EFFECTS OF CHRONIC AND EARLY GOITROGENIC STIMULATION ON THYROIDAL METABOLISM IN THE CHICKEN AND IN THE PROGENY

BY

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A THESIS SUBMITTED IN PARTIAL FULFILMENT OF
THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE

in the Department
of

POULTRY SCIENCE

We accept this thesis as conforming to the
required standard

THE UNIVERSITY OF BRITISH COLUMBIA

August, 1975

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ABSTRACT

White Leghorn pullets were fed goitrogenic rapeseed meal or soybean meal during either or both the growing period and the laying period. Rapeseed meal was fed at a level of 17.5 percent of the starter-grower diet and 19.0 percent of the layer diet. Control rations contained 11.0 and 13.1 percent of soybean meal, respectively. The responses to the dietary treatments were studied when the birds were over 90 weeks of age. Parameters measured included: weights, histology and iodine contents of the thyroid glands, thyroidal I-131 uptake at different time intervals after administration of the radioiodine, distribution of injected doses of radioiodine in iodinated substances in the thyroid glands, iodine contents of egg yolks, and the thyroidal characteristics of the progeny of these birds at hatching.

Birds which were being fed rapeseed meal at the time the thyroid glands were examined showed effects which varied in magnitude according to the length of time over which rapeseed meal had been fed. Thyroid weights, follicle diameters, amounts of epithium and iodine contents of the thyroid glands of rapeseed meal-fed birds increased whereas coupling efficiency declined with time.

The effects of over-stimulation of the thyroid gland induced by the feeding of goitrogenic rapeseed meal during the growing period persisted for as long as 75 weeks after withdrawal of the source of goitrogen from the diet. Thyroid weights, thyroid iodine contents and radioiodine uptake were all increased in birds which had received goitrogen during the growing period. Follicle diameters were greater

and heights of epithelial cells were lower in the thyroid glands of these birds than in those of the control.

Eggs from rapeseed meal-fed birds were low in iodine. The thyroid glands of newly-hatched progeny from dams which had been fed goitrogenic rapeseed meal were observed to be hypertrophic. Since analysis of the eggs for goitrin was negative and analysis for isothiocyanates indicated similar concentrations in the eggs from birds fed rapeseed meal and from control birds fed soybean meal, the hypothesis was that the low concentration of iodine in the eggs of the goitrous birds was due to diversion of a large proportion of circulating iodine into the thyroid glands with the result that the amounts reaching the developing ova were reduced.

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ACKNOWLEDGEMENTS

The author gratefully acknowledges the supervision and encouragement of Professor B.E. March in accord with her particular contribution to my academic development leading to the completion of this work, and also her many helpful advices in areas outside the academic field.

A full measure of appreciation is also extended to the other members of my Thesis Committee:

Dr. W.D. Kitts	Chairman, Departments of Animal Science and Poultry Science
Dr. R.C. Fitzsimmons	Department of Poultry Science
Dr. C.R. Krishnamurti	Department of Animal Science
Dr. A.M. Perks	Department of Zoology

In particular, Dr. A.M. Perks must receive special mention in accord with his help and concern in my programme.

Special appreciation is given to Dr. J. Biely (Research Professor, Department of Poultry Science) for his many interesting and stimulating ideas that provided food for thought, and to Mr. R. Soong (Poultry Nutrition Laboratory) for his frequent assistance when needed.

INTRODUCTION

The thyroid gland is one of the first endocrine organs studied by man, and the effects of its hormones on man and animals have long been known. Many aspects of its metabolism, however, still remain unclear. Although many investigations have been made on the formation of the thyroid hormones, both *in vitro* and *in vivo*, the exact mechanism whereby thyroid hormonogenesis takes place under physiological conditions is still uncertain.

The picture becomes more complicated when the thyroid gland is inhibited by substances such as goitrogens. Thyroid function and hormone biosynthesis is either affected or blocked, dependent on the mode of action of the goitrogen concerned. While numerous studies have been made on the effects of the antithyroid compounds on thyroid functions of experimental animals, only a few of these investigations have examined the effects of chronic goitrogenic stimulation on the thyroid gland. Much less is known of any possible persistence of effects of early stimulations by goitrogens when they are later withdrawn.

In the present study, rapeseed meal was fed to chickens either just for the growing period or over a long period of time, and the effects of the dietary treatments on the thyroid glands were examined. Rapeseed is one of the most valuable oilseed crops in Canada, both for its potential in the production of edible and industrial oils and in the manufacture of high protein meals suitable for supplementing animal diets. It has been indicated in the literature that the mode of action of the rapeseed meal goitrogens is different from that of other anti-

thyroid compounds. Therefore, although it is now possible to manufacture rapeseed meals with a negligible goitrogen content, the effects of chronic stimulation by goitrogenic rapeseed meal on thyroid hormonogenesis, compared to other antithyroid compounds such as propylthiouracil and thiourea, are still worthy of investigation from the academic standpoint.

In addition, the possibility that effects of early stimulation of the thyroid gland may persist after withdrawal of the goitrogen warrants further studies in its own right.

REVIEW OF LITERATURE

BIOSYNTHESIS AND RELEASE OF THYROID HORMONES

The importance of iodine in the prevention of goiter has long been recognized (Hart and Steenbeck, 1918; Kalkus, 1920; Welch, 1928) and iodine metabolism in the body has been thoroughly reviewed (Wilgus et al, 1953; Chaikoff and Taurog, 1948; Hemken, 1970; Koutras, 1972). In the alimentary tract, dietary iodide is readily absorbed into the circulation and is mainly concentrated within the thyroid. The role of the thyroid, then, is to incorporate inorganic iodide into thyroid hormones and to maintain a sufficient release of these hormones to the periphery tissues.

Intrathyroidal biosynthesis of thyroid hormones has been extensively reviewed (Plaskett et al, 1963; Rapoport and DeGroot, 1971; Davies, 1972; Ermans et al, 1972; DeGroot et al, 1972; Mason and Wilkinson, 1973). Therefore in the present discussion, we will review only the more recent developments in the physiology of thyroid hormone biosynthesis, with special attention to such areas that may perhaps be affected by goitrogenic agents.

It is generally agreed that formation of thyroid hormones involves the following intermediary steps (DeGroot et al, 1972): 1) transport and concentration of iodide into the thyroid, 2) synthesis of thyroglobulin, 3) generation of an oxidizing agent, probably hydrogen peroxide, 4) oxidation of iodide to a reactive higher valence state, 5) binding of iodine to tyrosine present in thyroglobulin, 6) formation of iodothyronines in thyroglobulin by coupling of iodotyrosyl resides, 7) storage and proteolysis of thyroglobulin, and 8) release of thyroid hormones to the circulation.

Iodide trapping by the thyroid is dependent on adquate potassium concentration (Wolff and Halmi, 1963) and is blocked by inhibitors of or agents that uncouple oxidative phosphorylation such as 2,4-dinitrophenol, cyanide, cardiac glycosides and anoxia (Wyngaarden et al, 1953; Freinkel and Ingbar, 1955; Wolff, 1964). Therefore it is probably an active process requiring energy related with the Na^+,K^+-ATPase-dependent transport system (Turkington, 1962). Much attention has been focussed on the site of this iodide pump mechanism, which is probably a membrane receptor site located in the peripheral border of the follicular cells of the thyroid (Halmi, 1961; Pitt-Rivers and Trotter, 1953; Tong et al, 1962). It has been suggested that a lecithin phospholipid is a constituent of the receptor site (Vilkki and Jaakonmaki, 1966) and a role of Fe^{++} has also been implicated (Dhopeshwarkar, 1968).

Thyroglobulin, like other proteins, is synthesized in the polyribosomes of the endoplasmic reticulum of the thyroid cells (Nadler et al, 1964; Thomson and Goldberg, 1968); it then travels down to the apical region of the cells to be accumulated in the colloid (Whur et al, 1969). It is a glycoprotein with a molecular weight of about 650,000 (Goldberg and Leed, 1965; Robbins and Rall, 1960; Rall et al, 1960) and a sedimentation coefficient of 19S when iodinated. The site of its iodination has been the topic of much extensive studies (Edelhoch, 1962; Nunez, 1966; Lissitzky et al, 1969), and it is probably at the border of the apical membrane of the thyroid cell and the colloid. The distribution of various iodoamino acids in the thyroid tissue is largely determined by the iodination level of the thyroglobulin (DeCrombrugghe et al, 1967; Ermans et al, 1968; Valenta et al, 1968).

Many questions pertaining to the mechanism of thyroid hormone formation are still unsolved; they have recently have discussed thoroughly by DeGroot et al (1972). It is widely accepted that in vivo iodinations require generation of H_2O_2 which, together with O_2 are the only biological oxidizing agents with an oxidation-reduction potential above that of the $2I^- \leftrightarrow I_2$ couple, i.e. +0.535 volts (Bernard and DeGroot, 1969; Ahn and Rosenberg, 1970). Although cytoplasmic DPNH oxidase (Rossi et al, 1969) and monoamine oxidase (Fischer et al, 1966, 1968) activity have been suggested to be the origin of H_2O_2, the cofactor NADPH is likely to be involved in thyroidal H_2O_2 production, possibly via reaction with NADPH-cytochrome C reductase (DeGroot and Davis, 1961; Nagasaka et al, 1971).

The particulate-bound enzyme, peroxidase, has been isolated from thyroid tissues of a number of species (Alexander, 1959; Hosoya and Morrison, 1967; Mahoney and Igo, 1966; Ljunggren and Adeson, 1968; Nagasaka and DeGroot, 1971). It is a heme protein containing a prosthetic group similar to Protoporphyrin IX (Taurog et al, 1970). In addition to utilizing H_2O_2 for peroxidation of iodide to iodine -- probably to its free radical form, $I^{\emptyset}$ (Klebanoff et al, 1962; Yip and Hadley, 1966) -- peroxidase has also been held responsible for catalyzing the organification of iodine to tyrosine present in thyroglobulin. DeGroot et al (1972) envisage a "ping-pong" mechanism of organification, which presumably involves formation of free radicals of iodide and tyrosine by peroxidase complexed with H_2O_2. Union of the iodine and tyrosine free radicals to form iodotyrosine returns the enzyme to its original unoxidized state.

The synthesis of iodotyrosines is a rapid process (DeGroot and Davis, 1961). Distribution of radioiodine between monoiodotyrosine (MIT) and diiodotyrosine (DIT) reaches a constant level after variable lengths

of time, and the constant MIT/DIT ratio in physiological conditions is consonant with a precursor-product relationship between the two iodotyrosines (Plaskett et al, 1963a). When the iodine content of the thyroid tissue is reduced, however, there appears a marked increase in the MIT/DIT ratio (Querido et al, 1957; Ermans et al, 1961), which, as mentioned earlier, is dependent on the degree of iodination of thyroglobulin (Ermans et al, 1968).

The 'coupling' of iodotyrosines to intrathyroglobulin iodothyronines, as first proposed by Harington and Barger (1927), is a much slower process (Pitt-Rivers, 1962; Plaskett et al, 1963b). Although it has been achieved in vitro (Pitt-Rivers, 1948; Yip and Klebanoff, 1952), its in vivo mechanism is still uncertain. The fact that free thyronine is not found in nature leaves two possible routes whereby iodothyronines can be formed in thyroglobulin (DeGroot et al, 1972): 1) by intramolecular rearrangement, i.e. conformational changes within the thyroglobulin which allow an iodotyrosyl free radical (produced by an oxidizing agent such as peroxidase-I^0) to be transferred to another iodotyrosyl group to form iodothyronine; or 2) by oxidative-deamination of DIT to form hydroxyphenolpyruvate (DIHPPA),peroxidation of the enol form of which leads to the formation of a DIHPPA-peroxide, which can then be coupled with another DIT molecule in thyroglobulin to form thyroxine (Toi et al, 1963, 1965). Despite evidence of the presence of necessary enzymes in the thyroid tisuse (Igo et al, 1968; Blasi et al, 1969a,b), the extent of these pathways in vivo thyroid hormone formation is still uncertain.

Thus,thyroid hormone is stored in the follicular lumen, as a constituent of the thyroglobulin polypeptide. Recent electronmicrographical data show, in addition to colloid droplets, two functionally different

types of vesicles in the apical regions of thyroid follicle cells (Seljelid et al, 1970): endocytotic and exocytotic. Exocytotic vesicles may be involved in the transport of newly synthesized thyroglobulin into the follicle lumen for storage, while the endocytotic vesicles may be responsible for the re-entrance of thyroglobulin into the cell for release to the circulation. Furthermore, there is evident for the "last come, first served" principle of iodine turnover in the thyroid pool (Schneider, 1964), which is supported by the structural and functional heterogeneity among the follicles (Nadler, 1954; Studer et al, 1972).

Once re-entered into the thyroid cell, release of the thyroglobulin is achieved by proteolysis by acidic proteases and peptidases contained in the lysosomes(Wollman et al, 1964; Seljelid, 1968). The liberated iodotyrosines are deionated by iodotyrosine deiodinase within the thyroid cell, while the iodothyronines are secreted into the circulation to affect target tissues.

Almost every intermediary step in thyroid hormone biosynthesis and release is regulated one way or the other by thyrotropin (TSH) from the adenohypophysis. An excellent review of the action of thyrotropin on thyroid metabolism is given by Dumont (1971). In addition to the extrathyroidal classical negative feedback control by thyroid hormones and the hypothalamic control by TSH-releasing hormone (TRF) via the hypothalamohypophyseal portal system (Schally et al, 1969; Burgus and Guillemin, 1970), there seems to be an intrathyroidal autonomous mechanism whereby thyroidal iodine metabolism is regulated (Wolff and Chaikoff, 1948; Socolow et al, 1968; Barakat and Ingbar, 1965; Golman et al, 1966; Studer and Greer, 1968). It also appears to alter thyroid hormone secretion, independently of TSH supply (Green and Ingbar, 1962; Halmi, 1961; Onaya and Halmi, 1967). Solomon and Dowling (1960) hypothesized that autoregulation of thyroidal iodide

transport allows an iodide-deficient animal to maintain its intrathyroidal hormone stores. The accumulated supply may then be released by the extra TSH secreted in response to lowered iodothyronine levels in the circulation.

Analyses of distribution of iodoamino acids in thyroid tissues has shown little difference between the avian and the mammalian glands (Shellabarger and Pitt-Rivers, 1958; Mellen and Wentworth, 1959). Hence a diversity of mechanisms has not been recognized for thyroid hormone biosynthesis in the different species. However, it should be noted that while in mammals L-triiodothyronine (T_3) has a higher potency than an equimolar quantity of L-thyroxine (T_4), the iodothyronines have different relative potencies in birds (Shellabarger, 1955; Hutchins and Newcomer, 1966; Newcomer, 1957; Robbins and Rall, 1957). Furthermore, the finding by Tata and Shellabarger (1959) that thyroxine-binding globulin is absent in chicken blood probably explains the failure of protein-bound iodine (PBI) as a criterion of measurement of thyroid hormone metabolism in the bird.

EFFECTS OF GOITROGENS ON THYROID HORMONE BIOSYNTHESIS

Antithyroidal substances and natural goitrogens have been extensively reviewed (VanEtten, 1969). The former include, among others, inorganic ions such as iodide,(large doses of which inhibit thyroid hormone synthesis in the rat -- the so-called Wolff-Chaikoff effect), the thionamide drugs such as thiourea and thiouracil, and the sulfonamides such as para-aminobenzoic acid and sulfanilamide (Aswood, 1943; Astwood et al, 1945). Among the naturally occurring goitrogens, (-)-5-vinyl-2-thio-oxazolidone is of particular importance because of its possible role in the pathogenesis of endemic goiter and will be discussed in more detail.

Disturbances or inhibition of thyroid hormone biosynthesis due to defects of iodine metabolism can be classified as follows (Blizzard, 1960): 1) defect in trapping of inorganic iodide from the circulation; 2) inability to oxidize iodide to an active form; 3) inability to halogenate tyrosine in presence of elemental iodine; 4) inability to couple iodinated tyrosines to form iodothyronines; 5) inability to hydrolyze thyroglobulin; 6) abnormal formation or release of thyroglobulin or "thyroglobulin-like" substances; 7) inability to deiodinate free iodotyrosines for reutilization. One or more of these intermediate steps of thyroid hormone biosynthesis can be inhibited by a goitrogen. For example, the thionamides such as thiouracil appear to interfere with the organic binding of intrathyroidal iodide (Iino, 1961), thus competing with the iodide while the peroxidase is acting (DeGroot and Davis, 1962). The sulfonamides interfere with organification of iodine without inhibiting the trapping of iodide (Milne and Greer, 1962). That their mechanisms of action are different is evident by the observation that addition of iodine reduces goiter size in animals treated with thionamides but increase thyroid hypertrophy in animals treated with sulfonamides (MacKenzie and MacKenzie, 1943).

In a systematic study in which N-iodosuccinimide was used as a model for the active iodine of the thyroid gland (Jirousek and Soodak, 1973), all classes of goitrogens tested were found to react. The effects of the various goitrogens on <u>in vitro</u> biosynthesis of thyroid hormones have been delineated (Iino, 1961). Their ability to completely inhibit DIT formation in decreasing order are: 1-methyl-2-mercaptoimidazole (MIA); thiouracil (TU); propylthiouracil (PTU) and (-)-5-vinyl-2-thio-oxazolidone (goitrin); thiourea and methylthiouracil (MTU). Their ability to inhibit

MIT formation in decreasing order are: MIA; PTU, MTU, TU and thiourea; goitrin. All of them seem to show similar mechanisms of action except goitrin. This is in agreement with evidence presented in a following section with regard to goitrogenicity of rapeseed meal.

Many studies have been focused on the enzyme systems in the thyroid gland, notably on thyroidal peridase (Serif and Kirkwood, 1958) and its possible interaction with antithyroid compounds. Though perchlorate appears to have no effect on the enzyme, it has been shown that azide, cyanide, thiouracil, thiocyanate, p-aminobenzoate, and 3-amono-1,2,4-triazole are potent inhibitors of iodide peroxidase (Alemander, 1959), and that some naturally occurring goitrogens including goitrin may also possess inhibitory activity on the various thyroid enzymes such as peroxidase, cytochrome C oxidase and DPNH-linked iodinating activity (Langer and Michajlovskij, 1972).

Insufficient proteolysis of the thyroglobulin and suppression of lysosomal enzyme activities have also been reported as causes of goiter in man (Beckers and DeVisscher, 1962) and mice (Itkiawa and Kawada, 1974).

RAPESEED MEAL AS A SOURCE OF GOITROGEN

The seed meals from rape (Brassica napus L.) and turnip rape (B. campestris L.), after the oils have been expelled or solvent-extracted, are high protein-containing materials and used in animal feedstuffs (Bowland et al, 1965; Clandinin and Robblee, 1966; Clandinin, 1967; Bell and Belzile, 1965; Rutkowski, 1971).

Practically all plants of the family Cruciferae, to which the genus Brassica belongs, which have been investigated contain glucosinolates (VanEtten 1969). In Brassica plants, twelve different glucosinolates are known to occur and are distributed in almost all parts of the plants (Josefsson, 1967). Myrosinase is present in the tissues of these glucosinolate-producing plants as well as in gastrointestinal tract of animals (Greer, 1962 a,b). It is probably an -SH dependent enzyme since it is inactivated by inhibitors of that chemical radical (Sandberg and Holly, 1932).

Glucosinolates are biologically inactive. Destruction of the plant cellular structure allows the myrosinase enzyme (or enzyme system) to hydrolyze the glucosinolates, liberating glucose and bisulphate, while activating the compounds into one of three groups: goitrin and related nitriles; organic isothiocyanates; inorganic thiocyanate (Ettlinger and Lundeen, 1957).

The goitrogenic properties of rapeseed meal are largely attributed to (-)-5-vinyl-oxazolidinethione (goitrin), a hydrolysis product of its precursors, progoitrin and epi-progoitrin. It is probably not preformed in the glucosinolate molecule but arises from cyclization following myrosinase action (Clandinin et al, 1959).

The mechanism of goitrin influence on the thyroid gland is far from being understood. Rutkowski (1971) stated that goitrin blocks the irreversible mechanism connected with organic binding of iodine in the thyroid, thus partially suppressing thyroid hormone biosynthesis. This, however, is not borne out by reports from other investigators (Matsumoto et al, 1969; Akiba and Matsumoto, 1971). Clandinin et al (1961) described

a histopathological picture of the rapeseed meal-treated chicken thyroid gland, which shows considerable hypertrophy and hyperplasia of parenchymal elements and almost complete loss of follicular colloid. This is further supported by the photomicrographic findings of Rutkowski (1971), in which desquamation of epithelium compressed follicles in small restricted areas and decrease in colloid could be readily observed in thyroids of chickens after four weeks of rapeseed meal feeding.

The isothiocyanates and thiocyanates have a considerably lower goitrogenic effect than the oxazolidinethiones (Fertman and Curtis, 1951; Gmelin and Virtanen, 1960). They block iodine accumulation into the thyroid gland, but their effect could be removed by addition of iodine to the ration (Rutkowski, 1971). A synergetic influence of the oxazolidinethiones, isothiocyanates and thiocyanates may be possible (the so-called "Brassica factor"), however, since it has been shown (Langer, 1966) that the effect of the mixture of goitrogens was considerably higher than the sum of results obtained in administrating them individually.

EFFECTS OF GOITRIN ON THYROID METABOLISM OF THE CHICKEN

The problems involved in the feeding of rapeseed meal to livestock have been extensively reviewed (Bell, 1955, 1957; Manns and Bowland, 1963; Manns et al, 1963). Thyroid hypertrophy has invariably been reported in domestic animals fed rapeseed meal (Turner, 1946; Blakely and Anderson, 1948 a,b; Witz et al, 1950; Klain et al, 1956) and has been ascribed to the effect of the known goitrogenic compounds of rapeseed: (-)-5-vinyl-2-thiooxazolidone (Astwood et al, 1949; Carroll, 1949) and allyl or crotonyl isothiocyanates (Dow and Allen, 1954).

(-)-5-vinyl-2-thio-oxazolidone (goitrin) is the most potent natural goitrogenic compound isolated so far (Langer et al, 1971). It was first identified in rapeseed by Astwood et al (1949 a,b) and Carroll (1949) as L-5-vinyl-2-thiooxazolidone and later renamed (-)-5-2-oxazolidinethione. Its synthetic analogue, (±)-5-vinyl-2-oxazolidinethione, was first made available by Ettlinger (1950).

The work of Clandinin (1962) and Clandinin et al (1966) in the effects of synthetic goitrin in thyroid metabolism in the growing chick suggests the following sequence of events: initially, iodine uptake is greatly reduced and colloid stores in the glands are rapidly depleted. Chick growth rate is depressed. Rapid enlargement of the glands follows with accompanying hypertrophy and hyperplasia. Iodine uptake increases. Colloid stores return and the gland takes on a more normal appearance. Hence it would appear that chicks fed the goitrogen eventually reach physiological equilibrium at increased thyroid-to-body weight ratios.

When comparing the goitrogenic effects of goitrin, 1-methyl-2-mercaptoimidazole (methimazole) and 6-methyl-thiouracil (methiocil) in growing chicks, Matsumoto et al (1968) reported that the chicks which received 0.05% goitrin showed thyroid enlargement, the highest thyroidal uptake of I-131 and the slow release of I-131, which was in marked contrast to the methimazole- or methiocil-fed chicks. They concluded that the incorporation of blood I-131 by the thyroid gland is not inhibited and that, from the slow release of I-131 and the depressed thyroid hormone in blood, some step of thyroid hormone biosynthesis and furthermore secretion of thyroid hormone into blood or deiodination of some iodoamino acids in thyroid gland may be inhibited by the treatment with goitrin.

That the goitrogenic effects of goitrin are different from those of propylthiouracil (PTU) in the growing chick was further studied by Matsumoto et al (1969) and Akiba and Matsumoto (1971). These investigators showed that while the PTU chicks do not maintain incorporated I-131 in the thyroid gland but release it into the blood easily, the goitrin-fed chick is promoted to maintain a large amount of incorporated I-131 in the gland and appears to inhibit its release. Furthermore, since the percentage of iodothyronines (T_3 and T_4) in this latter group also decreased to one-third of the control chicks in their distribution of radioactive iodinated substances found in thyroid homogenates, and that the highest percentage was observed in MIT (65%) and the MIT/DIT ratio was higher than that of the control group, it is quite possible that goitrin almost never inhibits the monoiodination of tyrosine, but it inhibits the coupling of MIT to synthesize DIT. The finding of increased MIT percentage is in agreement with results of Iino et al (1961) and Greer et al (1962) in rats given small doses of PTU.

Reports on the effect of PTU or thiouracil on the intrathyroidal metabolism of I-131 are numerous. For example, thiouracil inhibits the organic binding of iodine in rat thyroid in vivo (Astwood and Bissel, 1944) and in vitro (Franklin et al, 1944), PTU blocks the iodination of tyrosine (Pitt-Rivers, 1948; Mayberry and Astwood, 1960) and inhibits the synthesis and coupling of DIT (Richard and Ingbar, 1959). Thus it appears that PTU inhibits these steps of thyroid hormone biosynthesis: oxidation of iodide, iodination of tyrosine, and iodothyronine synthesis from iodotyrosines. The most distinct difference between effects of goitrin and PTU is that the amount of iodothyronines synthesized in the gland is markedly depressed

in PTU-fed chicks but is increased in goitrin-fed chicks while the plasma level of thyroid hormone is depressed in both groups (Akiba and Matsumoto, 1971). It is suggested that the hydrolysis of thyroglobulin and secretion of thyroid hormone from the gland may be decreased or at least not enhanced by goitrin treatment in growing chicks.

Moreover, in their study of thyroid function of chicks after withdrawal of goitrin from the diet following twenty-one days of feeding, Akiba and Matsumato (1973) reported a rapid restoration of normal thyroid metabolism and hormone biosynthesis, despite hypertrophy of thyroid gland which persists for relatively longer time. After withdrawal of goitrin, plasma PBI-131 was radically elevated and a little increase could be demonstrated. This so-called rebound phenomenon is similar to goitrogen withdrawal in other laboratory animals such as mice, rats and guinea pigs (D'Angelo et al, 1951, 1954; Lipner et al, 1959; Studer and Greer, 1967; Langer, 1968) and can be explained by an overstimulation of TSH on the unblocked thyroid gland after release of goitrogenic effects by goitrogen withdrawal (D'Angelo, 1961, 1969). The gradual involution in thyroid weight after goitrin withdrawal is in agreement with that reported for PTU withdrawal in chicks (Akiba et al, 1971), rats (Shimoda, 1960) and guinea pigs (D'Angelo et al, 1954).

EFFECTS OF LONG-TERM ADMINISTRATION OF RAPESEED MEAL ON BIRDS AND PROGENY

Although a considerable amount of work has been carried out on the feeding of rapeseed meal to young animals, relatively few experiments have been performed wherein the goitrogens are fed chronically (Peltola,

1965; Summers et al, 1969; Vogt et al, 1969; Clandinin and Robblee, 1970; March et al, 1972). In an experiment where rapeseed meal was used as a protein supplement in the diets of laying hens for 252 days (Jackson, 1969), the histological picture of the thyroid glands was not identical with that obtained in growing chicks (Clandinin and Bayly, 1960). No evidence of cellular infiltration was obtained, thus supporting the view that the initial effect of the rapeseed goitrogens are compensated for by thyroid enlargement, and the chronically-treated birds have normal physiological function with regard to the egg-laying process.

Some investigators reported the presence of so-called Psammoma bodies in the thyroids of chronically stimulated animals (Axelrad and Leblond, 1955; Wollman, 1961; Grimm et al, 1970), which are probably dense aggregations of thyroprotein related to the observation of radioactivity with a very long biological half-life in thyroids described by Van Middlesworth (1965). Follis (1965), however, could not find these Psammoma bodies in thyroids of hamster, mice or monkeys given goitrogens for prolonged periods.

It appears that goitrin is not transferred for deposition in the egg (Rutkowski, 1971; March et al, 1972). The thyroid glands of newly-hatched progeny of dams fed rapeseed meal, however, showed hypertrophy, which was greater when the diet contained the higher level of rapeseed meal (March et al, 1972). Also these chicks at hatching were slightly smaller than those from birds fed soybean meal control diet. This is in agreement with the adverse effects of rapeseed meal in progeny of other species such as gilts (Bell and Belzile, 1965).

Experiment 1: EFFECTS OF CHRONIC AND EARLY STIMULATION BY GOITROGENIC RSM ON WEIGHT AND HISTOLOGY OF THYROID GLAND

It has long been known that the feeding of RSM to the fowl causes thyroid hypertrophy and hyperplasia. However, since most of the histological data are on growing chicks, little is known about the alteration of chronic RSM stimulation on thyroid histology. Furthermore, the extent to which adaptation of the thyroid gland is possible via the mechanism of compensation has not been established. When compensatory functions of the hypothalamus-pituitary-thyroid axis in the fowl cease and atrophy of the gland begins have not been accurately assessed. It is not clear if involution does occur in the early-stimulated thyroid glands when goitrogenic stimulation is alleviated. Hence the following long-term experiment was conducted to elucidate these effects on thyroid weight and histology.

Materials and Methods:

In this and all the following experiments (with the exceptions of experiments 5 and 6B), the birds used were on an experiment designed to compare production and mortality of birds fed soybean meal (SBM) and rapeseed meal (RSM) as the source of supplementary protein in growing and/or laying diets (March et al, 1975).

Day-old White Leghorn chicks of two strains (Shaver chicks and DeKalb chicks) were reared in batteries and fed starting and growing diets containing either SBM or RSM as indicated in Table 1. The starting diets were fed from 0-8 weeks and the growing diets from 8-23 weeks. The birds were vaccinated against Marek's disease, Newcastle disease and infectious bronchitis. Causes of mortality were recorded.

At 23 weeks of age the birds were transferred to community cages and fed the laying diets (Table 1) containing either SBM or RSM. The laying diets were fed according to the following cross-over design. One half the birds were continued on the protein supplement which they had received during the growing period and the other half were transferred to the alternate protein supplement. The treatments were designated as S-S, R-S, R-R and S-R, the first letter of each pair indicating soybean meal or rapeseed meal in the growing diet and the second letter indicating soybean meal or rapeseed meal in the laying diet. Six hundred birds were distributed into 24 lots of 25 birds each, in community cages (2 strains x 4 treatments x 3 replicates).

At 91 weeks, forty birds of the Shaver strain (10 from each treatment) were weighed and killed. The thyroid glands were dissected free from connective tissue and fat, weighed, and immediately fixed in buffered 10% formalin for subsequent histological preparation (Histology Laboratory, Zoology Department, U.B.C.). The glands were embedded in paraffin, sectioned at a thickness of 5 μ, and every 20th section of the left gland was stained with haematoxylin and eosin. The central sections of each gland were chosen for measurements of epithelial height and volume proportion of thyroidal tissues using a micrometer fitted to the light microscope.

At 99 weeks of age, forty birds of the DeKalb strain were individually weighed and killed, and their thyroid glands were removed for measurement of weights only.

All experimental data of this and the following experiments were subjected to analysis of variance and Duncan's multiple range tests (Snedecor and Cochran, 1956).

Goitrogenicity of the RSM fed in the experiment at the time the birds were killed was determined by a separate experiment, in which day-old White Leghorn cockerels were fed either a SBM- or RSM-supplemented diet (Table 1) similar in composition to those fed in experiment 1. Six chicks from each treatment at 1 week and twelve chicks from each treatment at 3 weeks were killed to determine the weight of the thyroid glands.

Results:

Goitrogenicity of the present lot of RSM (Span) was established by the hypertrophy of the thyroid glands shown by the RSM-fed chicks at 1 week or 3 weeks of treatment (Table 2).

The average thyroid weights (mg/100 g body weight) of the Shaver and DeKalb birds at 91 and 99 weeks of age are presented in Table 3. The R-R birds showed the most thyroidal enlargement over that of the control (10 to 13 times increase), while the R-S birds also had double the thyroid size of the S-S birds.

Histological data of the thyroid glands of the Shaver birds at 91 weeks are presented in Table 4. Total percentage thyroid epithelium in the R-R birds was less than that in the control (15.1% vs 24.4%), while the average epithelial height of the R-R thyroids (10.65 μ) was higher than that (7.13 μ) for the control. Similarly, the R-S thyroids showed a decrease in percentage epithelium (14.8%) but the average epithelial height (5.61 μ) was also lower than that of the control.

Consistent with the appearance of enlarged glands, the average follicle diameters of the RSM-treated thyroid glands were also increased over that of the control, the largest follicles being in the R-R thyroids. Again, the average follicle diameter of the R-S thyroids was also greater than that of the control (0.84 mm vs 0.54 mm).

It should be noted that, with all the histological parameters measured, the S-R thyroids were consistently intermediate between the R-R and the control glands.

Under light microscopy, the follicle lumens of the thyroids in the four treatments were filled with stained colloid. The R-R and S-R glands showed the appearance of colloid goiter. Desquamated cells were infrequent but did occur in the thyroid glands of the chronically RSM-fed birds. No evidence of atrophy was seen in these glands. Only in one or two occasions had thyroid cysts been found in the chronically RSM-stimulated birds.

Table 1: Percentage composition of diets fed in experiment 1 and RSM goitrogenicity test

	Experiment 1				RSM Goitrogenicity Test	
	Starter-Grower		Layer			
Code Letter	S	R	S	R	S	R
Ingredient						
Soybean meal	11.0	-	13.1	-	26.3	7.8
Rapeseed meal	-	17.5	-	19.0	-	30.0
Ground wheat	66.5	60.0	60.6	53.5	53.6	42.6
Ground corn	15.0	15.0	6.0	6.0	10.0	10.0
Ground oats	-	-	10.0	10.0	-	-
Dehydrated cereal grass	2.0	2.0	2.0	2.0	2.0	2.0
Bone meal	2.0	2.0	2.5	2.5	2.0	2.0
Limestone	1.0	1.0	4.75	4.75	1.3	1.9
Iodized salt	0.5	0.5	0.25	0.25	0.5	0.5
Feeding tallow	2.0	2.0	0.5	2.0	3.0	3.0
DL-Methionine	-	-	0.1	-	0.1	-
L-Lysine	-	-	-	-	-	0.1
Tricalcium phosphate	-	-	-	-	1.2	0.1
Micronutrients	*	*	**	**	*	*

*/kg: manganese sulphate 132 mg, choline chloride 1320 mg, niacin 27 mg, riboflavin 4.0 mg, calcium pantothenate 9.2 mg, folacin 0.55 mg, pyridoxine HCl 2.9 mg, biotin 0.09 mg, vitamin B_{12} 13.0 mcg, vitamin A 4400 I.U., vitamin D_3 660 I.U., Zn bacitracin 9.7 mg, oleandomycin 11.0 mg.

**/kg: manganese sulphate 198 mg, riboflavin 3.3 mg, vitamin B_{12} 3.3 mcg, vitamin A 6600 I.U., vitamin D_3 660 I.U.

Table 2: Comparison of average thyroid weights of White Leghorn cockerels fed SBM- or RSM-supplemented diets at 1 week or 3 weeks of treatment from hatching

Treatments	Average thyroid weights (mg/100 g body weight)	
	1 week	3 weeks
SBM - diet	5.8 (6)*	5.1 (12)
RSM - diet	9.4 (6)	9.9 (12)

* No. of determinations

Table 3: Average weights of thyroid glands in birds fed RSM or SBM for different periods of time in experiment 1

		EXPERIMENT 1	
		Strain A	Strain B
Dietary treat.	Ave. thyroid wt. at 58 weeks of age* mg/100 g body wt.	Ave. thyroid wt. at 91 weeks of age mg/100 g body wt.	Ave. thyroid wt. at 99 weeks of age mg/100 g body wt.
S-S	11.0^{aA}(12)**	10.7^{aA}(10)	11.2^{aA}(10)
R-S	27.1^{b} (12)	23.5^{aB}(10)	28.4^{aB}(10)
R-R	67.8^{d} (12)	112.5^{c} (10)	149.4^{c} (10)
S-R	44.9^{c} (12)	84.9^{b} (10)	103.4^{b} (10)
Thiouracil in starting diet until 4 wks of age:	17.7^{abB}(12)		
Level of significance	5%	1%	1%

* unpublished data from an earlier experiment in this laboratory

** number of determinations per treatment

abcd Values with the same superscript are not significantly different.

AB Significantly different at the 1% level of significance by separate comparison.

Table 4: Histological measurements on thyroid glands from birds (Shaver strain) in experiment 1 at 91 weeks of age.

Dietary treatment	Ave.* thyroid wt. g.	Ave. epithelial ht. μ	Ave. follicle diam. mm	% epithelium	Wt. epithelium mg	Ave. no. follicles across gland
S-S	0.19^{aA}	7.13^{a}	0.108^{a}	24.4^{c}	41.1^{a}	67
R-S	0.52^{aB}	5.72^{a}	0.167^{b}	14.8^{a}	76.5^{a}	51
R-R	2.30^{c}	10.65^{b}	0.227^{c}	15.1^{ab}	334.6^{b}	58
S-R	1.39^{b}	10.11^{b}	0.196^{bc}	20.1^{abc}	277.4^{b}	67
Level of significance	1%	1%	5%	5%	1%	

* five glands examined per treatment

^{abcd} values with the same superscript are not significantly different.

^{AB} significantly different at the 1% level of significance by separate comparison.

Experiment 2: EFFECTS OF CHRONIC AND EARLY STIMULATION BY GOITROGENIC RSM ON TOTAL IODINE CONTENT OF THE THYROID GLAND

Since the histological picture of the R-R and S-R thyroids showed the healthy appearance of colloid goiter instead of some degree of atrophy due to chronic RSM stimulation, the following experiment was conducted to determine the total iodide content of the thyroid tissue as affected by the treatments.

Materials and Methods:

Five Shaver birds of each of the four dietary treatments (S-S, R-S, R-R and S-R as in experiment 1) were killed and their thyroid glands removed and weighed. Each pair of glands was homogenized a Potter-Elvehjem glass-homogenizer to give a 25 ml suspension with deionized water.

The analytical method for the measurement of total iodine content was an adaptation of the Barker procedure for serum (Barker, 1948). The concentration of iodine was determined by measuring its catalytic effect on the rate of reaction between ceric ion and arsenious acid, which involved the conversion of the yellow ceric ion to the colorless cerous ion. The color of the ceric ion was determined photometrically twenty minutes after the reaction started, and the iodine conentration was related inversely to the logarithm of the optical density.

1 ml of the thyroid tissue homogenate was weighed into Kimax 16 x 125 mm culture tubes. 1 ml of 4N sodium carbonate was added to each tube and the content was mixed thoroughly by a shaker. The tubes were dried in a

an oven at 100°C to complete dryness, after which they were ashed in a muffle furnace at 625 ± 25°C for 4 hours. The tubes were then removed and allowed to cool.

Four ml of mixed acid solution (7N H_2SO_4 + 2N HCl; 1:1) were added to each tube. The contents were mixed and allowed to stand for approximately 10 minutes to dissolve the ash. Three ml of deionized water were added, the contents of the tube again mixed. 10 µl of this mixture was added to 8 ml of deionized water in another set of 12 ml glass-stoppered tubes. A bland and three iodine standards (.01, .05 and .10 µg) were analyzed in duplicate with each group of samples. All the tubes (standard, blank and sample) contained 8 ml of solution.

To each glass-stoppered tube (blanks, standards and samples) was added 0.5 ml of 0.1N sodium arsenite solution. The tubes were placed in a shaking water bath at 39 ± 1°C for 10 minutes to allow for thermal equilibrium. Ceric ammonium sulfate solution (.02N) was also warmed in the same water bath. Using a Centaur microliter pipette, 1 ml of ceric ammonium sulfate solution was added to each glass-stoppered tube at precisely 1 minute intervals. A stop watch was used to ensure accurate timing. The content of the tube was immediately shaken to ensure thorough mixing.

Twenty minutes after the ceric ammonium sulfate solution was added to the first tube, measurements of optical density at 420 nm were begun, using an Unicam SP1800 spectrophotometer. These readings were made at intervals of 1 minute in order that the lapsed time for all of the samples were constant.

Measurements of the optical density of the blank and the standard iodine solutions were plotted on semilog paper. The concentrations of iodine in the unknown samples were read from the graph and multiplied by the dilution

factor of 700 (to compensate for the 10 ul aliquot taken from the 7 ml acid solution of the ashed sample). Total iodine content in the pair of thyroid glands = iodine concentration in the sample x 25.

The iodine concentrations in the diets were also analyzed by the same procedure, as modified by Sunderman (1963). 0.1 to 0.2 g of the sample was weighed into culture tubes. After addition of 1 ml of 4N sodium carbonate and subsequent drying at 100°C, the samples were ashed at 625 ± 25°C for 12-16 hours. Upon cooling and mixing with 4 ml of mixed acid solution and 3 ml of deionized water, the contents of the tubes were passed through a Pyrex high form fritted glass filter of F(fine) porosity to remove any insoluble material. A 3 ml aliquot of the filtrate from each ashed sample was pipetted into another set of glass-stoppered tubes to which 5 ml of deionized were added to make up a total of 8 ml in each tube. Optical densities of the unknown samples were read off from a standard graph and multiplied by a dilution factor of 7/3 to compensate for the 3 ml aliquot taken from the 7 ml acid solution of the ashed sample.

Results:

The average absolute gland weights, iodine concentration and total iodine content of the thyroids of the birds after chronic dietary treatments are given in Table 5. The absolute thyroid weights were in the same order as the relative weights; the largest again being the R-R glands (2.08 g) which were 10 times the size of the control.

The iodine concentration (mg per g thyroid tissue) was lowest in R-R birds (0.89) compared to 2.65 mg/g of the control. However, because of the much enlarged size, the R-R thyroids had an average total iodine content of 1.72 mg in the pair of glands, while that in the control was only 0.55 mg. Likewise, the R-S thyroids had an accumulation of 1.25 mg iodine in a pair of glands, which was more than double that of the control. Again, the iodine concentration or total iodine content of the S-R thyroids were intermediate between those of the R-R and S-S glands.

The iodine concentrations of the respective diets are shown in Table 6. Both SBM- and RSM-supplemented rations had iodine values above that of 0.3 mg/kg, which is the NRC stated requirement for iodine by poultry.

Table 5: Iodine concentration in thyroid glands of birds fed RSM or SBM for different periods of time.

Dietary treatment	Ave. wt. of thyroid glands* g	Ave. iodine concentration in gland mg/g	Total iodine in gland mg
S-S	0.20 [a]	2.65 [a]	0.55 [a]
R-S	0.47 [a]	2.73 [a]	1.25 [b]
R-R	2.08 [c]	0.89 [b]	1.72 [c]
S-R	1.51 [b]	1.08 [b]	1.65 [c]
Level of significance	5%	1%	5%

* 5 determinations per treatment.

abcd values with the same superscript are not significantly different.

Table 6: Analyzed iodine concentrations in SBM- and RSM-supplemented diets fed to birds in experiments 1 and 6B.

Rations	Analyzed iodine concentrations mg/kg
In experiments 1 and 6B:	
SBM-supplemented	0.85
RSM-supplemented	0.91
In experiment 6B only:	
SBM-supplemented + KIO_3	1.10
RSB-supplemented + KIO_3	1.35

Experiment 3: EFFECTS ON UPTAKE OF A SINGLE DOSE OF I-131 BY THE THYROID GLAND 6 HOURS AND 22 HOURS AFTER ADMINISTRATION OF THE RADIOIODINE

Radioiodine has long been used in assessing thyroid iodine metabolism (Chaikoff and Taurog, 1948; Turner et al, 1959; Pitt-Rivers and Rall, 1961). Since the goitrous thyroid glands of the chronically RSM-stimulated birds appeared to be actively accumulating iodine (which could be three times as much as in the control glands), it would be of interest to compare the patterns of thyroidal radioiodine uptake among the birds of the different treatments. The following experiments was conducted to determine the percentage uptake of a dose of I-131 by the thyroid gland 6 and 22 hours after administration.

Materials and Methods:

(A) I-131 uptake at 6 hours after administration:

Five birds (Shaver strain) of each of the four treatments as in experiment 1 were moved to individual cages in a separate room reserved for radioisotope studies. The birds were given their respective diets and water ad libitum. I-131 was purchased from Atomic Energy of Canada, Ottawa, in the form of $Na^{131}I$ in sodium sulfate solution. 0.5 μCi of I-131 in 0.5 ml physiological saline was injected into the left brachial vein of each bird. The time of each injection was noted. At exactly 6 hours after each I-131 administration, the birds were killed. The thyroid glands were removed and weighed, and counted for radioactivity in a Nuclear-Chicago 8166 well-type scintillation counter. The count rates (cpm) were corrected for background and coincidence loss according to the equation in Wang and Willis (1965):

$$n = \frac{m}{1 - mT}$$

where n is the corrected cpm, m is the observed cpm, and T is the resolving time of the counting assembly. The corrected cpm was calculated back to injection (zero) time by correction with the decay constant of I-131, and expressed as a percentage of the corrected count rate of a standard original dose (0.5 μCi).

(B) I-131 uptake at 22 hours after administration:

This part of the experiment was carried out in conjunction with experiment 4.

Another twenty White Leghorn layers (Shaver), five of each treatment, from the same stock were separately caged. 50 uCi of I-131 in normal saline was injected into the left wing vein of each bird and the time of injection was recorded. The birds were given their respective diets and water ad libitum. Twenty-two hours after I-131 administration, the birds were killed, and their thyroids were removed and homogenized in a Potter-Elvehjem glass-homogenizer in Tris-HCl buffer to give a solution of tissue concentration 40 mg/ml. 100 ul of this suspension was taken for counting of radioactivity in a well-type scintillation counter. Again, the count rates were corrected for background, coincidence loss and decay, and finally expressed as percentages of the corrected cpm of the original dose (50 μCi) of radioiodine.

In addition to the thyroid glands, portions of the liver of each birds were excised, weighed and counted for radioactivity.

Results:

The average absolute thyroid weights, total percentage thyroid uptake, percentage uptake per 100 mg thyroid tissue, and estimated percentage uptake per unit epithelial weight of I-131 for both 6 h and 22 h intervals are presented in Table 7. The marked increase of absolute thyroid I-131 uptake by the chronically RSM-fed birds were compatible with their enhanced accumulation of thyroidal iodide as determined by chemical analysis. When the 'I-131 uptake/g thyroid' data were calculated with the 'average percentage epithelium' of the respective glands as determined in experiment 1, estimated values of 'I-131 uptake/weight epithelium' were obtained.

Furthermore, it can be noted that while total thyroid I-131 uptake had dropped from 6 hours to 22 hours in the S-S and R-S birds, it remained high in the R-R and S-R groups.

There did not appear to be any difference in the liver I-131 concentration 22 hours after administration of the radioiodine (Table 7) among the birds from the different dietary treatments.

Table 7: Concentrations of radioiodine in the thyroid gland and liver 6 and 22 hours after administration.

Dietary treatment	Total thyroid radioactivity % injected dose		Thyroid radioactivity % injected dose /100 mg thyroid		Estimated thy. radioactivity % injected dose /mg epithelium		Liver radioactivity at 22 h
	6 h	22 h	6 h	22 h	6 h	22 h	cpm/g
S-S	9.1 ^{a}	7.8 ^{a}	4.7 ^{b}	4.2 ^{bc}	.194	.172	556
R-S	13.6 ^{a}	10.9 ^{a}	2.3 ^{a}	3.6 ^{bc}	.156	.247	397
R-R	35.4 ^{b}	40.6 ^{b}	1.5 ^{a}	2.2 ^{a}	.104	.143	694
S-R	30.1 ^{b}	42.4 ^{b}	2.7 ^{a}	3.0 ^{ab}	.134	.147	382
Level of significance	1%	1%	1%	5%			

^{abcd} values with the same superscript are not significantly different.

Experiment 4: THE DISTRIBUTION OF A SINGLE DOSE OF I-131 IN IODINATED SUBSTANCES IN THYROID GLANDS OF SBM- OR RSM-FED BIRDS

Since the chronically RSM-stimulated thyroid glands showed such marked enhancement of I-131 uptake when compared to the control, it might be pertinent to know how this accumulated radioiodine is distributed among the various iodo-compounds in the thyroid tissue. The determination of labeled iodoamino acid distribution in thyroglobulins is currently a common measure of thyroid hormone biosynthesis under physiological or pathological conditions (Mellen and Wentworth, 1959; Gattereau et al, 1971; Kobayashi and Greer, 1971, 1973; Burgi et al, 1974; Akiba and Matsumoto, 1973; Sorimachi and Ui, 1974). The present experiment was carried out to determine the effects of chronic and early goitrogenic stimulation by RSM on the distribution of I-131 radioactivity in thyroid hydrolysates after pronase digestion.

Materials and Methods:

Five birds (same as in experiment 3B) of each of four treatments (S-S, R-S, R-R and S-R) fed experimental diets as in experiment 1 were separated from the rest of the population. 50 μCi of I-131 (Atomic Energy, Ottawa) in 0.5 ml normal saline was injected intravenously to each birds. Twenty-two hours after injection, the thyroid glands were removed. The glands were weighed and homogenized in a Potter-Elvehjem glass-homogenizer in ice-cold Tris(hydroxymethyl)aminomethane-HCl buffer, pH 8.6 (Lamas and DeEscobar, 1972), containing 10^{-3}M thiouracil (Studer and Greer, 1968), to give a solution of tissue concentration 40 mg/ml.

The method for the measurement of I-131-labeled metabolites in thyroid tissue followed was a modification of that of Agerbaek (1972).

Two ml of well-mixed homogenate, tissue concentration 40 mg/ml, was placed in a Thunberg tube and 4 mg Pronase (subtilopeptidase-A, K and K Fine Chemicals) plus 2 drops of toluene as bacteriostatic agent were added. The tubes were evacuated and flushed with purified N_2 several times, nitrogen finally being allowed to remain in the tube at atmospheric pressure. During the evacuation procedure it was necessary to tap the tube briskly to control frothing. The digestion took place in the stoppered tube under nitrogen placed in a shaking water bath at 37°C for 21-24 hours.

Forty µl of digest suspension was removed immediately after vigorous shaking of the digestion tube and was applied on Whatmann 3 MM chromatography paper strips (4.5 cm wide). Application took place in a stream of cold, atmospheric air after which the chromatography paper was suspended in an airproof and temperature isolated jar where it was equilibrated with solvent for about 12 hours before chromatography was started. Freshly prepared chromatography solvent n-butanol (redistilled)-ethanol-ammonia 1N (5:1:2) was used. The ascending chromatogram was developed for about 16 hours at room temperature in the dark (Lamas and DeEscarbar, 1972). The chromatogram was then sprayed lightly with ferrichloride-potassium ferricyanide-arsenic acid (Gmelin and Virtanen, 1959) so that the chromatographic process could be visually monitored. The sections of the chromatograms corresponding to MIT, DIT, T_4 and T_3, together with the origin and intermediate areas, were excised and counted in a well-type scintillation counter. Origin I-131, as used in this study, referred not only to radioactivity remaining at the site

of application, but to all I-131 radioactivity up to the DIT band. The I-131 corresponding to each individual iodoamino acid was expressed as a percentage of the total I-131 radioactivity on the chromatogram.

A reference chromatogram was also obtained by spotting 1 ul of a standard solution (approximately 0.6%) of stable KI, MIT, DIT, T_3 and T_4 (Sigma Chemicals) together with a small amount of digest. The pattern of elution of these compounds in the chromatogram was identical to that given by Agerbaek (1972).

Several different solvent systems were tested such as n-butanol-acetic acid-water (4:1:5) used by Kobayashi and Greer (1971), collidine-3N ammonium hydroxide (3;1) used by Inoue and Taurog (1967), n-butanol-absolute ethanol-0.25N ammonium hydroxide (5:1:2) used by Kobayashi and Greer (1971), and n-butanol-ethanol-ethanol-ammonia 0.4N (5:1:2) used by Agerbaek (1972), before a final decision was made on n-butanol-ethan-1 N ammonia (5:1:2) which had been used by Mouriz et al (1966) and Lamas and DeEscobar (1972). Moreover, it was found necessary to redistill n-butanol and to use a freshly-prepared solvent system for each chromatography run.

Results:

Effects of the different dietary treatments upon the distribution of radioactive iodinated substances found in thyroid glands 22 hours after injection of I-131 are presented in Table 8. A quantitatively reversed relationship between the percentages of radioactivity in the iodotyrosines of the chronically RSM-stimulated thyroid tissues was evident: In the S-S and R-S thyroids, the majority of the I-131 was found in the DIT band, whereas in the R-R and S-R chromatograms, most of the radioactivity was located at the

MIT band. There was accordingly an increase in the MIT/DIT ratios of the RSM-stimulated thyroids. Furthermore, it appeared that RSM-stimulated birds had more radioactivity residing in the iodide band and at the origin, which probably represented degradative loss or heterogeneity of labeled iodoprotein with regard to hydrolysis (Inoue and Taurog, 1967; Kobayashi and Greer, 1975).

Upon spraying with ferrichloride-potassium ferricyanide-arsenic acid, the bands in the chromatograms appeared clearly visible except for T_3. Despite questions as to the presence of triiodothyronine in thyroglobulin of the aves (Vlijm, 1958; Sorimachi and Ui, 1974), I-131 radioactivity was definitely located in the region corresponding to the T_3 band of the reference chromatogram. The amounts of T_3 and T_4 present were, however, relatively small and have therefore been combined in the table (Table 8).

There appeared to be an additional band in all the chromatograms of the S-S and R-S groups, which was absent from the R-R and S-R patterns (Figure 1). This band was close but distinctly separated from the I^- band, between the I^- and the DIT levels. In the absence of indentification, it was designated by (?) in Table 8. It might be a hitherto unrecognized iodoamino acid (diiodothyronine, T_2?) in the chromatogram or a more experimental artefact of the chromatography system.

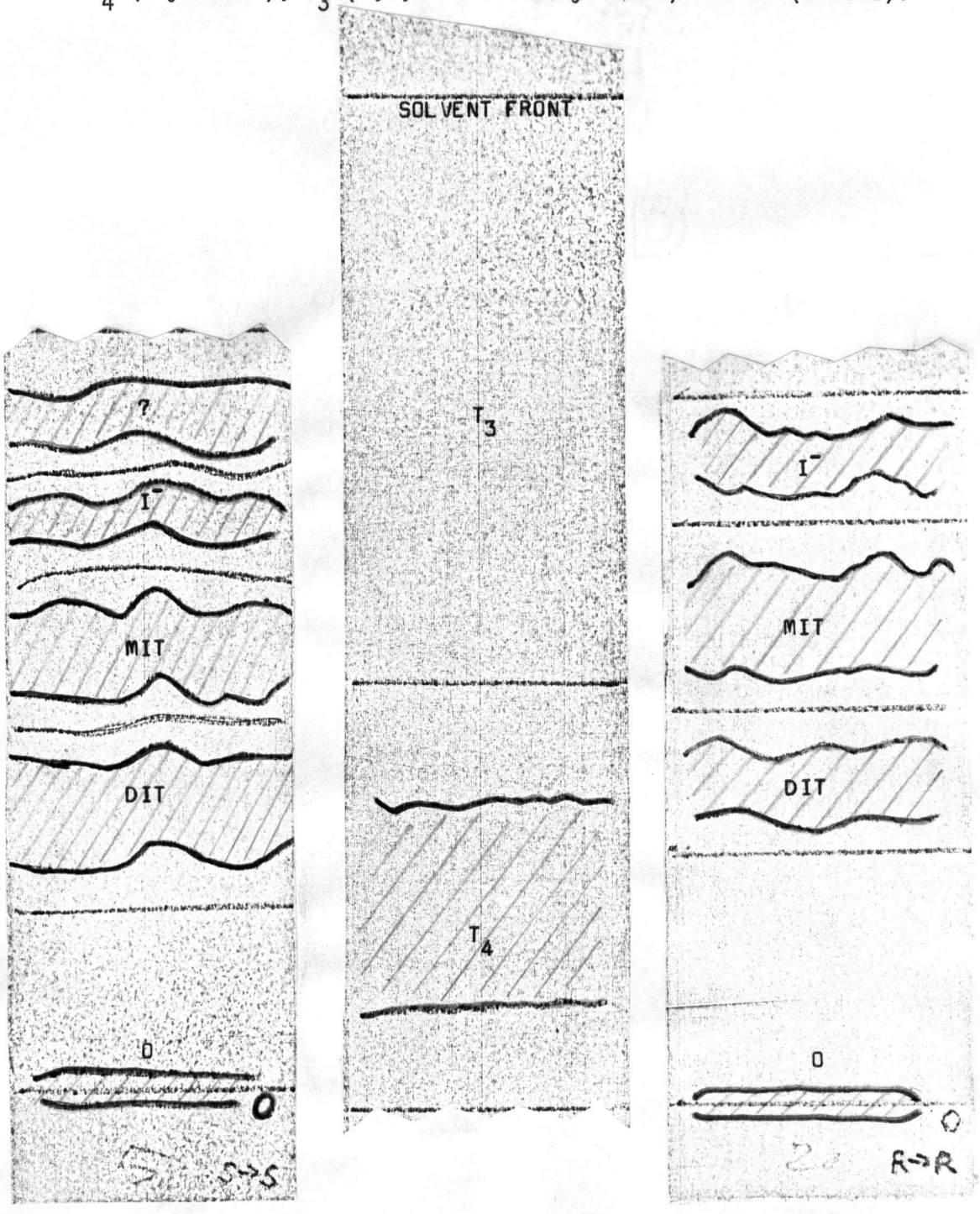
Coupling efficiency, as calculated by $T_3 + T_4/(MIT/DIT)$, was lower for the RSM-stimulated birds. Once again, as consistent with the other parameters of thyroid metabolism measured, the order of inhibition in thyroid hormone biosynthesis, as represented by the pattern of distribution of labeled metabolites in the thyroid tissues, appeared in the following order: R-R>S-R>R-S>S-S.

Table 8: Distribution, 22 hours after administration, of a single dose of radioiodine into thyroidal compounds.

Dietary treatment	Origin*	% of total thyroidal activity					MIT/DIT	$\frac{T3+T4}{MIT/DIT}$
		I^-	MIT	DIT	?	T_3 & T_4		
S-S	5.0	1.2	40.7	47.9	1.9	3.3	0.85	3.89
R-S	5.3	1.4	42.7	45.3	2.0	3.3	0.95	3.43
R-R	8.3	2.8	49.1	36.8	-	3.0	1.34	2.28
S-R	6.4	2.1	49.0	39.5	-	2.9	1.27	2.39

* Represents total radioactivity from line of application to band of DIT.

Figure 1: Paper chromatograms of thyroidal metabolites of birds on different dietary treatments. Chromatographic solvent: n-butanol-ethanol-ammonia 1N (5:1:2). Abbreviations used: O (origin), DIT (3,5-diiodotyrosine), MIT (3-monoiodotyrosine), T_4 (thyroxine), T_3 (3,5,3'-triiodothyronine) and I^- (iodide).



S - S
R - S

R - R
S - R

Experiment 5: EFFECTS OF GOITROGENIC RSM ON SUSCEPTIBILITY TO HYDROLYSIS OF THYROGLOBULIN

Heterogeneity of the thyroid follicles has often been discussed in the literature with regard to the rate of iodine turnover (Sneider, 1964), and heterogeneity of the iodoproteins in the thyroid gland have long been recognized (Tarutani and Ui, 1969a). More recently, it has been suggested that thyroglobulins stored in the colloid may perhaps also be heterogenous with regard to the release of thyroid hormones (Simon et al, 1966; Tarutani and Ui, 1969b; Nunez et al, 1966; Koyayashi and Greer, 1971; Kohler et al, 1971; Rosenberg et al, 1964).

Since our finding (experiment 4) of a larger percentage of I-131 radioactivity residing in the I^- and origin regions of the chromatograms of the RSM-stimulated thyroid tissues, it was considered relevent to test whether there was indeed a difference in the susceptibility to proteolysis among the iodoproteins of the various treatment groups. The following experiment was designed to demonstrate any heterogeneity in the thyroid tissues of RSM- and SBM-fed birds with reference to the rate of hydrolysis by pronase.

Materials and Methods:

White Leghorn cockerels, 8 weeks of age, fed the SBM- or RSM-supplemented diets same as those for the RSM goitrogenicity test in experiment 1 were used. Six birds each of the two treatments (SBM and RSM) were put in separate cages and given water and their respective diets ad libitum. 50 μCi of I-131 in 0.5 ml normal saline was injected into the left brachial vein of each. Twenty-two hours after radioiodine administration, three birds

from each treatment were killed and their thyroids removed. Each pair of glands was homogenized in the same manner as in experiment 4, and subjected to pronase digestion in a water bath at 37°C. Four hours after its commencement hydrolysis of six samples was stopped (three from each treatment) and 40 µl of the digest suspension was transferred for chromatography as described in experiment 4. The other six samples were similarly treated after 24 hours of pronase digestion.

Results:

Percent distribution of radioactivity in thyroid tissues of SBM- or RSM-treated cockerels after four or twenty-four hours of pronase digestion are given on Table 9. That digestion was not complete by 4 hours was evident by the high percentage of radioactivity remaining at the origin of the chromatograms. However, there was no difference in the percentages of radioactivity remaining at the origin. After 24 hours of hydrolysis, the MIT/DIT ratio of the RSM-fed birds was markedly increased over that of the control. There also did not appear to be any difference in the percentages of I-131 radioactivity corresponding to the iodothyronines or the 'origin' bands in the chromatograms of the thyroid tissue of the RSM- or SBM-fed birds.

The distribution of radioactivity in iodinated substances in the thyroid tissue of young cockerels in the present experiment was comparable to that reported for growing chicks by other investigators (e.g. Akiba and Matsumoto, 1973).

Table 9: Distribution, 22 hours after administration, of a single dose of radioiodine into thyroidal compounds measured in hydrolyzates after different times of pronase digestion.

Dietary treatment	Origin	% of total thyroidal radioactivity I^-	MIT	DIT	T_3 & T_4	MIT/DIT
After 4 hours pronase hydrolysis:						
SBM	16.2	6.1	29.7	41.3	6.7	0.72
RSM	15.0	6.5	43.8	24.3	10.4	1.81
After 24 hours pronase hydrolysis:						
SBM	7.0	6.0	33.2	46.8	9.0	0.72
RSM	8.1	7.2	44.3	28.2	12.2	1.58

Experiment 6A: EFFECTS OF GOITROGENIC RSM ON THE IODINE CONTENT OF EGG YOLK

A direct relationship between the iodine content of the dam's diet and the iodine concentration in the egg has long been known (Hercus and Roberts, 1927; Simpson and Strand, 1930; Wilder et al, 1933; Asmundson et al, 1936) and the effects of iodine deficiency on embryo development and hatchability have been clearly established (Hollander and Riddle, 1943, 1946; Wilgus et al, 1953; Bennett and Adams, 1952; Vidal, 1952; Adams and Buss, 1952; Kingsbury et al, 1955; Romanoff and Laufer, 1956; Perdomo et al, 1966). Thyroid histology of the newly-hatched progeny showed hypertrophy of the epithelium and a lack of colloid, which could be reversed by addition of potassium iodide to the dam's diet.

Thyroidal hypertrophy was reported from this laboratory in the progeny from dams chronically fed a RSM-supplemented diet (March et al, 1972). The goitrous condition was alleviated when the young chicks were fed a diet normally supplemented with iodine. The resemblance between the progeny from the chronically RSM-treated birds to those from dams fed an iodine-deficient diet led to the assumption of an iodine deficiency in the eggs of the former group. This was further supported by the failure to demonstrate any deposition of goitrin in the eggs of the RSM-fed birds or any difference in isothiocyanate content of the eggs of these birds when compared to control (March et al, 1972).

Analysis of iodine content of the diets, however, did not reveal any deficiency of this element in the RSM-supplemented ration (experiment 1). Since the egg production and hatchability of these birds fed chronically with

the RSM-supplemented diet were affected (March et al, 1975) and the progeny from them consistently showed thyroid hypertrophy at hatching (experiment 8), the following experiment was conducted to determine iodine concentration in the eggs from the different treatments and hopefully to elucidate the materno-embryonic relationship in the iodine metabolism of the chronically stimulated birds.

Materials and Methods:

Five eggs from each of two replicate pens of the birds fed the diets of experiment 1 were collected and their yolks were pooled. Quadriplicate samples (0.1 - 0.2 g) of the yolk pools were ashed with 1 ml 4N sodium carbonate and assayed for iodine according to the modified Barker procedure as described in experiment 2 for the assay of iodine in the diets.

Despite long hours of ashing at 625 $\pm$ 25°C, the ash in the tubes still appeared gray instead of white. This was expected (probably due to the high lipid content of the yolk) and the incineration was deemed adequate to put all of the organic iodine into extractable inorganic form (Barker et al, 1951). It was found necessary, however, to filter the ash and acid mixture to eliminate fluctuations in the optical density readings due to undissolved particles. This could be efficiently carried out in a fritted-glass filter connected to a water pump. If this was not available and filter paper was used, it would be necessary to correct for the absorbance due to the filtrate. Precision of the assay system depended largely on how this problem was overcomed.

The decision to analyze only the yolk of eggs for iodine and not the albumin was based on preliminary findings in this laboratory (unpublished data) that iodine was mostly concentrated in the yolk (Also: Romanoff and

Romanoff, 1949). Total iodine content was preferred to protein-bound iodide(PBI) measurement because the latter might not be an appropriate parameter in estimating avian iodine metabolism.

Results:

Iodine concentrations in the yolks of eggs from birds on the different treatments are shown in Table 10. The egg yolks from the control birds contained 0.27 µg/g of iodine. This value is in agreement with average values given in the literature (Romanoff and Romanoff, 1949). The data clearly established that the chronically RSM-treated birds produce eggs with a lower iodine concentration. The yolks of R-R eggs contained less than 0.09 µg/g iodine, a value which was estimated from the lower limit of the working range of the assay under the described experimental conditions. A larger yolk sample from this pool would not be appropriate because of the difficulty that might be encountered during the incineration process.

Again the iodine concentration in the S-R egg yolks was intermediate between that of the R-R and S-S egg yolks. The concentration in the R-S eggs was not statistically different from that of the control.

Table 10: Iodine concentrations in egg yolks from birds on different dietary treatments in experiment 6A.

Dietary treatment	Iodine concentration in egg yolk, ppb
S-S	273 [a]
R-S	286 [a]
R-R	< 85
S-R	144 [b]

[ab] values with the same superscript are not statistically different, $p<0.01$

Experiment 6B: EFFECTS OF INCREASED DIETARY IODINE INTAKE ON IODINE DEPOSITION IN EGG YOLKS OF SBM- AND RSM-FED BIRDS

The findings that the chronically RSM-stimulated birds produced eggs with a significantly lower iodine content (experiment 6A), despite apparently comparable dietary iodine intake (experiment 1) compared to the control, led to the hypothesis that the peripheral blood circulation of these birds were also low in iodine concentration. Since measurement of serum PBI content would not be an appropriate parameter in the aves, it was postulated that by increasing the dietary intake of iodine, the RSM-stimulated birds would show a corresponding increase in the deposition of this element in their eggs. The following experiment was conducted to elucidate this point.

Materials and Methods:

One hundred and ninety-six Babcock layers, 44 weeks of age, were divided into four groups and individually caged. They were given one of the following experimental diets:

R-1: SBM-supplemented diet as in experiment 1;

R-2: R-1 plus potassium iodate (0.3 mg iodine/kg);

R-3: RSM-supplemented diet as in experiment 1;

R-4: R-2 plus potassium iodate (0.3 mg iodine/kg).

When the birds had been on experiment for 3 weeks, six eggs from each treatment were collected from birds of comparable laying performance. The yolks were weighed and individually assayed for iodine by the Barker

procedure as described in experiment 6A with one further modification: six-ml aliquots from the 7 ml mixed acid solutions containing the ashed samples were pipetted for the assay instead of 3 ml aliquots, in order that the presumably low iodine concentration of the RSM-treated yolks could also be measured.

The iodine content of the diets were again determined.

Results:

Effects of a further addition of 0.3 mg I/kg ration to the SBM- and RSM-supplemented diets are shown in Table 11. Values for both iodine concentration and total iodine content in the yolks are given. Even in this short-term feeding study, less iodine was deposited in the eggs of the RSM-fed birds than in those of the SBM-fed birds (41 ng/g vs 105 ng/g). When the dietary iodine level was increased by an additional 0.3 mg per kg ration, in the form of potassium iodate, the respective iodine concentration of the SBM- or RSM-treated egg yolks were also elevated correspondingly. The difference between the total iodine content of the R-2 and R-4 eggs was highly significant ($p<0.01$), indicating that even at an elevated dietary iodine uptake, while the SBM-fed birds showed a drastic increase in their egg iodine accumulation, a significantly small amount of this element was deposited in the eggs of the RSM-stimulated birds.

The iodine concentrations of the different diets are also shown in Table 6. The addition of 0.3 mg I/kg ration in the form of potassium iodate was recovered during the iodine assay, thus validating the analytical

procedure used in this and the previous experiments. It should be noted that small samples (0.1 - 0.2 g) only were used for the assay procedure, so that further modifications of the method (such as addition of more than 1 ml 4N sodium carbonate) would not be necessary that might interfere with the precision of the assay.

Table 11: Iodine concentrations in egg yolks from birds on different dietary treatments in experiment 6B.

Dietary treatment	Iodine concentrations in egg yolk, ppb
SBM - diet	105 ^{aA}
SBM - diet plus KIO_3	242 ^{b}
RSM - diet	41 ^{aB}
RSM - diet plus KIO_3	73 ^{a}

^{ab} values with the same superscript are not statistically different, $p<0.01$.

^{AB} significantly different by separate comparison, $p<0.01$.

Experiment 7: OVARIAN UPTAKE OF A SINGLE DOSE OF I-131 BY DEVELOPING FOLLICLES

The growth of the avian ovarian follicle has been extensively studies (e.g. Stieve, 1918; Warren and Conrad, 1939; Romanoff, 1943). Experiments with isotopic tracers have shown that the levels of dietary iodine could affect the development of ova of laying hens (Marcilese et al, 1968). Since the total iodine content was found to be lower in the RSM-fed birds (experiments 6A and 6B), the following experiment was conducted to determine the relative radioiodine uptake of these birds as compared to the control.

Materials and Methods:

The same birds of experiment 4 were employed. Twenty-two hours after the administration of 50 μCi of I-131, the birds were killed and the five largest ova were removed. Preliminary tests in which the labeled ova were plunged into boiling water, cut up and counted piece by piece revealed that most of the radioactivity of the newly-deposited I-131 was concentrated to a ring towards the periphery of the ovum. Therefore in the present experiment where a hundred (5 ova x 5 birds x 4 treatments) ovarian follicles were involved, each ovum was mixed thoroughly by repeated stirring with a glass rod, and 0.1 to 0.5 g aliquots of the mixed yolks were pipetted to tubes for counting in a well-type scintillation counter. Individual weights of the ova were recorded, and total I-131 uptake was expressed as a percentage of the count rate of the original dose. Moreover, permeability of the ova to I-131 was also calculated, according to the equation of Smith (1959) which expressed the activity of the yolk relative to the surface area of the follicle and the time since injection, i.e. permeability = mμCi/cm^2/h.

Results:

The values for total percent I-131 uptake by the five largest ova in an average of 5 birds per treatment are presented in Table 12. Since the net transfer rate of yolk materials into the developing yolk was not linear, the data on the permeability to I-131 and the surface area of the ovarian follicles were transformed to the logarithmic function and analyzed by multiple regression to give the plot shown in Figure 2. The regression equations and R^2 values were computed as follows:

Treatments	Regression Equations	R^2
S-S	$\log Y = -1.1958 + 2.7086 \log X - 0.0983 X$	0.78
R-S	$\log Y = -0.9731 + 2.1800 \log X - 0.0785 X$	0.75
R-R	$\log Y = -1.4432 + 2.7948 \log X - 0.1093 X$	0.83
S-R	$\log Y = -1.2304 + 2.1641 \log X - 0.0857 X$	0.63

Table 12 also compares the data obtained when laying birds were injected with radioiodine and the radioactivity of the thyroid glands and the five largest ova in the ovary measured after 22 hours. Radioiodine concentrations in the thyroid glands of the RSM-fed birds were approximately four times those in the SBM-fed birds. Average radioiodine concentration appeared higher in the thyroids of the SBM-fed birds that had been fed RSM during the growing period than in the birds that were fed SBM throughout life. Radioiodine concentration was lower in the ova from the RSM-fed birds than in the SBM-fed birds. The ova from SBM-fed birds contained less radioiodine when the birds had been fed RSM during the growing period and ova from the RSM-fed birds contained less radioiodine when the birds had been fed SBM during the growing period. The amounts of radioiodine transported into the individual

ova relative to the surface area of the ova in the birds on the different treatments are shown in Figure 2.

The transfer rate of I-131 per unit of follicle surface area was maximal in the 10 to 12 cm^2 stage and decreased more-or-less linearly until ovulation. This is in agreement with changes in the functional capacity of the follicular wall, which is not uniform but reaches an early maximum and then declines (Smith, 1959). The rate of uptake of I-131 by developing ova of laying hens was recently determined by Pena et al (1975), which conformed well with our present finding.

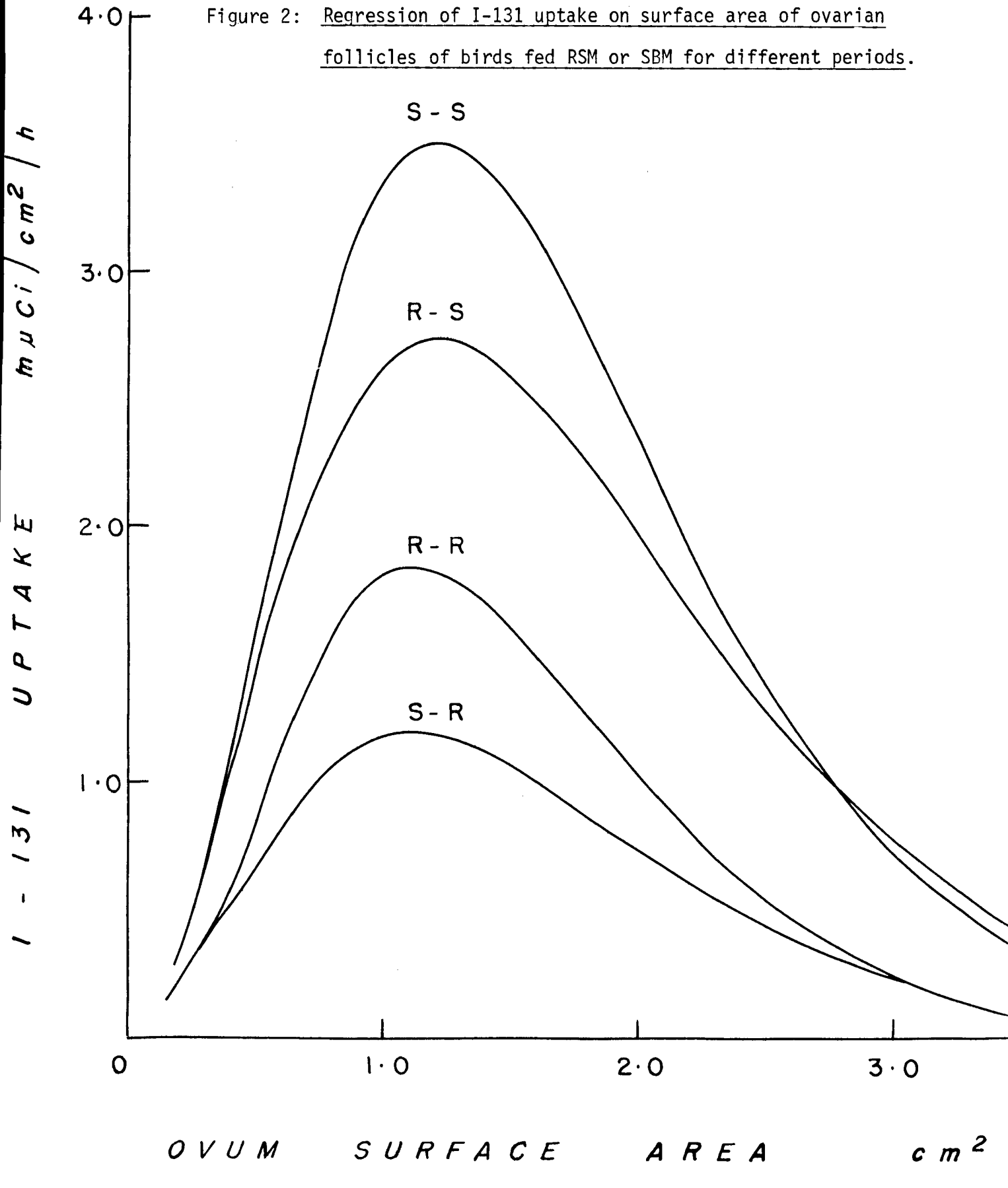
Table 12: Radioiodine concentrations in the thyroid glands and developing ovarian follicles 22 hours after administration of a single dose of radioiodine to birds which had been fed RSM or SBM for different periods of time.

Dietary treatment	Ave. radioiodine in thyroid gland % of injected dose	Ave. total radio-iodine in 5 largest ovarian follicles % of injected dose*
S-S	7.8 [a]	6.7 [a]
R-S	10.9 [a]	5.8 [a]
R-R	40.6 [b]	2.8 [b]
S-R	42.4 [b]	2.6 [b]

* average of determinations on five birds of each treatment.

[ab] values with the same superscript are not significantly different, $p<0.01$.

Figure 2: Regression of I-131 uptake on surface area of ovarian follicles of birds fed RSM or SBM for different periods.



Experiment 8: EFFECTS OF CHRONIC AND EARLY GOITROGENIC STIMULATION BY RSM IN THE DAM ON EMBRYONIC THYROID DEVELOPMENT

Since the iodine concentrations in eggs from RSM-stimulated birds were found to be lower than in eggs from the control birds (experiment 6A), and that the rates of deposition of this element in the developing ova of the same birds were lower than of those in the S-S birds as shown by the radioisotope study (experiment 6B), it would be reasonable to postulate thyroid hypertrophy when the progeny from these birds were hatched. The thyroid glands of newly-hatched progeny from dams fed goitrogenic RSM have been previously reported to be goitrous from this laboratory (March et al, 1972). The present experiment was performed to confirm this previous finding and also to elucidate any differences in the histological picture of the thyroid glands of the progeny from the chronically- or early-RSM-stimulated dams.

Materials and Methods:

Seventy-five birds each of the four treatments, 91 weeks of age, fed the same diets of experiment 1 were artifically inseminated and, after 4 days, eggs were collected and incubated. At hatching, 20 chicks from each treatment were killed and their thyroid glands removed and weighed. Thyroid glands from another 5 chicks per treatment were fixed in buffered 10% formalin for histological study as in experiment 1.

Results:

Thyroid weights of the progeny from each treatment are given in Table 13. Although not statistically significant, the weights were in the same order of magnitude as in the previous test conducted on progeny of dams 77 weeks of age. In this former test, the thyroid glands from the progeny of the R-R and S-R groups were significantly larger than those of the control ($p<0.01$).

The histological characteristics of the thyroid glands of newly-hatched progeny of birds fed RSM- or SBM-supplemented diets for different periods are given in Table 14. The percentages of epithelium in the thyroids of progeny from the RSM-fed dams were greater than from the progeny of the SBM-fed dams. The values for absolute epithelial weights were calculated by multiplying percentages epithelium with the average absolute thyroid weights of the respective groups. The average absolute weight of the thyroidal epithial tissue was greater in the progeny of the SBM-fed dams when the birds had been fed RSM during the growing period. Furthermore, the average absolute weight of the thyroidal epithelial tissue was lower in the progeny of the RSM-fed dams when the birds had been fed SBM during the growing period. The increased average follicular diameter with lesser number of follicles across the gland in the thyroids of the progeny from the chronically RSM-stimulated dams indicated follicle hypertrophy in these glands.

The histological characteristics in the thyroid glands of newly-hatched progeny and their dams are compared in Table 15.

Table 13: Thyroid weights at hatching of progeny birds fed RSM or SBM for different periods in experiment 8.

Dietary treatment	Ave. thyroid wt. of progeny when dams were 77 wks. of age * mg/100 body wt.	Ave. thyroid wt. of progeny when dams were 91 wks. of age (expt. 8) mg/100 body wt.
S-S	11.7 [a] (74)**	13.8 [a] (20)
R-S	12.6 [a] (75)	14.1 [a] (20)
R-R	16.1 [b] (98)	15.9 [a] (20)
S-R	14.3 [c] (73)	14.0 [a] (20)

* unpublished data from an earlier determination on the same population.

** number of determinations in parenthesis.

[abc] values with the same superscript are not significantly different, $p<0.01$.

Table 14: Thyroidal characteristics in newly-hatched progeny of birds fed RSM or SBM for different periods in experiment 8.

Dietary treatment	% epithelium	Ave. ht. epithelium μ	Ave. wt. epithelium mg	Ave. no. follicles across the gland	Ave. follicle diameter μ
S-S	43.0[b] (5)*	3.86 [a]	2.55	57	31.7
R-S	49.2[ab] (5)	4.76 [ab]	3.10	48	35.1
R-R	55.8[a] (5)	4.71 [ab]	4.03	47	39.6
S-R	55.1[a] (5)	5.09 [b]	3.41	51	31.9

* number of thyroid glands examined histologically.

[ab] values with the same superscript are not significantly different, $p<0.05$.

Table 15: Comparison of thyroidal characteristics of dams and progeny on different dietary treatments.

Dietary treatment	Ave. absolute thyroid wt		% epithelium		Ave. wt. epithelium		Ave. ht. epithelium	
	Dam	Progeny	Dam	Progeny	Dam	Progeny	Dam	Progeny
	g	mg	%	%	mg	mg	μ	μ
S-S	0.19	5.94	24.4	43.0	41.1	2.55	7.13	3.86
R-S	0.52	6.30	14.8	49.2	76.5	3.10	5.72	4.76
R-R	2.30	7.21	15.1	55.8	334.6	4.03	10.65	4.71
S-R	1.39	6.19	20.1	55.1	277.4	3.41	10.11	5.09

DISCUSSION

THYROIDAL RESPONSE TO CHRONIC GOITROGENIC STIMULATION BY RSM

The long-term effects of continuous feeding of goitrogenic RSM resulted in continuous increase in the size of the thyroid gland and in disruption of the epithelial tissues. The thyroid glands of the chronically RSM-fed birds were heavier when measurements were made at 91 and 99 weeks of age (experiment 1) than when the measurements were made at only 58 weeks of age in a previous experiment (Table 3). Although the birds were able to maintain production and secretion of thyroid hormone the compensatory increase in thyroid secretion was not accomplished with a fixed number of cells. The large amount of colloid present in the glands of the RSM-fed birds should not be considered contrary to the findings of Clandinin et al (1966) or Matusmoto et al (1968) since in these experiments the birds had been fed goitrin for a few weeks only when histological studies were made.

It has long been known that under continuous stimulation the epithelial cells are unable to maintain the hyperactive state and eventually become disorganised in the follicle with desquamation and disintegration being evident (Marine, 1935). Generally the amount of colloid in such over-stimulated glands is very small and the follicles become reduced in size as cells eventually die. However, this result follows because the cause of overactivity is usually a deficiency of iodine or the inability of the thyroid to trap the iodine or to produce the hormones. This apparently is not the case in the present study of the chronic goitrogenic effects of RSM.

It has already been mentioned that defects of thyroidal iodine metabolism may be caused by goitrogenic disturbances at any intermediary step or steps of thyroid hormone biosyntheis and/or secretion.

Thyroidal iodine uptake seems to be enhanced in the RSM-fed birds when compared to S-S controls. This is supported by the iodine concentrations in the thyroid glands of these birds, measurement of thyroidal I-131 uptake at different time intervals after administration of the radioiodine, as well as by the histological appearance of these tissues. Although the average iodine concentration in the thyroids of the chronically RSM-fed birds is less than half of that of the control (0.89 mg/g in R-R and 1.08 mg/g in S-R group), the absolute iodine content of these goitrous glands are more than three times that of the control (experiment 2). Similarly, the relative thyroid I-131 uptake (thyroid radioactivity percentage of injected dose/100gm thyroid) is low for the RSM-fed birds, but the total I-131 uptake in the thyroid glands of the latter groups is dramatically increased to three- or four-fold (experiment 3). This conforms with the finding of Matsumoto et al (1968) on the effects of goitrin in growing chicks. According to these investigators, in addition to maintaining a large amount of incorporated I-131 in the thyroid gland, the goitrin-fed chicks also show a slow thyroidal release of the injected radioiodine. This may perhaps explain our finding that in birds of chronic RSM treatments uptake of a single dose of radioiodine at 22 hours was greater than at 6 hours. This was in contrast to the birds on treatments S-S and R-S in which radioactivity had, by 22 hours, declined from the amount present 6 hours after injection. The time of maximum radioactivity, following administration of radioiodine, which is determined by the rate of release of

iodine relative to the rate of uptake, occurred later in the RSM-fed birds than in the SBM-fed birds.

Related to the active process of iodine accumulation of the thyroid gland of RSM-fed birds is its histological picture. The functional hyperactivity is demonstrated by the massive content of epithelium in their hypertrophic follicles. The average epithelial height of these glands is also significantly higher than that of the control. The colloid in the follicular lumen of these glands appear to be brightly stained, which is in agreement with their high iodine content. Although the occurrence of epithelial cells sloughing off into the lumen are higher in the chronically RSM-fed birds than in the control, no sign of follicular atrophy can be found. Thus it can be said that these birds are hyperactive in the thyroidal clearance of iodine from the blood circulation.

Goitrogenic inhibition of conversion of iodide accumulated by the thyroid into its active form and further to MIT and DIT has been extensively investigated (Stanbury et al, 1955; Haddad and Sidbury, 1959; Glayton et al, 1958; Floyd et al, 1960; Parker and Beierwaltes, 1961; Baschieri et al, 1963). According to these investigators, such an iodide organization defect is characterized by a predominance of inorganic iodine and scarcity of iodoamino acids when the thyroid tissue hydrolyzed, possibly due to failure of iodination of tyrosine by thyroidal peroxidase. In our present investigation in which the pre-labeled thyroid glands of the chronically RSM-fed birds were hydrolyzed and the iodoamino acids fractionated (experiment 4), the fractions of MIT and DIT were large and there was little inorganic iodine 22 hours after administration of radioiodine. Thus the iodide organization process is probably not disturbed in the RSM-fed birds.

That thyroid hormone biosynthesis is indeed inhibited in the RSM-fed birds is evident by the results obtained on fractionation of the iodoamino acids in thyroid hydrolysates. The distribution of I-131 radioactivity among various thyroid metabolites shows that the proportion of radioiodine present as MIT, 22 hours after administration, is greater in the RSM-fed birds. The subsequent increase in MIT/DIT ratio is held commonly as an indication of defect(s) in thyroid hormonogenesis. Coupling efficiency i.e. $T_3 + T_4/(MIT/DIT)$, as a measure of the combined rates at which diodotyrosine and thyroid hormones are produced is, in order, SS>RS>SR>RR and is inversely proportional to the total amount of iodine in the thyroid gland. However, since the percentages of thyroid iodothyronines in the RSM-fed birds are not lower to any significant extent when compared to the control, it is not likely that the coupling mechanism of MIT and DIT to iodothyronines is inhibited per se by the feeding of goitrogenic RSM. This seems to be in agreement with the finding of Akiba and Matsumoto (1971) that although the synthetic activity of the iodothyronines in goitrin-fed chicks is about two-third of the control at 24 hours after dosage of radioiodine, the amount of iodothyronines synthesized in these chicks is about two times that of the control. In the present study, the slightly lower percentages of thyroidal iodothyronines in the RSM-fed birds, when multiplied by the 10- to 13-fold increase in the weights of these glands over the control, will give estimated iodothyronine values larger than that of the control.

An inhibitory site would appear to be at the formation level of iodotyrosines, since it is shown that the MIT/DIT ratio is increased in the thyroids of the RSM-fed birds, while the coupling deficiency in the R-R and S-R groups is also depressed. Whatever the site of inhibition, however, it appears that these chronically stimulated birds have fully compensated for the defect by thyroid hypertrophy and hyperplasia, most likely involving the

secretions of the hypothalamus-pituitary-thyroid axis. An unreported experiment using the chick method of TSH bioassay (Bates, 1962) and histological examination of the pituitary glands of these birds failed to be conclusive in this respect. Further investigations in this area are necessary before the neurohormonal relationships controlling thyroid metabolism of the RSM-fed birds will be elucidated.

In the normal thyroid, synthesis and secretion are taking place under "steady state" conditions near the border of the epithelial cell in the follicles. This equilibrium is disturbed almost immediately after the introduction of a goitrogen. Under the influence of the early rise in TSH, new fractions of thyroglobulin take part in the hormonal exchange and the I-131 mixes with a different pool than in the "resting", iodine-rich thyroids. Thyroid secretion under these conditions of iodine deficiency could thus follow rather complicated rules.

Therefore, a more probable site of inhibition is at the release and secretion of iodothyronines into the circulation, and this is supported by the work of Akiba and Matsumoto (1971) on the effect of goitrin on the thyroid hormone biosynthesis in growing chicks. Particularly, it is presumed that the secretion of thyroid hormone from the gland by hydrolysis of thyroglobulin is depressed or that there is no increment of hydrolysis of thyroglobulin by the apparent increased secretion of TSH from the pituitary caused by the feeding of goitrin. Under these circumstances the thyroid glands were capable of continuous hyperplasia for the duration of the experiment. The situation may be similar to that when a small amount of thyroxine is administered to birds in which thyroid hormone production is blocked by administration of thiouracil. Shultze and Turner (1945) found that the goitrogenicity of thiouracil is enhanced when a small amount of thyroxine is administered simultaneously with the thiouracil. In the present study, the hydrolysis rate by extraneous

pronase does not appear to be any slower in the thyroid lobes of the RSM-fed birds than the control (experiment 5). How this can be applied to endogenous proteolysis of thyroglobulin by thyroid enzymes, however, is undetermined. Hence the inhibitory effect of goitrogenic RSM on the release of hormones from thyroglobulin and subsequent secretion from the thyroid gland remains a possibility.

Due to a defect in iodotyrosine deiodinase, MIT or DIT may not be deiodinated and a large amount of iodotyrosine may be lost in the urine, resulting in a disturbance in reutilization of inorganic iodine in iodide organization (Stanbury, 1957; Stanbury and Morris, 1958). Such a possible effect by goitrogenic RSM on the deiodination process has not been investigated in the present study. Although counting of excised pieces of liver 22 hours after administration of radioiodine has not revealed any notable difference in the I-131 concentration of the livers of the birds on the different dietary treatments, the relative rates of removal of iodinated compounds by these organs cannot be known from these data. However, a defect in the deiodination process due to the feeding of goitrogenic RSM is unlikely, at least for triiodothyronine. Unpublished data from this laboratory has shown that when a single dose of I-131-labeled T_3 was administered into RSM-fed birds, the rate of I-131 turnover, measured by radioactivity of the excreta 24 hours or 48 hours after injection, is not different from that of the control. Hence the deiodination capacity in the RSM-fed birds does not appear to be inhibited.

In chronic experiments with hamster, it was found that in severe iodine deficiency a part of the stored radioactivity in the thyroid could not be discharged even after TSH stimulation and PTU treatment. This indicated altered, abnormally strong, protein binding of the intrathyroidal iodoamino acids under certain conditions (Van Middlesworth, 1965; Follis, 1965). In the present case, the possibility that the defects in thyroid function due to

formation of abnormal iodoprotein in the thyroid gland is unlikely. This abnormal iodoprotein other than thyroglobulin in the thyroid is water-soluble and butanol-insoluble, so that on paper chromatography with butanol, it will not migrate from the origin (Stanbury and McGirr, 1957; DeGroot et al, 1958; DeGroot and Stanbury, 1959; Van Wyk et al, 1962). No such protein was detected in the thyroid glands of the present study. The percentages of radioactivity remaining at the origin on the chromatograms of the thyroid hydrolysates are similar and within normal ranges for all birds on the four dietary treatments.

It is therefore hypothesized that chronic feeding of goitrogenic RSM to birds may interfere with thyroid function, probably at an intermediary step involving the formation of iodotyrosines and/or the release or secretion of the thyroid hormones. One or more enzyme systems such as thyroid peroxidase in the thyroid gland may be involved. Disorders of these systems have been considered to cause disturbances of thyroid hormone production and provoke compensatory enlargement of the thyroid with high uptake of iodine (Fukase et al, 1967). Thus, in the chronically RSM-fed birds, a disturbance of thyroid hormone biosynthesis and/or release due to an intrinsic enzyme alteration is strongly suggested. The histological picture and radioiodine metabolism in the thyroids of these birds suggest hyperactivity, and this conforms with the hypothesis that increases in enzyme activities tend to occur when the enzymes are stimulated over a period of time (such as that may occur in chronic RSM treatment), and that once the enzyme pattern changes, the patterns of substrate disposition tend to become fixed (Chance et al, 1965). This idea on the "biochemical imprinting" of metabolic experience in cells signifies adaptive increases in enzyme activities that occur along specific metabolic pathways when there is a large and sustained increase in substrate traffic through them, and may well explain the hyperfunction of the thyroid of the

R-R and S-R birds which continuously accumulate iodine of the circulation for organification. The experience of accomodating large amounts of substrate is often reflected in a characteristic imprint on the enzyme pattern of the cell. Moreover, changes in enzyme pattern probably constitute a long-range metabolic control device which is super-imposed on other regulatory mechanisms such as the hypothalamus-pituitary-thyroid axis or the feedback mechanisms, which operate within a much shorter time scale.

PERSISTENCE OF EFFECTS OF EARLY GOITROGENIC STIMULATION

The various parameters of thyroid activity show that the effects of early goitrogenic stimulation of the thyroid gland remained apparent in later life. Thus thyroid glands remained enlarged in birds which had been fed RSM during the growing period and in birds given thiouracil for only the first four weeks of life (Table 3). Thyroid glands examined from birds 67 weeks after withdrawal of RSM from the diet had the histological appearance of colloid goiter. In these birds the thyroid follicles were larger and the height of the epithelial cells lower. This persistence of effect of early over-stimulation of the thyroid is in agreement with the findings of Bakke et al (1970) in the rat. In the rat brief perinatal hypothyroidism induced by administration of propylthiouracil to the pregnant dam and/or to the neonate resulted in a persistently enlarged thyroid gland with larger follicles and lower epithelial height. These investigators reported also that perinatal hypothyroidism caused a delay in puberty. Earlier experiments in this laboratory has revealed no evidence that the ingestion of goitrin by chickens up to 24 weeks of age affected the time of sexual maturity or reproductive capacity when RSM was withdrawn at this time (March et al, 1975). Thyroidal enlargement was related to the length of time over which the goitrogenic RSM

was fed. The follicle size and proportion of epithelial tissue were lower in the thyroid glands of the R-R birds than in the S-R birds at 91 weeks of age.

The total amount of iodine in the gland of the R-S birds was greater than that in the control S-S birds but the concentration per unit weight of gland was similar (experiment 2). Consistent with this observation was the greater total uptake of a single dose of radioiodine by the thyroid glands of the R-S birds but a lower uptake when expressed relative to the weight of gland (experiment 3).

Therefore it can be said that, although the size of the thyroid gland is permanently affected by early goitrogen intake, the thyroid activity is normal. In the enlarged gland the epithelial tissue responds normally to thyrotropin and the capability for thyroid hormone synthesis to meet the requirements of the birds is not adversely affected. The presence of increased numbers of epithelial cells in the thyroid glands of the birds fed goitrogenic RSM during the growing period makes it possible, once the inhibition of thyroid hormone production and/or secretion is withdrawn, for the hyperplastic gland to meet the requirements of the body for thyroid hormone at a lower level of activity (i.e. at a lower level of thyrotropin stimulation). It has been shown that thyroidal epithelial cells, following elimination of thyrotropin stimulation, continue to trap iodine and to secrete thyroid hormone into the follicular lumen for some time at least, whereas the release of thyroid hormone into the circulation stops almost immediately (Björkman et al, 1974). The fact that total iodine was higher and that there were more thyronines present in the glands of RSM-fed birds than in SBM-fed birds indicates that endocytosis and release of thyroid hormone into the circulation were retarded in the former. In R-S birds withdrawal of goitrin at 24 weeks of age

was followed by colloid goiter because the iodine concentration by the gland continued at a faster rate than did the release of iodine from the gland. This is different from the finding of Akiba and Matsumoto (1973) on the complete recovery of thyroid function within a short time after withdrawal of goitrin in growing chicks.

A persistent difference in iodine metabolism induced by the feeding of RSM to birds during the growing period was still evident more than a year later in the lesser amount of a single dose of radioiodine deposited in the developing ova of these birds compared to control birds which had neven been fed a goitrogenic diet. This aspect of the study (to be discussed later) provides additional evidence that the thyroid gland maintains a heightened capacity for uptake of iodine as a result of early over-stimulation.

The mechanism of the persistence of early stimulation by goitrogens of the thyroid gland is unknown. The Wolff-Chaikoff effect, which commonly explains the blocking effect of intrathyroidal iodide on biosynthesis of the thyroid hormones when iodine-deficient rats are re-fed a high iodine diet, is characterized by a very high iodide percentage of intrathyroidal radioactivity, high labeled MIT/DIT ratio, and absence of labeled iodothyronines. Normally this blocking effect of large concentrations of iodide on hormone synthesis is a transient phenomenon. There is probably an autonomous adaptation of the transport mechanism to the high level of iodide in which there is a rapid loss of thyroidal capacity to concentrate iodide. In the R-S birds in the present study, is it possible that this adaptation mechanism is impaired by goitrogenic RSM so that colloid goiter results? Although the Wolff-Chaikoff effect is not evident in the thyroid hydrolyzates of these birds, defects in the autoregulation system in these glands may still remain a possible mechanism whereby effects of early goitrogenic stimulation may persist.

It is therefore concluded that the effects of early goitrogenic stimulation of the thyroid gland persist after withdrawal of the goitrogen. The thyroid gland which has been over-stimulated contains larger than normal numbers of epithelial cells and accordingly less activity of individual cells is required to maintain normal levels of hormone secretion.

EFFECTS OF ALTERATIONS IN MATERNAL THYROID METABOLISM ON EMBRYONIC THYROID DEVELOPMENT IN THE CHICK

Since eggs laid by birds fed RSM contained less iodine than when SBM was fed (experiment 6A), the hypothesis was tested that, in the goitrous birds, the increased thyroidal uptake of dietary iodine from the circulation limited the amount available for deposition in the developing ova. To eliminate the possibility that some component of RSM interfered with absorption of dietary iodine from the intestine the distribution of injected radioiodine between the ovarian follicles and the thyroid gland was compared in the birds fed the SBM and RSM diets (experiment 7). The data in Table 12 clearly show that the developing ova in the RSM-fed birds contained less of the injected radioiodine than ova in the SBM-fed birds. It is apparent from Figure 2 that the rate of iodine uptake by the developing follicle is proportional to the total rate of transfer of all yolk components from the circulation into the follicle. The amount of iodine present in the follicle is therefore a function of both the rate of the follicular growth and the concentration of iodine in the plasma. In contrast to the developing ova, the thyroid glands of the RSM-fed birds contained more radioiodine 22 hours after injection than did those of the SBM-fed birds. Details of the distribution of the radioactivity among different iodinated thyroidal compounds have already been discussed.

Preliminary tests in this laboratory (unpublished data) have indicated that most of the iodine present in eggs is in its inorganic form. If this is true, it will be in agreement with the postulation that when more iodine is trapped by the thyroid gland from the extrathyroidal inorganic pool, less of this element will be available for deposition in the developing ova. To test this hypothesis, it is thought that if the dietary iodine intake of the RSM-fed birds is increased to over that can be accumulated by the thyroid gland, the net deposition of this element in the eggs will be further enhanced despite the inhibitory effect of the goitrogen in thyroid metabolism. Results from experiment 6B seem to bear this out.

Thyroid weights at hatching were compared in the progeny of chickens that had been fed goitrogenic RSM and SBM respectively in the growing and/or laying diets (experiment 8). The thyroid glands were heaviest in the progeny of birds which had been fed RSM in both the growing and the laying diets. There was, however, a carryover of the effect of feeding RSM during the growing period when the birds were shifted to a SBM diet at maturity. Although the average weights of the glands of the progeny of dams receiving SBM in the laying and fed RSM during the growing period were not significantly heavier than in those from dams fed SBM throughout life, the proportion of epithelium in the glands was greater from the RSM-fed birds. In other words, the stimulus to the thyroid gland was evidenced by an increase in the ratio of epithelial tissue to colloid within the thyroid glands but was not sufficient to result in increased thyroid weight. On the other hand, progeny from dams fed RSM during the laying period only, had smaller thyroid glands than did those fed RSM throughout life. The percentage of epithelial tissue was similar in the glands of the progeny from these two treatments.

SUMMARY AND CONCLUSIONS

From the data of the various experiments discussed above, the following conclusions concerning the chronic effects of feeding goitrogenic RSM to birds can be drawn.

Analyses of the thyroid glands showed that the iodine content of the RSM-fed birds (experiment 2) was significantly higher than that in the control. The chronically stimulated (R-R and S-R) birds had a three-fold increase in their thyroidal iodine content while even the R-S group (which had only been fed goitrogenic RSM during the growing period) had over twice the thyroidal iodine content of the control birds. This was mainly due to the goitrous state of the RSM-stimulated glands. The chronically-stimulated thyroid glands showed a seven- to ten-fold hypertrophy, while the R-S group had an average thyroid weight double that of the control (experiment 1).

Histological data (experiment 1) confirmed the actively functioning state of the thyroid glands of the birds on chronic RSM and/or SBM treatments. The thyroids of the R-R and S-R birds showed colloid goiter rather than atrophy that would sometimes be expected from chronic goitrogenic treatments, coupled with increased average epithelial heights and follicle diameters in the thyroid glands of birds in these groups. This gave support to the hypothetical picture of a stimulated thyroid gland of the chronically RSM-stimulated birds actively accumulating iodine from the blood.

On the other hand, the thyroid glands of the birds fed chronically with goitrogenic RSM were found to be accumulating radioiodine, when I-131 uptake was measured at different time intervals after administration of a

single dose intraveneously (experiment 3). This four- to five-fold increase in thyroidal radioiodine uptake could account for the decrease in iodine deposition in the eggs of the RSM-fed birds.

The site of inhibition by goitrogenic RSM on intrathyroidal iodine metabolism of the chronically treated birds was investigated (experiment 4). It was found that although there appeared to be inhibition at an intermediary step(s) in thyroid hormone biosynthesis as demonstrated by an alteration of the iodotyrosine ratio, the production of iodothyronines was estimated to be adequate as judged from their percentages in the goitrous thyroid glands of the RSM-fed birds. A more probable inhibitory site might be at the release mechanism of these hormones from the thyroglobulin. An experiment (experiment 5) to determine the respective rates of thyroglobulin hydrolysis by the extraneous enzyme pronase failed to reveal any difference between the SBM- and RSM-fed birds. However, the possibility that there might be a difference in the thyroglobulin proteolysis rates by endogenous thyroid enzymes between the two groups of birds is still open to further investigation.

From the above data, it was hypothesized that the goitrous thyroid glands of the chronically RSM-fed birds were actively accumulating iodine from the circulation because of a possible inhibitory step in thyroid hormone biosynthesis and/or release, so that less of the element was available for deposition to the eggs.

The yolk of eggs from chronically RSM-fed birds was indeed found to be significantly lower in total iodine content than those from the control (experiment 6A). Since the iodine concentration in the albumin was regarded as too low to be of significance in the present issue, it was concluded that

the chronically RSM-fed birds deposited less iodine in their eggs. This was further supported by a radioiodine study (experiment 7) which demonstrated a decrease in the deposition of I-131 in the developing ovarian follicles of these birds compared to the control. Since there was no inhibition by goitrogenic RSM on iodine absorption in the gastrointestinal tract because the radioiodine was administered intravenously, this difference in iodine deposition in the eggs of the RSM- and SBM-fed birds could not be due to a difference in iodine levels in dietary intake. The RSM-supplemented diet actually contained a slightly higher concentration of iodine than the SBM-supplemented ration fed to the birds (experiment 2).

That this decrease of iodine deposition in the eggs of the RSM-fed birds could be due to a more rapid excretion of this element was also unlikely, since unpublished data from this laboratory showed that there was practically no difference in the deiodination or excretion rate of when extraneous radioactive triiodothyronine was administered to the SBM- or RSM-fed birds.

Thyroidal hypertrophy and altered thyroidal characteristics were found in the progeny of the RSM-fed dams. It is concluded therefore that the thyroidal enlargement observed in the progeny of chicks from dams fed goitrogenic RSM is due to a low concentration of iodine in the eggs. The low concentration of iodine in the eggs is caused by a diversion of a high proportion of dietary iodine into the thyroid gland with the result that amounts reaching the developing ova are reduced.

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A P P E N D I X

Table 1(A): Experiment 1: Statistical analysis comparing the average weights of thyroid glands in White Leghorn pullets (Shaver strain) fed RSM and/or SBM, 91 weeks of age.

i) Analysis of variance:

Source of variation	df	MS	F	P
Among groups	3	23719.7	48.0	<0.01
Within groups	36	493.9		
Total	39			

ii)Duncan's multiple range test:

Dietary treatment	S-S	R-S	S-R	R-R
Ave. thyroid wt. mg/100 body wt.	10.7	23.5	84.9	112.5
Duncan's comparison P<0.01	a	a	b	c

iii) Separate comparison of S-S and R-S groups:

Source of variation	df	MS	F	P
Among groups	1	819.2	39.4	<0.01
Within groups	18	20.8		
Total	19			

Table 2(A). Experiment 1: Statistical analysis comparing the average weights of thyroid glands in White Leghorn pullets (DeKalb strain) fed RSM and/or SBM, 99 weeks of age.

i) Analysis of variance:

Source of variation	df	MS	F	P
Among groups	3	35005.0	20.7	<0.01
Within groups	36	1693.0		
Total	39			

ii) Duncan's multiple range test:

Dietary treatment	S-S	R-S	S-R	R-R
Ave. thyroid wt. mg/100 g body wt.	11.2	28.4	103.4	149.4
Duncan's comparison $P<0.01$	a	a	b	c

iii) Separate comparison of S-S and R-S groups:

Source of variation	df	MS	F	P
Among groups	1	1477.5	69.7	<0.01
Within groups	18	21.2		
Total	19			

Table 3(A). Experiment 1: Statistical analysis comparing the average thyroid weights (g) of White Leghorn pullets (Shaver strain) at 91 weeks of age.

i) Analysis of variance:

Source of variation	df	MS	F	P
Among groups	3	4.5067	44.4	< 0.01
Within groups	16	0.1016		
Total	19			

ii) Duncan's multiple range test:

Dietary treatment	S-S	R-S	S-R	R-R
Ave. thyroid wt.(g)	0.1877	0.5175	1.3930	2.3027
Duncan's comparison $P < 0.01$	a	a	b	c

iii) Separate comparison of S-S and R-S groups:

Source of variation	df	MS	F	P
Among groups	1	0.2720	11.29	< 0.01
Within groups	8	0.0241		
Total	9			

Table 4(A). Experiment 1: Statistical analysis comparing the average epithelial heights in thyroid glands of White Leghorn pullets at 91 weeks of age.

i) Analysis of variance:

Source of variation	df	MS	F	P
Among groups	3	28.050	11.53	<0.01
Within gorups	16	2.433		
Total	19			

ii) Duncan's multiple range test:

Dietary treatment	R-S	S-S	S-R	R-R
Ave. epithelial heights, μ	5.61	7.13	10.11	10.65
Duncan's comparison $P < 0.01$	a	a	b	b

Table 5(A). Experiment 1: Statistical analysis comparing the average follicle diameter in thyroid glands of White Leghorn pullets at 91 weeks of age.

i) Analysis of variance:

Source of variation	df	MS	F	P
Among groups	3	12905	11.44	<0.01
Within groups	16	1128		
Total	19			

ii) Duncan's multiple range test:

Dietary treatment	S-S	R-S	S-R	R-R
Ave. follicle diameter, mm	.108	.167	.196	.227
Duncan's comparison $P< 0.05$	a	b	bc	c

Table 6(A). Experiment 1: Statistical analysis comparing the percentage epithelium in thyroid glands of White Leghorn pullets at 91 weeks of age.

i) Analysis of variance:

Source of variation	df	MS	F	P
Among groups	3	111.44	2.99	<0.05
Within groups	16	37.32		
Total	19			

ii) Duncan's multiple range test:

Dietary treatment	R-S	R-R	S-R	S-S
Percentage epithelium	14.2	15.1	20.1	24.4
Duncan's comparison $P < 0.05$	a	ab	abc	c

Table 7(A). Experiment 1: Statistical analysis comparing the average epithelial weights in thyroid glands of White Leghorn pullets at 91 weeks of age.

i) Analysis of variance:

Source of variation	df	MS	F	P
Among groups	3	105615	18.92	<0.01
Within groups	16	5580		
Total	19			

ii) Duncan's multiple range test:

Dietary treatment	S-S	R-S	S-R	R-R
Weight of epithelium, mg	41.1	76.5	277.4	334.6
Duncan's comparison P < 0.01	a	a	b	b

Table 8(A). Experiment 2: Statistical analysis comparing the average weights of thyroid glands of White Leghorn pullets fed RSM or SBM for different periods of time.

i) Analysis of variance:

Source of variation	df	MS	F	P
Among groups	3	3.8623	23.09	<0.01
Within groups	16	0.1673		
Total	19			

ii) Duncan's multiple range test:

Dietary treatment	S-S	R-S	S-R	R-R
Ave. thyroid weights, g	0.2039	0.4733	1.5115	2.9781
Duncan's comparison $P<0.05$	a	a	b	c

Table 9(A). Experiment 2: Statistical analysis comparing the average iodine concentrations in thyroid glands of White Leghorn pullets.

i) Analysis of variance:

Source of variation	df	MS	F	P
Among groups	3	4.879	29.25	<0.01
Within groups	16	0.1668		
Total	19			

ii) Duncan's multiple range test:

Dietary treatment	R-R	S-R	S-S	R-S
Ave. iodine concentration, mg/g	0.89	1.08	2.65	2.73
Duncan's comparison $P< 0.01$	a	a	b	b

Table 10(A). Experiment 2: Statistical analysis comparing the total iodine contents in thyroid glands of White Leghorn pullets.

i) Analysis of variance:

Source of variation	df	MS	F	P
Among groups	3	1.4305	25.8	<0.01
Within groups	16	0.0554		
Total	19			

ii) Duncan's multiple range test:

Dietary treatment	S-S	R-S	S-R	R-R
Total iodine in gland, mg	0.55	1.25	1.65	1.72
Duncan's comparison $P<0.05$	a	b	c	c

Table 11(A). Experiment 3: Statistical analysis comparing the total thyroid radioactivity 6 hours after administration of the radioiodine.

i) Analysis of variance:

Source of variation	df	MS	F	P
Among groups	3	851.00	18.4	< 0.01
Within groups	16	46.26		
Total	19			

ii) Duncan's multiple range test:

Dietary treatment	S-S	R-S	S-R	R-R
Percentage of injected dose	9.08	13.55	30.14	35.36
Duncan's comparison P< 0.01	a	a	b	b

Table 12(A). Experiment 3: Statistical analysis comparing the total thyroid radioactivity 22 hours after administration of the radioiodine.

i) Analysis of variance:

Source of variation	df	MS	F	P
Among groups	3	1731.54	40.5	<0.01
Within groups	16	42.72		
Total	19			

ii) Duncan's multiple range test:

Dietary treatment	S-S	R-S	R-R	S-R
Percentage of injected dose	7.83	10.87	40.57	42.40
Duncan's comparison $P<0.01$	a	a	b	b

Table 13(A). Experiment 3: Statistical analysis comparing the thyroid radioactivity (% injected dose/g thyroid) 6 hours after administration of the radioiodine.

i) Analysis of variance:

Source of variation	df	MS	F	P
Among groups	3	933.54	10.40	< 0.01
Within groups	16	89.73		
Total	19			

ii) Duncan's multiple range test:

Dietary treatment	R-R	R-S	S-R	S-S
% injected dose per g thyroid	15.41	23.13	26.98	47.18
Duncan's comparison $P < 0.01$	a	a	a	b

Table 14(A). Experiment 3: Statistical analysis comparing the thyroid radioactivity (% injected dose/g thyroid) 22 hours after administration of the radioiodine.

i) Analysis of variance:

Source of variation	df	MS	F	P
Among groups	3	376.34	3.88	<0.05
Within groups	16	96.91		
Total	19			

ii) Duncan's multiple range test:

Dietary treatment	R-R	S-R	R-S	S-S
% injected dose per g thyroid	21.61	29.55	35.58	41.96
Duncan's comparison $P<0.05$	a	ab	bc	bc

Table 15(A). Experiment 6A: Statistical analysis comparing the iodine concentrations in egg yolks from birds on different dietary treatments.

i) Analysis of variance:

Source of variation	df	MS	F	P
Among groups	2	50473	22.02	<0.01
Within groups	18	2292		
Total	20			

ii) Duncan's multiple range test:

Dietary treatment	S-R	S-S	R-S
Iodine conc. in egg yolk, ppb	144	273	286
Duncan's comparison P< 0.01	a	b	b

Table 16(A). Experiment 6B: Statistical analysis comparing the iodine concentrations in egg yolks from birds on different dietary treatments.

i) Analysis of variance:

Source of variation	df	MS	F	P
Among groups	3	43093	4.321	<0.05
Within groups	20	9973		
Total	23			

ii) Duncan's multiple range test:

Dietary treatment	RSM	RSM+I	SBM	SBM+I
Iodine conc. in egg yolk, ppb	41	73	105	241
Duncan's comparison $P<0.01$	a	a	a	b

iii)Separation comparison of SMB and RSM groups:

Source of variation	df	MS	F	P
Among groups	1	12352	10.78	<0.01
Within groups	10	1145		
Total	11			

Table 17(A). Experiment 7: Statistical analysis comparing the average total radioiodine in 5 largest ovarian follicles 22 hours after administration.

i) Analysis of variance:

Source of variation	df	MS	F	P
Among groups	3	21.5	39.09	< 0.01
Within groups	16	0.55		
Total	19			

ii) Duncan's multiple range test:

Dietary treatment	S-R	R-R	R-S	S-S
% injected dose in 5 largest ovarian follicles	2.624	2.830	5.796	6.717
Duncan's comparison P< 0.01	a	a	b	b

REGRESSION ANALYSIS, THE DEPENDENT VARIABLE IS LOG PERM

CONTRIBUTION TO MULTIPLE REGRESSION

INDEPENDENT VARIABLE	REGRESSION COEFFICIENT	STANDARD ERROR OF THE REGR. COEFF.	PARTIAL COEFFICIENT OF DETERMINATION	VARIANCE RATIO (F)
S.AREA	-0.982563E-01	0.111586E-01	68.20	77.536
LOG AREA	0.270855E+01	0.328604E+00	-10.27	67.940

INTERCEPT = -0.119575E+01
STANDARD ERROR OF ESTIMATE = 0.163484E+00
RESIDUAL VARIANCE = 0.267270E-01
MULTIPLE CORRELATION COEFFICIENT = 0.88278
R SQUARED = 0.77931
VARIANCE RATIO (F) = 38.844 WITH 2 AND 22 DEGREES OF FREEDOM
THE VARIABLE TO BE OMITTED IS = LOG AREA

REGRESSION ANALYSIS, THE DEPENDENT VARIABLE IS LOG PERM

CONTRIBUTION TO MULTIPLE REGRESSION

INDEPENDENT VARIABLE	REGRESSION COEFFICIENT	STANDARD ERROR OF THE REGR. COEFF.	PARTIAL COEFFICIENT OF DETERMINATION	VARIANCE RATIO (F)
S.AREA	-0.108918E-01	0.689903E-02	9.78	2.492

INTERCEPT = 0.391614E+00
STANDARD ERROR OF ESTIMATE = 0.323288E+00
RESIDUAL VARIANCE = 0.104515E+00
MULTIPLE CORRELATION COEFFICIENT = 0.31269
R SQUARED = 0.09777
VARIANCE RATIO (F) = 2.492 WITH 1 AND 23 DEGREES OF FREEDOM

REGRESSION ANALYSIS, THE DEPENDENT VARIABLE IS PERM

CONTRIBUTION TO MULTIPLE REGRESSION

INDEPENDENT VARIABLE	REGRESSION COEFFICIENT	STANDARD ERROR OF THE REGR. COEFF.	PARTIAL COEFFICIENT OF DETERMINATION	VARIANCE RATIO (F)
S.AREA	0.712840E+00	0.147068E+00	-275.26	23.494
AREA**2	-0.388821E-01	0.923148E-02	695.36	17.740
AREA**3	0.562208E-03	0.171290E-03	-347.54	10.773

INTERCEPT = -0.768565E+00
STANDARD ERROR OF ESTIMATE = 0.573134E+00
RESIDUAL VARIANCE = 0.328482E+00
MULTIPLE CORRELATION COEFFICIENT = 0.85183
R SQUARED = 0.72562
VARIANCE RATIO (F) = 18.512 WITH 3 AND 21 DEGREES OF FREEDOM
THE VARIABLE TO BE OMITTED IS = AREA**3

Table 18(A). Experiment 6: Regression analysis.

REGRESSION ANALYSIS, THE DEPENDENT VARIABLE IS LOG PERM

CONTRIBUTION TO MULTIPLE REGRESSION

INDEPENDENT VARIABLE	REGRESSION COEFFICIENT	STANDARD ERROR OF THE REGR. COEFF.	PARTIAL COEFFICIENT OF DETERMINATION	VARIANCE RATIO (F)
S.AREA	-0.785429E-01	0.106521E-01	4.11	54.368
LOG AREA	0.218004E+01	0.276988E+00	71.23	61.060

INTERCEPT = -0.973106E+00
STANDARD ERROR OF ESTIMATE = 0.156303E+00
RESIDUAL VARIANCE = 0.244307E-01
MULTIPLE CORRELATION COEFFICIENT = 0.86795
R SQUARED = 0.75335
VARIANCE RATIO (F) = 30.543 WITH 2 AND 20 DEGREES OF FREEDOM
THE VARIABLE TO BE OMITTED IS = S.AREA

REGRESSION ANALYSIS, THE DEPENDENT VARIABLE IS LOG PERM

CONTRIBUTION TO MULTIPLE REGRESSION

INDEPENDENT VARIABLE	REGRESSION COEFFICIENT	STANDARD ERROR OF THE REGR. COEFF.	PARTIAL COEFFICIENT OF DETERMINATION	VARIANCE RATIO (F)
LOG AREA	0.253555E+00	0.184100E+00	8.28	1.897

INTERCEPT = -0.801994E-01
STANDARD ERROR OF ESTIMATE = 0.294138E+00
RESIDUAL VARIANCE = 0.865171E-01
MULTIPLE CORRELATION COEFFICIENT = 0.28783
R SQUARED = 0.08284
VARIANCE RATIO (F) = 1.897 WITH 1 AND 21 DEGREES OF FREEDOM

REGRESSION ANALYSIS, THE DEPENDENT VARIABLE IS PERM

CONTRIBUTION TO MULTIPLE REGRESSION

INDEPENDENT VARIABLE	REGRESSION COEFFICIENT	STANDARD ERROR OF THE REGR. COEFF.	PARTIAL COEFFICIENT OF DETERMINATION	VARIANCE RATIO (F)
S.AREA	0.561084E+00	0.213811E+00	-44.06	6.886
AREA**2	-0.278921E-01	0.152627E-01	197.35	3.340
AREA**3	0.352982E-03	0.313132E-03	-99.53	1.271

INTERCEPT = -0.592955E+00
STANDARD ERROR OF ESTIMATE = 0.786107E+00
RESIDUAL VARIANCE = 0.617964E+00
MULTIPLE CORRELATION COEFFICIENT = 0.73312
R SQUARED = 0.53747
VARIANCE RATIO (F) = 7.359 WITH 3 AND 19 DEGREES OF FREEDOM
THE VARIABLE TO BE OMITTED IS = AREA**3

REGRESSION ANALYSIS, THE DEPENDENT VARIABLE IS LOG PERM

CONTRIBUTION TO MULTIPLE REGRESSION

INDEPENDENT VARIABLE	REGRESSION COEFFICIENT	STANDARD ERROR OF THE REGR. COEFF.	PARTIAL COEFFICIENT OF DETERMINATION	VARIANCE RATIO (F)
S.AREA	-0.857045E-01	0.141453E-01	37.95	36.710
LOG AREA	0.216407E+01	0.363090E+00	25.20	35.523

INTERCEPT = -0.123046E+01
STANDARD ERROR OF ESTIMATE = 0.250678E+00
RESIDUAL VARIANCE = 0.628394E-01
MULTIPLE CORRELATION COEFFICIENT = 0.79467
R SQUARED = 0.63150
VARIANCE RATIO (F) = 18.851 WITH 2 AND 22 DEGREES OF FREEDOM
THE VARIABLE TO BE OMITTED IS = LOG AREA

REGRESSION ANALYSIS, THE DEPENDENT VARIABLE IS LOG PERM

CONTRIBUTION TO MULTIPLE REGRESSION

INDEPENDENT VARIABLE	REGRESSION COEFFICIENT	STANDARD ERROR OF THE REGR. COEFF.	PARTIAL COEFFICIENT OF DETERMINATION	VARIANCE RATIO (F)
S.AREA	-0.823994E-02	0.882869E-02	3.65	0.871

INTERCEPT = -0.117142E+00
STANDARD ERROR OF ESTIMATE = 0.396438E+00
RESIDUAL VARIANCE = 0.157163E+00
MULTIPLE CORRELATION COEFFICIENT = 0.19103
R SQUARED = 0.03649
VARIANCE RATIO (F) = 0.871 WITH 1 AND 23 DEGREES OF FREEDOM

REGRESSION ANALYSIS, THE DEPENDENT VARIABLE IS PERM

CONTRIBUTION TO MULTIPLE REGRESSION

INDEPENDENT VARIABLE	REGRESSION COEFFICIENT	STANDARD ERROR OF THE REGR. COEFF.	PARTIAL COEFFICIENT OF DETERMINATION	VARIANCE RATIO (F)
S.AREA	0.216527E+00	0.857160E-01	-120.55	6.381
AREA**2	-0.114076E-01	0.598289E-02	324.45	3.636
AREA**3	0.151429E-03	0.119723E-03	-153.19	1.600

INTERCEPT = -0.625384E-01
STANDARD ERROR OF ESTIMATE = 0.369837E+00
RESIDUAL VARIANCE = 0.136780E+00
MULTIPLE CORRELATION COEFFICIENT = 0.71212
R SQUARED = 0.50711
VARIANCE RATIO (F) = 7.202 WITH 3 AND 21 DEGREES OF FREEDOM
THE VARIABLE TO BE OMITTED IS = AREA**3

Table 20(A). Experiment 6: Regression analysis.

REGRESSION ANALYSIS, THE DEPENDENT VARIABLE IS LOG PERM

CONTRIBUTION TO MULTIPLE REGRESSION

INDEPENDENT VARIABLE	REGRESSION COEFFICIENT	STANDARD ERROR OF THE REGR. COEFF.	PARTIAL COEFFICIENT OF DETERMINATION	VARIANCE RATIO (F)
S.AREA	-0.109314E+00	0.106617E-01	66.95	105.123
LOG AREA	0.279482E+01	0.281865E+00	15.80	98.317

INTERCEPT = -0.144323E+01
STANDARD ERROR OF ESTIMATE = 0.159572E+00
RESIDUAL VARIANCE = 0.254633E-01
MULTIPLE CORRELATION COEFFICIENT = 0.90968
R SQUARED = 0.82752
VARIANCE RATIO (F) = 52.777 WITH 2 AND 22 DEGREES OF FREEDOM
THE VARIABLE TO BE OMITTED IS = LOG AREA

REGRESSION ANALYSIS, THE DEPENDENT VARIABLE IS LOG PERM

CONTRIBUTION TO MULTIPLE REGRESSION

INDEPENDENT VARIABLE	REGRESSION COEFFICIENT	STANDARD ERROR OF THE REGR. COEFF.	PARTIAL COEFFICIENT OF DETERMINATION	VARIANCE RATIO (F)
S.AREA	-0.926432E-02	0.787605E-02	5.67	1.384

INTERCEPT = 0.200225E-01
STANDARD ERROR OF ESTIMATE = 0.364969E+00
RESIDUAL VARIANCE = 0.133202E+00
MULTIPLE CORRELATION COEFFICIENT = 0.23821
R SQUARED = 0.05674
VARIANCE RATIO (F) = 1.384 WITH 1 AND 23 DEGREES OF FREEDOM

REGRESSION ANALYSIS, THE DEPENDENT VARIABLE IS PERM

CONTRIBUTION TO MULTIPLE REGRESSION

INDEPENDENT VARIABLE	REGRESSION COEFFICIENT	STANDARD ERROR OF THE REGR. COEFF.	PARTIAL COEFFICIENT OF DETERMINATION	VARIANCE RATIO (F)
S.AREA	0.443172E+00	0.114627E+00	-248.18	14.947
AREA**2	-0.263608E-01	0.814305E-02	641.31	10.480
AREA**3	0.416808E-03	0.167228E-03	-327.68	6.212

INTERCEPT = -0.542931E+00
STANDARD ERROR OF ESTIMATE = 0.383969E+00
RESIDUAL VARIANCE = 0.147432E+00
MULTIPLE CORRELATION COEFFICIENT = 0.80780
R SQUARED = 0.65254
VARIANCE RATIO (F) = 13.146 WITH 3 AND 21 DEGREES OF FREEDOM
THE VARIABLE TO BE OMITTED IS = AREA**3

Table 21(A). Experiment 6: Regression analysis.

Table 22(A). Experiment 8: Statistical analysis comparing the average thyroid weights of progeny when dams were 91 weeks of age.

i) Analysis of variance:

Source of variation	df	MS	F	P
Among groups	3	.0000175	0.99	n.s.
Within groups	76	.0000177		
Total	79			

Table 23(A). Experiment 8: Statistical analysis comparing the average percentage epithelium in thyroid glands of progeny at hatching.

i) Analysis of variance:

Source of variation	df	MS	F	P
Among groups	3	179.50	3.77	<0.05
Within groups	16	47.58		
Total	19			

ii) Duncan's multiple range test:

Dietary treatment of dam	S-S	R-S	S-R	R-R
% thyroid epithelium in progeny	43.0	49.2	55.1	55.8
Duncan's comparison $P< 0.05$	a	ab	b	b

Table 24(A). Experiment 8: Statistical analysis comparing the average heights of thyroid epithelium of progeny of brids fed RSM or SBM for different periods.

i) Analysis of variance:

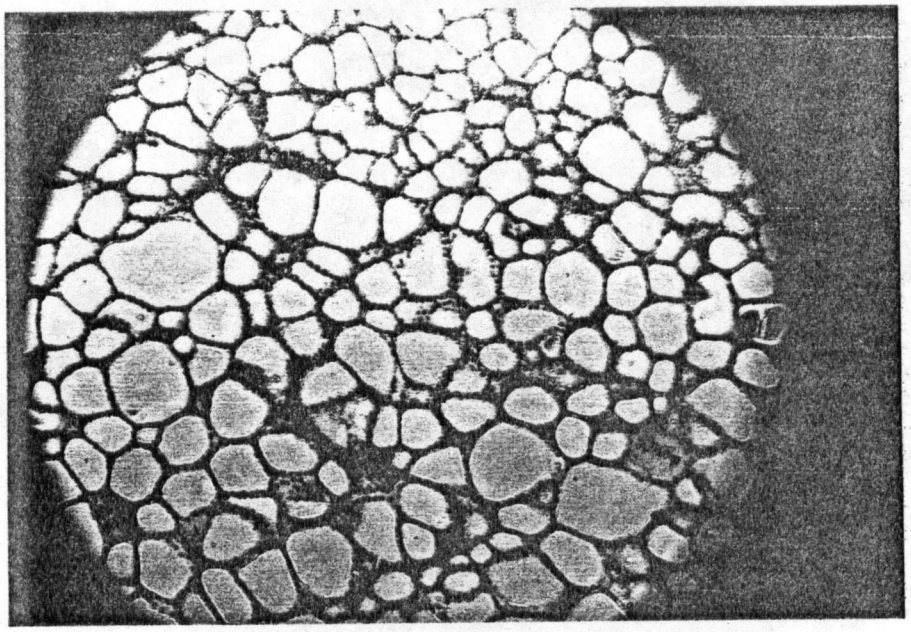
Source of variation	df	MS	F	P
Among groups	3	1.369	2.894	< 0.10
Within groups	16	0.473		
Total	19			

ii) Duncan's multiple range test:

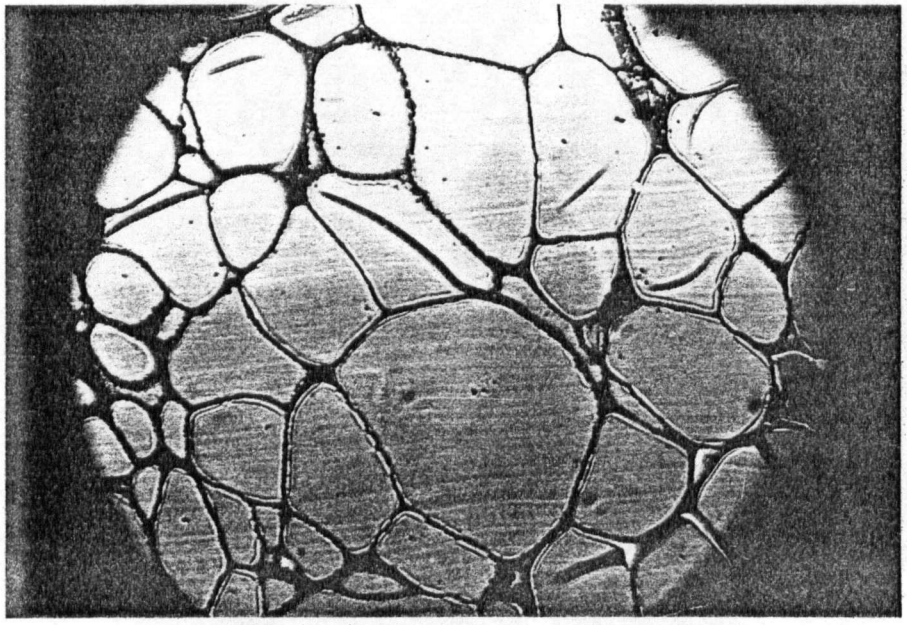
Dietary treatment of dam	S-S	R-R	R-S	S-R
Ave. heights of epithelium, μ	3.864	4.708	4.761	5.092
Duncan's comparison $P < 0.05$	a	ab	ab	b

Photomicrographs: (160x) Thyroid gland

S-S

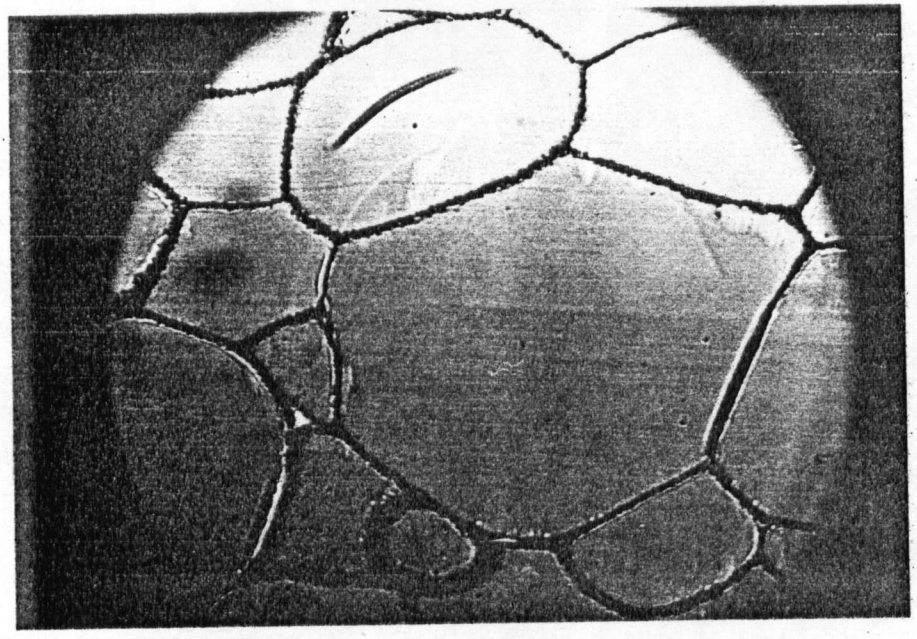


R-S



Photmicrographs: (160x) Thyroid gland

R-R



S-R