloride in biological fluids. The determination is based on the coulometric principle of titration. A silver anode when placed in the unknown sample generates silver ions. These ions combine with the chloride ions in the unknown solution. After precipitation of all of the chloride ions present the excess free silver ions produced are sensed as a current at the indicator electrode. All action stops at this "amperometric endpoint" and the digital readout displays the actual chloride concentration of the sample in mEq/L.

This instrument is internally calibrated to the Faraday constant and requires no external standard. However, the user can override the internal constant and calibrate to an external standard or to compensate the reading for repetitive pipetting errors.

APPENDIX G

Coulter Counter Model Z
Theory of Operation

The Coulter Counter is an electronic self-zeroing counter used to count cells or particles suspended in solution. These particles are sized and counted when passed through an apertura (orifice) with a specific path of current flow for a given length of time. When these particles (cells) pass through the aperture they displace an equal volume of electrolyte and the resistance in the path of current is changed. This change results in corresponding current and voltage changes. The magnitude of this change is directly proportional to the volumetric size of the particle (cell). Thus the number of changes within a specific length of time is proportional to the number of particles or cells within the suspension.

Each current pulse, which is representative of each particle counted, is detected, amplified, and sent to the threshold card. The threshold card does the actual sizing, depending upon the upper and lower limits set with

the threshold controls. The count pulses are then sent to the readout display.

APPENDIX H

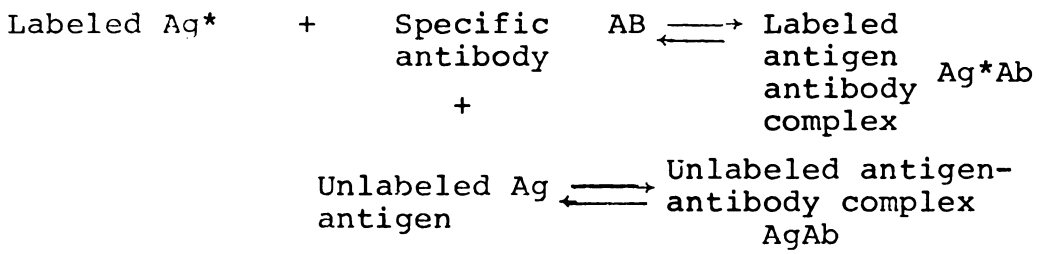
Hemoglobinometer
Theory of Operation

The sample is placed in a flow-through cuvette and a beam of light is passed through the fluid sample into a photo-electric measuring device. A reference solution (blank) is automatically introduced into the cuvette and the amount of light transmission is measured. This information is stored, electronically, as a point of reference. The diluted and lysed blood sample (unknown) is placed in the cuvette and the amount of light transmission is again measured in the same manner. The reading of the unknown is the compared to the reading of the blank, and the hemoglobin concentration is computed and displayed.

APPENDIX I

T_3, T_4, Reverse T_3 and T_4-Binding Index Determinations

The competition between a radioactive (tracer) and a non-radioactive (standard or sample) antigen for a limited fixed amount of antibody. This relationship is illustrated as:



The labeled and unlabeled antigens compete for specific binding sites on the antibody. As the amount of unlabeled antigen is increased, a decrease in amounts of the labeled antigen bound to the antibody is seen. Thus, by holding the concentration of labeled antigen and antibody constant, while increasing the concentration of unlabeled antigen, one can obtain a progressive response which is plotted as a standard curve.

$\underline{T_3}$

To each tube 600 µl buffer tracer (see Appendix I), 100 µl of sample and 100 µl of antibody[1] was added. Each tube was then vortex and then incubated in a 37°C water bath for 30 minutes. After incubation the tubes were placed in a 0-4°C water bath for a minimum of one hour (may be as long as overnight). Next, 0.5 ml of a charcoal solution (Appendix J) was added per tube and left for ten minutes to absorb all of the free T_3 in the solution. The tubes were then centrifuged for ten minutes at 4000 rpms leaving the antibody complex in the supernatant. This supernatant was poured off and the charcoal with T_3 was counted on a 4 channel automatic gamma counter, Micromedic model 28154[2]. The unknowns were computed using four standards[3], 0, 1.5, 1.0 and 4.0 nanagrams per ml. The procedure was monitored by checking two in-house controls, an equine and a canine.

$\underline{T_4}$

To each tube 600 µl buffer tracer (see Appendix K), 30 µl of sample and 100 µl of antibody (see Appendix L)

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[1]Wien Laboratories, Inc., P.O. Box 227, Succasunna, NJ 07876. For each bottle of antibody, 7.5 ml of T-protein buffer is added.

[2]Micromedic Systems, 1312 Meridian Street, Huntsville, AL 35810

[3]Industrial Nuclear, 9641 Lockland, St. Louis, MO 63114

was added. Each tube was then incubated in a 37°C water bath for 20 minutes, then placed in a cold (0-4°C) bath for a minimum of two hours (to a maximum of overnight). After the cold bath 0.5 ml of a charcoal solution (Appendix J) was added and left for ten minutes. The tubes were centrifuged, as with T_3, and the supernatant poured off. The charcoal was counted as with the T_3 Procedure. For T_4 five standards were used, 0, 6.25, 12.5, 25 and 50 ng per ml. The same two in-house controls were used.

For both T_3 and T_4 assays a nonspecific binding blank was counted then subtracted from the total counts/tube measured on all other samples. The blank, which contained the buffer tracer (T_3 or T_4) with added charcoal, determined how much of the hot T_3 or T_4 was brought down into the charcoal.

Assays proceed as follows, standards ran first, then controls, unknown samples and controls were then repeated.

T_4-Binding Index

To each tube 600 ml buffer tracer (see Appendix K) with enough hot T_4 to produce 10,000 counts/tube/minute rather than 50,000 counts, and 10 ml of samples was added. Each tube was then vortex and incubated in a

37°C water bath for 20 minutes, then placed in a cold (0-4°C) bath for overnight (14 to 20 hours). After the cold bath 0.5 ml of T_4 charcoal solution (Appendix J) was added and immediately centrifuged at 4000 rpm for ten minutes. The supernatant was poured off and the charcoal counted for one minute. One in-house canine control was used, which ranged from five to 11 percent and five standard tubes that consisted of equine values 1, 10, 26, 50 and 100 percent.

$\underline{rT_3}$

This test determines the amount of 3, 3', 5'-triiodothyronine in the serum. The test is based on the RIA explained before and uses a limited, fixed amount of antibody (anti - rT_3) which binds to the radio-labeled antigen molecule (tracer) added in a constant amount. The reaction is in competitive inhibition by the unlabeled antigen (standard or sample) to be assayed. The postulated reaction is:

$$\underset{\text{unlabeled}}{2\ Ag} + \underset{\text{labeled}}{2\ Ag^*} + Ab \rightleftharpoons \underset{\text{bound}}{Ab\begin{matrix}Ag^* \\ Ag\end{matrix}} + \underset{\text{free}}{\begin{matrix}Ag^* \\ Ag\end{matrix}}$$

After the reaction has reached equilibrium the antibody-antigen complex is insolubilized and precipitated by adding a constant amount of polyethylene glycol (PEG)

solution. This precipitate (bound fraction) was counted after pouring off the supernatant. The general procedure is shown in Table Il. Tubes were then vortex and incubated for three hours at 15^{o}-22^{o}C (room temperature). To each tube 1.0 ml PEG was added, then vortex and centrifuged at 2^{o}-4^{o}C for 15 minutes at 3000 rpm. The supernatant was immediately decanted and the radioactivity of each tube was counted.

Table II. Order of addition and reagents used in the determination of rT_3.

The following reagents were added to the groups in the order listed:				
Reagent[2]	NSB[b]	Zero standard maximum binding	Standard	Serum Sample
Barbital buffer	0.2[c]	0.1	---	0.1
Thyronine free serum	0.1	0.1	0.1	---
rT_3 standard	---	---	0.1	---
Sample	---	---	---	---
rT_3 125I	0.1	0.1	0.1	0.1
Anti-rT_3	---	0.1	0.1	0.1

[a]All reagents from Serono Laboratories, Inc., Braintree, MA 02184

[b]Non-specific binding tubes

[c]All quantities are ml

APPENDIX J

Buffer Tracer for T_3 Determination

To one liter of B-2 Barbital buffer[1], at a pH of 8.6 with an ionic strength of 0.075, five grams of bovine gamma globulin is added.

Into 100 ml of this buffer 75 mg of ANS[2] is added which breaks down the globulin - T_3 complex to free T_3 and globulin. To this mixture is added enough radioactive counts of hot T_3 to equal 10,000 counts per tube, ^{125}I is used as the marker.

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[1]Beckman Instruments, Inc., Fullerton, CA 92634

[2]8-anilino-1-naphthalene sulfonic acid. Sigma Chemical Co., P.O. Box 14508, St. Louis, MO 63178.

APPENDIX K

Charcoal Solution

		%
T_4 buffer or	Barbital buffer (for T_3)	97.25
	Dextran	0.25
	Activated charcoal	2.50
		100.00

APPENDIX L

Buffer Tracer for T_4 Determination

Phosphate buffer:

23.34 g dibasic sodium phosphate ($Na_2HPO_4.7H_2O$)

1.81 g monobasic sodium phosphate

8.60 g NaCl

0.20 g Na azide

Add to these chemicals, distilled water in a quantity sufficient to make one liter. Set pH to 7.6.

Take 100 ml of phosphate buffer, add 100 mg ANS (listed in Appendix H), and enough hot T_4 to equal 50,000 counts per tube, ^{125}I is used as the marker.

APPENDIX M

Antibody for T_4 Determination

To each bottle of anitbody is added 30 ml of T_4 protein buffer.

T_4 protein buffer

23.34 g diabasic sodium phosphate ($Na_2HPO_4.7H_2O$)
1.81 g monobasic sodium phosphate
8.60 g NaCl
2.50 g gelatin
3.80 g EDTA
0.20 g Na azide

Add to these chemicals distilled water in a quantity sufficent to make one liter. Stir gently over low heat until solubilized, set pH to 7.6.

BIBLIOGRAPHY

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Albright, E. C., R. W. Heninger and F. C. Larson. 1965. Effect of cold-induced hyperthyroidism on iodine-containing compounds of the extra-thyroidal tissues. In Current Topics in Thyroid Research. Cassano, C. and M. Andreoli editors. Academic Press, Inc., NY.

Alexander, N. M. 1959. Iodine peroxidase in rat thyroid and salivary glands and its inhibition by antithyroid compounds. J. Biol. Chem., 234:1530-1533.

Alexander, N. M. 1961. The mechanism of iodination reactions in thyroid glands. Endocrinology, 69:671-679.

Alexander, W. D., R. McG. Harden, M. T. Harrison and J. Shimmins. 1967. Some aspects of the absorption and concentration of iodide by the alimentary tract in man. Proc. Nutr. Soc., 26:62-67.

Allcroft, R., J. Scarnell and S. L. Hignett. 1954. A preliminary report on hypothyroidism in cattle and its possible relationship with reproductive disorders. Vet Rec., 66:367-371.

Ammerman, C. B., L. R. Arrington, A. C. Warnick, J. L. Edwards, R. L. Shirley and G. K. Davis. 1964. Erproduction and lactation in rats fed excessive iodine. J. Nutr., 84:107-112.

Andrews, F. N., W. M. Beeson, E. R. Barrick and C. Harper. 1947. The influence of thiouracil and thiourea on the growth and fattening of lambs. J. Anim. Sci., 6:3-11.

Arrington, L. R., R. N. Taylor, Jr., C. B. Ammerman and R. L. Shirley. 1965. Effects of excess dietary iodine upon rabbits, hamster, rats and swine. J. Nutr.,87:394-398.

Arrington, L. R., R. A. Santa Cruz, R. H. Harms and H. R. Wilson. 1967. Effects of excess dietary iodine upon pullets and laying hens. J. Nutr., 92:325-330.

Ashbacher, P. W. 1968. Thyroid physiology in lambs as affected by iodine supplementation of the pregnant ewe's diet. J. Anim. Sci., 27:127-130.

Asher, S. J., R. J. Aulerich, R. K. Ringer and H. Kitchen. 1976. Seasonal and age variations which occur in the blood parameters of ranch mink. Fur Rancher, 4(4):4,6&9.

Asling, C. W. and E. S. Evans. 1963. Maintenance of skeletal growth and maturation in thyroidectomized rats by injection of iodide. Endocrinology, 72:283-291.

Asmundson, V. S., H. J. Almquist and A. A. Klose. 1936. Effect of different froms of iodine on laying hens. J. Nutr., 12:1-14.

Aulerich, R. J. and R. K. Ringer. 1970. Some effects of chlorinated hydrocarbon pesticides on mink. Amer. Fur Breeder, 43(6):10-11.

Aulerich, R. J. and R. K. Ringer. 1979. Toxic effects of dietary polybrominated biphenyls on mink. Arch. Environ. Contam. Toxicol., 8:487-498.

Aulerich, R. J. and P. J. Schaible. 1966. The influence of thyroproterin on growth and furring in mink. Quart. Bull. Mich. Agr. Expt. Stat., 49:211-215.

Aulerich, R. J., R. K. Ringer, H. L. Seagran and W. G. Youatt. 1971. Effects of feeding coho salmon and other Great Lakes fish on mink reproduction. Can. J. Zool., 49:611-616.

Aulerich, R. J., R. K. Ringer and S. Iwamoto. 1973. Reproductive failure and mortality in mink fed on Great Lakes fish. J. Reprod. Fert., Suppl., 19:365-376.

Aulerich, R. J., R. K. Ringer and S. Iwamoto. 1974. Effects of idetary mercury on mink. Arch. Environ. Contam. Toxicol., 2:43-52.

Aulerich, R. J., R. K. Ringer and G. R. Hartsough. 1978. Effect of iodine on reproductive performance of female mink. Theriogenology, 9:295-302.

Baker, H. J. and J. R. Lindsey. 1968. Equine goiter due to excess dietary iodine. JAVMA, 153:1618-1630.

Barker, S. B. and H. M. Klitgaard. 1952. Metabolism of tissues excised from thyroxine-injected rats. Amer. J. Physiol., 170:81-86.

Barua, J., R. G. Cragle and J. K. Miller. 1964. Sites of gastrointesinal-blood passage of iodide and thyroxine in young cattle. J. Dairy Sci., 47:539-541.

Belzile, R. J., L. S. Poliquin and J. D. Jones. 1974. Nutritive value of rapeseed flour for mink: effects on live performance, nutrient utilization, thyroid function and pelt quality. Can. J. Anim. Sci., 54; 639-644.

Berestov, V. 1971. Biokhimisa I norfologiya krovi pushnylh zverei. (Biochemistry and blood morphology of fur bearing animals). Translated from Russian, prepared for ARS, U. S. Dept. Agr. and Nat. Sci. Found., Wash. D. C. (TT-74-53001) By Mrs. Geti Saad. From 17. Kareliya pp. 291.

Bernal, J. and S. refetoff. 1977. The action of thyroid hormone. Clin. Endocrinol., 6:227-249.

Berthier, C. and T. Lamarchand-Béraud. 1978. Importance of thyroid iodine and cyclic AMP, and TSH concentrations on goiter formation in rats. Acta Endocrinol., 89:567-579.

Bjorkman, U., R. Elholm, L.-G. Elmqvist, L. E. Ericson, A. Melander and S. Smeds. 1974. Induced unidirectional transport of protein ioto the thyroid follicular lumen. Endocrinology, 95:1506-1517.

Black, D. J. G., R. C. Jennings, T. R. Mossis and J. A. Palgrave. 1954. A depressant effect of a white fish meal on hatchability. Proc. 10th World's Poultry Congr., 121-125.

Black, D. J. G., T. R. Morris and J. A. Palgrave. 1957. The value of grass sward for breeding hens. Poultry Sci., 36:780-788.

Blaxter, K. L. 1948. Severe experimental hyperthyroidism in the ruminant. II. Physiological effects. J. Agr. Sci., 38:20-27.

Boeynaems, J. M., N. Galand and J. E. Dumont. 1979. Inhibition by iodide of the cholinergic stimulation of prostaglandin synthesis in dog thyroid. Endocrinology, 105:996-1000.

Bray, G. A. and H. M. Goodman. 1968. Metabolism of adipose tissue from normal and hypothyroid rats. Endocrinology, 82:860-864.

Brown-Grant, K. 1961. Extrathyroidal iodine concentrating mechanisms. Physiol Rev., 41:189-213.

Bruce, H. M. and H. A. Sloviter. 1957. Effects of destruction of thyroid tissue by radioactive iodine on reproduction in mice. J. Endocrinol., 15:72-82.

Burke, G. 1968. Effects of inorganic iodide on thyroid stimulation in vitro. Endocrinology, 82:1170-1178.

Byrne, J. J. 1974. Influences of polychlorinated biphenyl administration on reproduction and thyroid function in mink (Mustela vison). Ph. D. dissertation, Physiol. Dept., MSU.

Byrne, J. J., E. P. Reineke, R. K. Ringer and R. Aulerich. 1975. Influences of polyshlorinated biphenyl mixture (Aroclor 1254) administration of reproduction and thyroid function in mink (Mustela vison). Fed. Proc., 34:1379 (Abst.).

Capan, C. C. 1978. In: Tumors in Domestic Animals. Ed. J. E. Moulton. Univ. Calif. Press. CA.

Cheville, N. F. 1976. Cell Pathology. The Iowa State Univ. Press. Ames, IA.

Chopra, I. J., D. A. Fisher, D. H. Solomon and G. N. Beall. 1973. Thyroxine and triiodothyronine in human thyroid. J. Clin. Endocrinol., 36:311-320.

Chopra, I. J. 1974. A radioimmunoassay for measurement of 3,3',5'-triiodothyronine (reverse T_3). J. Clin. Invest. 54:583-592.

Chopra, I. J., U. Chopra, S. R. Smith, M. Reza and D. H. Solomon. 1975. Reciprocal changes in serum concentration of 3,3',5'-triiodothyronine (reverse T_3) and 3,3',5-triiodothyronine (T_3) in systemic illnesses. J. Clin. Endo. Met., 41:1043-1049.

Church, D. C. and W. G. Pond. 1974. Basic Animal Nutrition and Feeding. D. C. Curch Pub., Dist, by O & B Books, Corvallis, OR.

Cole, L. J. and F. B. Hutt. 1927. Further experiments in feeding thyroid to fowls. Poultry Sci., 7:60-66.

Convey, E. M., L. Chapin, J. S. Kesner, D. Hillman and A. R. Curtis. 1977. Serum thyrotropin and thyroxine after thyrotropin releasing hormone in dairy cows fed varying amounts of iodine. J. Dairy Sci., 60:975-980.

Convey, E. M., L. T. Chapin, J. W. Thomas, K. Leung and E. W. Swanson. 1978. Serum thyrotropin, thyroxine, and tri-iodothyronine in dairy cows fed varying amounts of iodine. J. Dairy Sci., 61:771-775.

Correa, P. and R. A. Welsh. 1960. The effect of excessive iodine intake on the thyroid gland of the rat. Arch. Path., 70:129/247-133/251.

DeGroot, L. J. and A. M. Davis. 1962a. Studies on the biosynthesis of iodotyrosines; a soluble thyroidal iodide-peroxidase tyrosineiodinase system. Endocrinology, 70: 492-504.

DeGroot, L. J. and A. M. Davis. 1962b. Studies on the biosynthesis of iodotyrosines: the relationship of peroxidase, catalase and cytochrome oxidase. Endocrinology, 70:505-510.

Drew, B., W. P. Barber and D. G. Williams. 1975. The effect of excess dietary iodine on pregnant mares and foals. Vet. Rec., 97:93-95.

Driscoll, J. 1978. Goiter in foals caused by excessive iodine. JAVMA, 173:858-859.

Duby, R. T. 1969. Physiological basis of reproduction in mink. Amer. Fur Breeder, 42(2):16-17.

Dyck, R. F., R. A. Bear, M. B. Goldstein and M. L. Halperin. 1979. Iodine/iodide toxic reaction: case report with emphasis on the nature of the metabolic acidosis. CMA J., 120:704-706.

Edelman, I. S. and F. Ismail-Beige. 1974. Thyroid thermogensis and active sodium transport. Rec. Prog. Horm. Res., 30:235-257.

Enders, R. K. 1952. Reproduction in the mink (Mustela vison). Proc. Amer. Phil. Soc., 96:691-755.

Enders, R. K. and A. C. Enders. 1963. Morphology of the female reproductive tract during delayed implantation in the mink. In: Delayed Implantation. A. C. Enders, Ed. : Univ. of Chicago Press, Chicago, IL.

Erickson, J. P. 1977. Iodine nutrition in sow reproduction and lactation. Research report 343. Farm Sci. Report of Swine Research. MSU Agr. Expt. Stat.

Erickson, J. P. 1978. A nutritional evaluation of iodine in the diet of sows (Sus domesticus). Ph. D. Dissertation. Dept. Anim. Husb., MSU.

Etling, N., F. Gehin-Fouque, J. P. Vielh and J. P. Gautray. 1979. The iodine content of amniotic fluid and placental transfer of iodinated drugs. Obstetrics and Gyn., 53:376-380.

Etta, K. M. 1971. Comparative studies of the relationship between serum thyroxine and thyroxine-binding globulin. Ph. D. dissertation. Dept. Physiol., MSU.

Feed Additive Compendium. 1975. Ethylenediamine Dihydriodide. Product information. 198-201.

Flamboe, E. E. and E. P. Reineke. 1959. Estimation of thyroid secretion rates in dairy goats and measurement of I^{131} uptake and release with redard to age, pregnancy, lactation, and season of the year. J. Anim. Sci., 18:1135-1148.

Fletch, S. M. and L. H. Karstad. 1972. Blood parameters of healthy mink. Can. J. Comp. Med., 36:275-281.

Food and Nutrition Board; 1970. National Academy of Science. Iodine Nutrition in the United States. Summary of a conference Oct. 31, 1970; Wash., D. C.

Frieden, E. and H. Lipner. 1971. Biochemical Endocrinology of the Vertebrates. Prentice-Hall Inc., Englewood Cliffs, NJ.

Fukuda, H., N. Yasuda and M. A. Greer. 1975a. Acute effects of thyroxine, triiodothyronine, and iodide on thyrotropin secretion. Encocrinology, 97:924-931.

Fukuda, H., N. Yasuda and M. A. Greer. 1975b. Changes in plasma thyrotrophin, thyroxine, and triiodothyronine after acute or chronic administration of iodide to iodine-deficient rats. Endocrinology, 97:1196-1204.

Galton, V. A. and R. Pitt-Rivers. 1959a. The effect of excessive iodine on the thyroid of the rat. Endocrinology, 64:835-839.

Galton, V. A. and R. Pitt-Rivers. 1959b. Thyroid-hormone metabolism in the kidney. Biochem. J., 72:314-318.

Goldfine, I. D., C. G. Simons and S. H. Ingbar. 1975a. Stimulation of the uptake of α-aminoisobutyric acid in rat thymocytes by L-triiodothyronine: a comparison with insulin and dibutryl cyclic AMP. Endocrinology, 96:802-805.

Goldfine, I. D., C. G. Simons, G. J. Smith and S. H. Ingbar. 1975b. Cycloleucine transport in isolated rat thymocytes: in vitro effects of triiodothyronine and thyroxine. Endocrinology, 96:1030-1037.

Goodman, H. M. and L. vanMiddlesworth. 1974. The thyroid. In; Medical Physiology. Vol 2. V. P. Montcastle ed., The C. V. Mosby Co., St. Louis, MO.

Greif, R. L. 1962. Thyroxine and the release of protein from rat liver mitochondria. Endocrinology, 70:511-516.

Gross, J. 1962. Mineral Metabolism. Vol. 2, Part b. Academic Press, NY.

Gross, J. and R. Pitt-Rivers. 1953. Identification of 3,5,3'-triiodothyronine. I. Isolation from thyroid gland and synthesis. Biochem. J., 53:654-651.

Gross, N. J. 1971. Control of mitochondrial turnover under the influence of thyroid hormone. J. Cell Biol., 48: 29-40.

Gustafsson, R., J. R. Tata, O. Lindberg and L. Ernster. 1965. The relationship between the structure and activity of rat skeletal muscle mitochondria after thyroidectomy and thyroid hormone treatment. J. Cell Biol., 26:555-578.

Haggard, D. L. 1978. Immunologic effects of experimental iodine toxicity in cattle. M.S. Thesis, Dept. Large Anim. Surg. & Med. MSU.

Haggard, D. L., H. D. Stow, G. H. Conner and C. K. Whitehair. 1978. Immunologic effects of experimental iodine toxicity in cattle. Abstracts 70th annual meeting, E. Lansing, MI. Amer. Soc. of Animl. Sci.;419.

Hansson, A. 1947. The physiology of reproduction in mink (Mustela vison, Screb.) with special reference to delayed implantation. Acta Zool., Stockh., 28:1-136.

Hartsough, G. R. 1965. Great Lakes fish now suspect as mink food. Amer. Fur Breeder, 38(10):25&27.

Hashizume, K., F. Akasu, K. Takazawa, W. Endo and T. Onaya. 1976. The inhibitory effect of acute administration of excess iodide on the formation of adenosine 3',5'-monophosphate induced by thyrotropin in mouse thyroid lobes. Endocrinology, 99:1463-1468.

Hemken, R. W. 1979. Factors that influence the iodine content of milk and meat: A review. J. Anim. Sci., 48: 981-985.

Hemken, R. W., J. H. Vandersall, M. A. Oskarsson and L. R. Fryman. 1972. Iodine intake related to milk iodine and performance of dairy cattle. J. Dairy Sci., 55:931-934.

Hennemann, G. 1979. Pathophysiology of peripheral thyroxine conversion. J. Endocrinol., 80(2):19P-20P.

Herd, P., S. S. Kaploy and D. R. Sanadi. 1974. On the origin and mechanism of action of a thyroxine-responsive protein. Endocrinology, 94:464-474.

Highman, B., S. H. Webster and M. E. Rice. 1955. Degeneration of retina and gastric parietal cells and other pathologic changes following administration of iodates. Fed. Proc., 14:407 (Abst.).

Hinkle, P. M., M. H. Perrone and T. L. Green. 1979. Thyroid hormone action in pitritary cells. J. Biol. Chem., 254:3907-3911.

Hoar, R. M., R. W. Goy and W. C. Young. 1957. Loci of action of thyroid hormone on reproduction in the female guinea pig. Endocrinology, 60:337-346.

Hoch, F. L. 1962. Biochemical actions of thyroid hormones. Physiol. Rev., 42:605-673.

Howell, R. E. 1979. Causes of early kit losses in finely-bred dark mink studied. Fur Rancher, 59(4):4,8&13.

Hunn, J. B. and E.P. Reineke. 1964. Influence of iodine intake on iodine distribution in trout. Proc. Soc. Expt. Biol. and Med., 115:91-93.

Ikeda, H. and S. Nagataki. 1976. Augmentation of thyrotropin responses to thyrotropin-releasing hormone following inorganic iodide. Endocrinol. Jap., 23:431-433.

Ingar. S. H. and L. E. Braverman. 1975. Active form of the thyroid hormone. Anua. Rev. of Med., 26:443-449.

Jarosz, S. and J. Berteczko. 1976. Influence of diets containing fish, meat by-products and various quality fat on the growth rate, fur quality and fertility in mink. Scientifur, Nov: 25-28, (Introl issue).

Katz, R., C. N. Tai, R. McConnell, R. Diener and D. Semonick. 1980. Dichloroacetate, sodium (DCA): 90-day oral toxicity in rats and dogs. Abstracts of papers, Soc. of Toxicol. 19th ann. meeting, Wash., D.C. March 9-13, 1980.

Kekki, M. 1964. Serum protein turnover in experimental hypo- and hyper-thyroidism. Acta Encocrinol., 91:1-139, (Suppl.).

Kindall, E. C. 1915. The isolation in crystalline form of the compound containing iodine which occurs in the thyroid; its chemical nature and physiological activity. Trans. Assoc. Amer. Physicians, 30:421-449.

Kennedy, A. H. 1935. Cytology of the blood of normal mink and raccoon. II. The numbers of the blood elements in normal mink. Can. J. Res., 12:484-494.

Kennedy, A. H. 1951. The mink in health and disease. Pub. by Fur Trade J. of Can., Toronto, Canada.

Kiiskinen, T. and J. Mäkelä. 1977. The results of mineral supplementation in the feeding of mink. Scientifur, 1(4):41-41.

Kubin, R. and M. M. Mason. 1948. Normal blood and urine values for mink. Cornell Vet., 38:79-85.

Lea, C. H., L. J. Parr and K. J. Carpenter. 1960. Chemical and nutritional changes in stored herring meal. 2. Brit. J. Nutr., 14:91-113.

Lengemann, F. W. 1963. ^{131}I concentrations in blood, milk, urine and faeces of dairy cows following a single dose of radio-iodine. J. Agr. Sci., 61:375-379.

Lengemann, F. W. 1965. Factors affecting iodine concentration in bovine milk. J. Dairy Sci., 48:197-202.

Levey, G. S., L. Skelton and S. E. Epstein. 1969. Decreased myocardial adenyl cyclase activety in hypothyroidism. J. Clin. Invest., 48: 2244-2250.

Lewis, M., R. Capiferri, J. R. Bourke, W. Ratcliff and D. C. Evered. 1979. Role of reverse tri-iodothyronine in feedback control of thyrotrophin secretion. J. Endocrinol., 80(2):20P-21P.

Liwendahl, K., E. Niskanen and A. Gordin. 1972. Thyroid function in rats administered excess iodide. Acta Endocrinol., 69:321-328.

Lo, C-S. and I. S. Edelman. 1976. Effect of triiodothyronine on the synthesis and degradation of renal cortical ($Na^+ + K^+$)-adenosine triphosphatase. J. Biol. Chem., 251:7834-7840.

Lo, C-S, T. R. August, U. A. Liberman and I. S. Edelman. 1976. Dependence of renal (Na^+ and K^+)-adenosine triphosphatase activity on thyroid status. J. Biol. Chem. 251:7826-7833.

Main, J. R. 1977. Iodine toxicosis in holstein heifer calves: Clinical and laboratory data. M.S. Thesis, Dept. Large Anim. Surg. & Med., MSU.

Magsood, M. 1951. Thyroxine therapy in male subfertility. Nature, 168:466-467.

March, B. E., J. Biely and H. L. A. Tarr. 1963. Nutrient composition and evaluation of British Columbia whole herring meal. J. Fish Res. Bd. Can., 20:229-238.

Marcilese, N. A., R. H. Harms, R. M. Valsecchi and L. R. Arrington. 1968. Iodine uptake by ova of hens given excess iodine and effect upon ova development. J. Nutr., 94:117-120.

McCauley, E. H., D. W. Johnson and I. Alhadji. 1972. Disease problems in cattle associated with rations containing high levels of iodide. Bov. Pract., 7:22-27.

McCauley, E. H,, J. G. Linn and R. D. Goodrich. 1973. Experimentally induced iodide toxicosis in lambs. Amer. J. Vet. Res., 134:65-70.

McDonald, R. J., G. W. McKay and J. D. Thompson, 1961. The use of organic iodine in the treatment of repeat breeder cows. Proc. IV th Int. Cong. Anim. Reprod., 3:679-681.

Miettinen, T. A. 1968. Mechanism of serum cholesterol reduction by thyroid hormones in hypothyroidism. J. Lab. Clin. Med., 71:537-547.

Miller, J. K. and E. W. Swanson. 1973. Metabolism of ethylenediaminedihydriodide and sodium or potassium iodide by dairy cows. J. Dairy Sci., 56:378-384.

Miyazawa, K., S. Motoyoshi and K. Usui. 1978. Nodular goiters of three mares and their foals, induced by feeding excessive amount of seaweed. Jap. J. Vet. Sci., 40:749-753.

Moberg, R. 1961. Possible influence of supplementary iodine administered by evaporation, on reproductive performance in cattle. Proc. IVth Int. Congr. Anim. Repro., 3: 682-685.

Moccia, R. D., J. F. Leatherland and R. A. Sonstegard. 1977. Increasing frequency of thyroid goiters in coho salmon (Oncorhynchus kisutch) in the Great Lakes. Science, 198:425-426.

MΦller, O. M. 1973. The progesterone concentrations in the peripheral plasma of the mink (Mustela vison) during pregnancy. J. Endocrinol., 56:121-132.

Nagataki, S., H. Uchimura, Y. Masuyama, K. Nako and K. Ito. 1972. Triiodothyronine and thyroxine in thyroid glands of euthyroid Japanese subject. J. Clin. Endocrinol. Metab., 35:18-23.

Narasimhalu, P., R. J. Belzile and M. Lepage. 1978. Effects of feeding raw meat or soybean meal on blood composition in mink (Mustela vison). Can. J. Anim. Sci., 58:191-197.

Newton, G. L. and A. J. Clawson. 1974. Iodine toxicity: physiological effects of elevated dietary iodine on pigs. J. Anim. Sci., 39:879-884.

Newton, G. L., E. R. Barrick, R. W. Harvey and M. B. Wise. 1974. Iodine toxicity: physiological effects of elevated dietary iodine on calves. J. Anim. Sci., 38: 449-455.

Niclolff, J. T., J. C. Low, J. H. Dussault and D. A. Fisher. 1972. Simultaneous measurement of thyroxine and triiodothyronine peripheral turnover kinetics in man. J. Clin. Invest., 51:473-480.

Ohashi, T., Y. Yukimura, R. Kibayashi, A. Sato, M. Ohtake, M. Kotani, T. Onaya and T. Yamada. 1973. An increase of thyroid hormone secretion in response to excess iodide in rats treated with thyroxine. Endocrinology, 93:880-886.

O'Hea, E. K. and G. A. Leveille, 1969. Lipogenesis and enzymatic activity of pig adipose tissue as influenced by dietary protein and fat. Fed. Proc., 28:687 (Abst.).

Ohtake, M., A. Sato, T. Onaya and T. Yamada. 1973. Effects of repeated administration of excess iodide on thyroid hormone secretion in rats, with special reference to the escape from the iodide effect. Endocrinology, 93: 343-347.

Onaya, T. and N. S. Halmi. 1967. Mechanism of the inhibitory effect of iodide on thyroidal ^{131}I release in the rat. Endocrinology, 81:643-648.

Onaya, T., T. Tomizawa, T. Yamada and K. Shichijo. 1966. Further studies on inhibitory effect of excess iodide on thyroidal hormone release in the rat. Endocrinology, 79:138-148.

Ousterhout, L. E., C. R. Grau and B. D. Lundholm. 1959. Biological availability of amino acids in fish meals and other protein sources. J. Nutr., 69:65-73.

Parrott, M. W., M. E. Johnston and P. W. Durbin. 1960. The effects of thyroid and parathyroid deficiency on repooduction in the rat. Endocrinology, 67:467-483.

Peacock, L. B. and H. M. Davison. 1957. Observations on iodide sensitivity. Annals of Allergy, 15:158-164.

Perdomo, J. T., R. H. Harms and L. R. Arrington. 1966. Effects of dietary iodine upon egg production, fertility and hatchability. Proc. Soc. Exp. Biol. Med., 122:758-760.

Pilbeam, T. E., P. W. Concannon and H. F. Travis. 1979. The annual reproductive cycle of mink (Mustela vison). J. Anim. Sci., 48:578-583.

Pisarev, M. A. and M. E. Itoiz. 1972. Action of KI on stimulated thyroid protein biosynthesis. Endocrinology, 90:1409-1412.

Pitt-Rivers, R. and W. R. Trotter. 1964. The thyroid gland. Vol. 1 and 2. Butterworth and Co., Ltd. London, England.

Potter, G. D., W. Tong and I. L. Chaikoff. 1959. The metabolism of I^{131} labeled iodine, thyroxine, and triiodothyronine in the mammary gland of the lactating rat. J. Biol. Chem., 234:350-354.

Power, H. E., K. A. Savagaon, B. E. March and J. Biely. 1969. Assessment of the nutrient content of Atlantic coast herring fish meals. Feedstuffs, Nov. 22, p.48.

Prosser, C. L. 1973. Comparative Animal Physiology. Prosser, C. L., ed., 3rd ed., W. B. Saunders Co., Philadelphia, PA.

Rall, J. E., R. Michel, J. Roche, O. Michel and S. Varrone. 1963. Action and metabolism of thyroid hormones and iodine-donating substances. I. Liver mitochondria. J. Biol. Chem., 238:1848-1854.

Rapoport, B., R. J. Adams and M. Rose. 1977. Cultured thyroid cell adenosine 3',5'-cyclic monophosphate response to thyrotropin: Loss and restoration of sensitivity to iodide inhibition. Endocrinology, 100:755-764.

Reineke, E. P. 1961. Factors affecting the secretion of iodine^{131} into milk of lactating goats. J. Dairy Sci., 44:937-942.

Reineke, E. P., H. F. Travis and P. E. Kifer. 1960. Thyroidal iodine^{131} turnover, thyroxine secretion rate, and thyroactive iodinated casein utilization in mink (Mustela vison). Amer. J. Vet. Res., 21:862-865.

Ringer, R. K., R. J. Aulerich and M. Zabik. 1972. Effect of dietary polychlorinated biphenyls on growth and reproduction of mink. 164th Nat'l Meeting A. M. Chem. Soc., 12(2):149-154.

Ringer, R. K., R. J. Aulerich, R. Pittman and E. A. Cogger. 1974. Cardiac output, blood pressure, blood volume, and other cardiovascular parameters in mink. J. Anim. Sci., 38:121-123.

Roberts, W. K. and R. J. Kirk. 1964. Digestibility and nitrogen utilization of raw fish and dry meals by mink. Amer. J. Vet. Res., 25:1746-1750.

Rosenberg, I. N. and C. H. Bastomsky. 1965. The Thyroid. Ann. Rev. Physiol., 27:71-106.

Rosenberg, L. L., M. K. Dimick and G. LaRoche. 1963a. Thyroid function in chickens and rats. Effect of iodine content of the diet and hypophysectomy on iodine metabolism in white leghorn cockerels and Long-Evans rats. Endocrinology, 72:749-758.

Rosenberg, L. L., G. LaRoche and M. K. Dimick, 1963b. Thyroid function in chichens and rats. Equilibration of injected iodide with existing thyroidal iodine in white leghorn cockerels. Endocrinology, 72:759-763.

Rotenberg, S. and G. Jørgensen. 1971. Some haematological indices in mink. Nort. Vet-Med., 23:361-366.

Rudolph, M., T. Sakurada, A. Vagenakis, S. Fang, L. Braverman and S. Ingbar. 1976. Demonstration of sequential mono-deiodination as the major pathway of iodothyronine metabolism. Clin. Res., 24:429A.

Saad, G. (translator). 1975. Feeding fur bearing animals (translated from Russian). TT 74-53383. 1972. N. Sh. Pyeryeldik, L. D. Milovanov and A. T. Erin. Ag. Res. Survey, U. S. Dept. of Ag. and Nat. Sci. Found.

Saberi, M. and R. D. Utiger. 1975. Augmentation of thyro-tropin responses to thyrotropin-releasing hormone following small decreases in serum thyroid hormone concentration. J. Clin. Endoc. Metab., 40:435-441.

Salter, W. T. 1940. The Endocrine Function of Iodine. Harvard University Press. Cambridge, MA.

Sawin, C. T., J. M. Hershman and M. Azukizawa. 1979. Relationship of iodide-induced increase in TSH re-sponse to TRH to changes in serum thyroid hormones. Hormone Res., 10:20-24.

Schadlow, A. R., M. I. Surks, H. L. Schwartz and J. H. Oppen-heimer. 1972. Specific triiodothyronine binding sites in the anterior pituitary of the rat. Science, 176: 1252-1254.

Schwartz, P., K. B. Wolfe and J. T. Beuthas. 1971. Spontaneous amyloidosis in mink. J. Comp. Path., 81:437-445.

Scott, M. L., M. C. Nesheim and R. J Young. 1969. Nutrition of the Chicken. M. L. Scott and Associates, Ithaca, NY.

Sherwin, J. R. and W. Tong. 1974. The actions of iodide and TSH on thyroid cells showing a dual control system for the iodide pump. Endocrinology, 94:1465-1474.

Sherwin, J. R. and W. Tong. 1975. Thyroidal autoregulation. Iodide-induced suppression of thyrotropin-stimulated cyclic AMP production and iodinating activity in thyroid cells. Biochim. et Biophys. Acta, 404:30-39.

Simon, C. 1963. The iodide pool of the thyroid studied by means of the isotopic equilibrium method and double labelling with ^{125}I and ^{131}I. Bioshim. et Biophys. Acta, 74:565-567.

Simpson, B. W. 1947. Iodine metabolism in relation to dietary calcium. Biochem. J., 41:xlv-xlvi.

Sinadinović, J., M. Krainčanić, G. Kostić and M. Jovanović. 1978. Retarded biosynthesis of thyroglobulin subunits and their polymerization in vitro after suppression of TSH secretion in rats by chronic administration of excess thyroxine in athe form of iodinated casein. Acta Encocrinol., 89:108-121.

Skrede, A. 1970a. Dietary blood in the prevention of fish-induced anaemia in mink. Acta Agr. Scand., 20:265-274.

Skrede, A. 1970b. Normal variations in the haemoglobin concentration in mink blood. Acta Agr. Scand., 20: 258-264.

Sleight, S. D., S. Mangkeewidjojo, B. T. Akoso and V. L. Sanger. 1978. Polybrominated biphenyl toxicosis in rats fed an iodine-deficient, iodine-adequate, or iodine-excess diet. Environ. Health Pers., 23:341-346.

Sodeman, W. A. and W. A. Sodeman. 1974. Pathologic Physiology: Mechanisms of Disease. 5th ed., W. B. Saunders Co., Philadelphia, PA.

Sonstegard, R. A. and J. F. Leatherland. 1979. Hypothyroidism in rats fed Great Lakes Coho Salmon. Bull. Environm. Contam. Toxicol., 22:779-784.

Spector, W. S., editor. 1956. Handbook of Biological Data, W. B. Saunders Co., Philadelphia, PA.

Stanbury, J. B. and V. Ramalingaswami. 1964. Nutrition. Vol. I. Macronutrients and nutrient elements. edited by G. H. Beaton. Academic Press, NY.

Stein, O. and J. Gross. 1962. Effect of thyroid hormone on protein biosynthesis by cell-free systems of liver. Proc. Soc. Exptl. Biol. Med., 109:817-820.

Sterling, K. 1979a. Thyroid hormone action at the cell level. Part I. The New Eng. J. Med., 300(3):117-123.

Sterling, K. 1979b. Thyroid hormone action at the cell level. Part II. The New Eng. J. Med., 300(4):173-177.

Sterling, K. and R. B. Chodos. 1956. Radiothyroxine turnover studies in myxedema thyrotoxicosis and hypermetabolism without endocrine disease. J. Clin. Invest., 35:806-813.

Sterling, K. and J. H. Lazarus. 1977. The thyroid and its control. Ann. Rev. Physiol., 39:349-371.

Stout, F. M. and J. Adair. 1970. Hepatotoxicosis in mink fed toxic herring meal. Anim. Nutr. & Health, Feb.;8-11.

Stout, R. M., J. Adair and J. E. oldfield. 1968. Hepatotoxicosis in mink associated with feeding toxic herring meal. Amer. Fur Breeder, 11(6):13-15.

Sugahara, M., D. H. Baker, B. G. Harmon and A. H. Jensen. 1969. Effect of excess levels of dietary crude protein on carcass development in swine. J. Anim. Sci., 29: 598-601.

Sullivan, T. W., B. D. Barnett, H. R. Bird, N. L. Karrick and L. McKee. 1960. Effect of origin, processing and storage on the unidentified growth factor activity of a variety of fish meals. Poultry Sci., 39:1037-1041.

Surks, M. I., A. R. Schadlow, J. M. Stock and J. H. Oppenheimer. 1973. Determination of iodothyronine absorption and conversion of L-thyroxine (T_4) to L-triiodothyronine (T_3) using turnover techniques. J. Clin. Invest., 52:805-811.

Swanison, E. W. 1972. Effect of dietary iodine on thyroxine secretion rate of lactating cows. J. Dairy Sci., 55: 1763-1767.

Talbot, J. M., K. D. Fisher and C. J. Carr. 1974. A review of the significance of untoward reactions to iodine in foods. Fed. Amer. Soc. Expt. Bio., PB-237 716, Dist. by: NITS, US Dept. of Commerce.

Taranov, G. S. 1976. Breeding results after feeding with large quantities of fish meal. Scientifur, 1(2):18-22.

Tata, J. R. and C. C. Widnell. 1966. Ribonucleic acid synthesis during the early action of thyroid hormones. Biochem. J., 98:604-620.

Tata, J. R., L. Ernster, O. Lindberg, E. Arrhenius, S. Pedersen and R. Hedman. 1963. The action of thyroid hormones at the cell level. Biochem. J., 86:408-428.

Taurog, A. 1970. Thyroid peroxidase and thyroxine biosynthesis. Rec. Prog. Horm. Res., 26:189-247.

Taurog, A. and E. M. Howells. 1966. Enzymatic iodination of tyrosine and thyroglobulin with chloroperoxidase. J. Biol. Chem., 241:1329-1339.

Taylor, R. N., L. R. Arrington, C. B. Ammerman, R. L. Shirley and C. K. Davis. 1964. Some effects of excess dietary iodine upon animals. J. Anim. Sci., 23:297 (Abst.).

Travis, H. F., C. F. Bassett, R. G. Warner and E. P. Reineke. 1966. Some effects of feeding products high in nat urally occurring thyroactive compounds upon reproduction of mink (Mustela vison). Amer. J. Vet. Res., 27:815-817.

Travis, H. F. and T. E. Pilbeam. 1978. A comparison of the physical and physiological characteristics of high and low-producing strains of dark mink. Prog. Report of Proj. Supported by the Mink Farmers Res. Found. Theinsville, WI. p. 1-11.

Travis, H. F., G. Sperling, C. F. Bassett and R. G. Warner. 1963. Gullet trimmings may cause severe reproduction losses. Amer. Fur Breeder, 36(2):11&38.

Turner, C. D. and J. T. Bagnara. 1971. General Endocrinology. W. B. Saunders Co. Philadelphia, PA.

Uchimura, H., S. M. Amir and S. H. Ingbar. 1979. Failure of organic iodine enrichment to influence the binding of Bovine thyrotropin to rat thyroid tissue. Endocrinology, 104:1207-1210.

Underwood, E. S. 1977. Trace Elements in Human and Animal Nutrition. 4th edition, Academic Press, Inc., NY.

Vagenakis, A. G., B. Rapoport, F. Azizi, G. I. Portnay, L. E. Braverman and S. H. Ingbar. 1974. Hyperresponse to thyrotropin-releasing hormone accompanying small decreases in serum thyroid hormone concentrations. J. Clin. Invest., 54:913-918.

Van Gelder, G. A. editor. 1976. Clinical and Diagnostic Veterinary Toxicology. 2nd edition. Kendall/hunt Publishing Co., Dubuque, IA.

Van Sande, J., G. Grenier, C. Willems and J. E. Dumont. 1975. Inhibition by iodide of the activation of the thyroid cyclic 3',5'-AMP system. Endocrinology, 96: 781-786.

Venge, O. 1959. Reproduction in the fox and mink. Anim. Breed. Abst., 27:129-145.

Wallace, C. E. 1975. Iodine toxicity in cattle. Georgia Vet., 27(6):25.

Webster, S. H., E. F. Stohlman and B. Highman. 1966. The toxicology of potassium and sodium iodates III. Acute and subacute oral toxicity of potassium iodate in dogs. Toxicol. and App. Pharmacol., 8:185-192.

Weiss, W. P. and L. Sokoloff. 1963. Reversal of thyroxine-induced hyper-metabolism by pruomycin. Science, 140: 1324-1326.

Werner, S. C. 1957. The Thyroid. 2nd edition. Hoeger-Haper Books, NY.

Wheeler, T. S., I. Hoffmann and C. L. Graham. 1948. The value of thyro-protein in starting, growing and laying rations. Poultry Sci., 27:103-111.

Widnell, C. C. and J. R. Tata. 1966. Additive effects of thyroid hormone, growth hormone and testosterone on deoxyribonucleic acid-dependent ribonucleic acid polymerase in rat-liver nuclei. Biochem. J., 98:621-629.

Wilson, B., E. Raghupathy, T. Tonoue and W. Tong. 1968. TSH-like actions of dibutyryl-cAMP on isolated bovine thyroid cells. Endocrinology, 83:877-884.

Wolff, J. 1964. Transport of iodide and other anions in the thyroid gland. Physiol. Rev., 44:45-90.

Wolff, J. 1969. Iodide goiter and the pharmacologic effects of excess iodide, Amer. J. Med., 47:101-124.

Wolff, J. and I. L. Chaiflff. 1948. Plasma inorganic iodide as a homeostatic regulator of thyroid function. J. Biol. Chem., 174:555-564.

Wood, A. J. 1962. Nutrient requirements of the mink. West. Fur Farmer, 1(April):10-11&29.

Wyngaarden, J. B., B. M. Wright and P. Ways. 1952. The effect of certain anions upon the accumulation and retention of iodide by the thyroid gland. Endocrinology, 50:537-549.

Yamada, T. and A. E. Lewis. 1968. Studies on the mechanism of the inhibitory action of excess iodide on the release of radioiodine from the rat thyroid gland. Endocrinology, 82:54-61.

Yamamoto, K., T. Onaya, T. Yamada and M. Kotani. 1972. Inhibitory effect of excess iodide on thyroid hormone release as measured by intracellular colloid droplets. Endocrinology, 90:986-991.

Yukimura, U., K. Ikejiri, A Lojima and T. Yamada. 1976. Effects of excess iodide and other anions on thyroid hormone secretion in normal or hypophysectomized rats treated with graded doses of thyroid hormone. Endocrinology, 99:541-548.

Zwillich, C. W., M. Matthay, D. E. Potts, R. Adler, F. Hofeldt and J. V. Weil. 1978. Thyrotoxicosis: Comparison of effects of thyroid ablation and beta-adrenergic blockade on metabolic rate and ventilatory control. J. Clin. Endocrinol. Metab., 46:491-500.