EFFECT OF SULFUR LEVELS IN TURNIPS
ON GOITROGENICITY IN THE
ALBINO RAT

by

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TURNIP GOITROGENICITY

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ABSTRACT

In a sand culture pot experiment, using five levels of sulfur in nutrient solution, the amount of sulfur in standard Hoagland-Arnon nutrient solution was too high for maximum turnip growth. The highest yield of turnip was obtained at the 1/9 S level. The sulfur concentrations of turnip roots and tops were almost the same ranging from 0.23 to 0.95 percent as the application rate of sulfur was increased.

Turnips raised in the green house containing 0.28 percent sulfur were used along with locally obtained turnips containing 0.49 percent sulfur in a goitrogenicity study with albino rats. The effects of turnip sulfur content, Ca, I and nitrate on thyroid uptake of radioactive iodine (I^{131}), body weight and thyroid weight was conducted. A central composite, rotatable design was used for this study.

Turnip sulfur generally increased percent I^{131} uptake per 100 mg thyroid at a low I and Ca levels while at high Ca level turnip sulfur had a strongly depressing effect. There was no turnip S - Ca interaction at high I level. At low levels of turnip sulfur and I, Ca increased I^{131} uptake. At low levels of I, but not at high levels, and at low nitrate the Ca effect on weight gain was depressing.

All the three goitrogens studied affected the thyroid enlargement significantly positively but the function of turnip sulfur on I^{131} uptake was different from that of Ca and nitrate. Increasing the levels of sulfur in turnips increased the goitrogenicity.

The relatively high percentage of equation sufficiency indicated that the method of using the partial cubic regression equation to characterize the response surfaces was effective.

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INTRODUCTION

Goiter is an endemic disease in Lebanon, especially in women and children. Although iodine deficiency is probably the primary cause, goitrogenic foods may contribute to the condition. It is not uncommon for clinicians to find patients with simple goiter who have had an apparently normally dietary intake of iodine, thus raising the possibility that factors other than iodine deficiency may be concerned in the production of endemic goiter.

One fact which has been established is that certain vegetables contain a goitrogen which interferes with thyroid function. Astwood _et al_. (6) found goitrogenic properties in cabbage, cauliflower, rape, rutabaga (yellow turnip), turnip and other foods, most of them members of the genus _Brassica_. The edible portion of white turnips and rutabaga yielded a detectable quantity of a compound which inhibited the uptake of radio-iodine by the thyroid in animals and human subjects. This compound exhibited goitrogenic activity even in the presence of iodine.

Marine _et al_. (45) have shown goiter formation in the rabbit after 10 to 15 days feeding with different varieties of cabbage. However, other workers expressed contrary views to the above finding. These contradictory views led to the belief that cabbage grown under different climatic conditions had different activity. Furthermore it has been shown that cabbage grown on low sulfur sand culture is less goitrogenic than that grown on high sulfur level (60). Hence, it was decided in this study to follow the correlation between plant goitrogenicity and sulfur utilization. In addition, since the design used made the study of interactions possible, other dietary factors known to affect thyroid function were studied. These included iodine, calcium and nitrate.

The purpose of the experiment reported here was to study the interrelationships and direct effects of turnip sulfur content and calcium, iodine and nitrate levels in the diet on thyroid weight, weight gain and radioactive iodine uptake in albino rats. Because of the exploratory nature of the study and the desirability of using several variables with each over a range of levels, the central composite, rotatable, incomplete factorial design was used. The use of this design with four variables in an animal experiment was not found in a search of the pertinent literature. Therefore, a secondary objective was to study the applicability of this type of design to small animal experimentation. Another phase of the study was to determine the effect of varying sulfur level in nutrient solution on the sulfur concentration and yield of turnips grown in sand culture.

REVIEW OF LITERATURE

The biosynthesis of thyroid hormones[1] from iodized tyrosine is a multistage reaction (40) whose end product is thyroxine. The thyroid gland is capable of absorbing iodide selectively from the blood plasma and concentrating it enormously. There are substances such as thiocyanate and perchlorate which disturb or inhibit this concentration process. As a result of a decrease in iodide uptake, the iodization processes slow down. A number of sulfur-containing food goitrogens, for example, thiourea, thiouracil and vinyl thiooxazolidone, obstruct oxidation of the iodide that iodizes tyrosine. The action of these goitrogens can not be stopped by increasing the amount of iodine in nutrition. The administration of thyroxine helps in thyroid disorders of this type. Since radioactive iodine became radily available, this isotope has been used extensively in studying the disturbances of the function of the thyroid gland in both humans and experimental animals.

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[1] The following abbreviations are used: MIT for monoiodotyrosine; DIT for diiodotyrosine; T_3 for 3, 5, 3-triiodothyronine; T_4 for thyroxine; TSH for thyrotropic or thyroid stimulating hormone.

Sulfur as a Plant Nutrient

The sulfur nutrition of turnips has not been studied very extensively because there are few cases of obvious sulfur deficiency in soil. This is probably due to the frequent addition of sulfur to the soil from various sources.

Sulfur has long been recognized as an essential plant nutrient. It has been well established, but not as widely recognized, that many plants require as much sulfur as phosphorus for growth and reproduction. Many grasses require relatively little sulfur, while certain legumes require about as much sulfur as phosphorus for growth and reproduction (50). Milton (50) further reported that, in general, the uptake of total sulfur increased by all annual grassland species as the rate of sulfur increased.

Iodine Function in Animal Body

Iodine in appropriate amounts, like iron, calcium, copper and many other chemical elements is essential throughout life.

Sebrell (59) reported that just as calcium is essential for formation of bones and iron is necessary to form hemoglobin for blood, so iodine is absolutely essential for formation of thyroxine. The thyroid normally contains from 15 to 20 mg of iodine. In normal adults a supply of about 75 mg of iodine per year will maintain the thyroid in normal condition.

Stanbury (66) has proposed that the mean daily intake of iodine by persons living in those parts of the world where endemic goiter does not occur lies above 75 to 200 ug. Most of this is in the inorganic form. The trapped iodide of the gland is oxidized, presumably by a specific oxidative system and attaches itself to the benzene ring of tyrosine residues which are present in peptide linkage within the substances of the gland (58). Thus, iodine is incorporated into thyroid hormone. Berson (11) has shown that iodine comprises two-thirds by weight of the thyroid hormone which regulates, in part at least, the metabolic activities of every cell in the animal body.

Cantarow and Schepartz (15) reported that the body normally contains about 20 to 30 mg of iodine, which is distributed approximately as follows: muscles 50 percent, thyroid 20 percent, skin 10 percent, skeleton 6 percent. The concentration in the thyroid is higher than in other tissues. This amount in all tissues diminishes when the intake is lowered, but the normal thyroid retains its capacity for trapping and storing iodine even under such circumstances.

Iodide circulating in the blood enters the thyroid by a mechanism known as the "iodide trap" which concentrates iodide to at least 25 times the plasma level. Once captured by the thyroid, iodide is oxidized by thyroid peroxidase to an active iodine (I_2) and is then attached to a tyrosine ring to produce MIT. The 5-position is also iodinated to form DIT. MIT and DIT are then oxidatively coupled to form T_3. Similarly, two DIT molecules are coupled to form thyroxine (T_4). T_3 and T_4 are stored as peptide linked residues within the thyroglobulin and these active hormones are released from the gland as required (56).

Rats deprived of iodine during pregnancy gave birth to young with heavier thyroids showing a greater uptake of I^{131} than normal young (20). Deficiency of iodine also inhibited reproduction. This increase in thyroid weight was probably the result of foetal deficiency of iodine.

Thyroid Function

The thyroid is one of the largest endocrine glands in the human body, weighing, normally between 15 and 25 gm. It is the first gland to develop both in the individual and in the evolution of the species and thus one needs to visualize the importance of its hormones on growth, development and metabolism (56). The thyroid hormone presumably stimulates the metabolism of all the tissues in the body, but certain conditions may be benefited by less stimulation, while others improve with more stimulation (74).

Cantarow and Schepartz (15) have summarized the important body functions influenced by thyroid activity as follows: metabolic rate (oxygen consumption); metabolism of lipids, carbohydrates, protein, electrolytes and water; vitamin requirement; temperature sensitivity; gastrointestinal activity; nervous system activity; muscle activity; reproduction and resistance to infection.

Rawson (56) reported that normal growth and development depends on the presence of adequate amounts of thyroid hormone. In physiological amounts, thyroxine promotes protein synthesis. However in excess, as in hyperthyroidism, catabolism is accelerated with increased nitrogen loss. With the excess of thyroxine, conversion of creatine to creatinine and formation of phosphocreatine are impaired.

Aschkenasy et al. (2) measured the thyroid function by uptake of labelled iodine and radioactive chromatography of plasma. Total iodine in thyroid was much less with low than with adequate intake of iodine. It was concluded that deficiency of iodine had much greater effect on thyroid function than has deficiency of protein.

Thyroid activity is controlled primarily by thyrotropic hormone (TSH) secreted by the anterior portion of the pituitary gland. It is generally believed that thyroxine exerts a "feed back" inhibition on the secretion of thyrotropic hormone (TSH). Thus, there is normally an inverse relationship between blood levels of TSH and thyroid hormone. The higher the level of thyroxine, the lower the level of TSH and vice versa. It is this inverse relationship that normally maintains the homeostasis of thyroid activity (69). Destruction or removal of the pituitary results in a reduced I^{131} uptake by thyroid; also it depresses the biosynthesis of thyroxine more than that of DIT (51, 58).

Lombardi (43) in an experiment with dogs used uptake of I^{131} by thyroid and the protein bound I^{131}(PBI^{131}) conversion ratio tests after 72 hours as indicators of thyroid function.

Food Goitrogens

The results of various studies demonstrated that there are present in plants and their seeds a number of substances which are actually or potentially goitrogenic for man, domestic animals and certain experimental animals.

It appears that there are at least two different classes of goitrogenic compounds. One produces goiter by preventing the uptake of iodine by thyroid; the thiocyanates are the example of this kind of substances. The other group produces goiter by blocking the organic binding of iodine. These are the substances for which the name "antithyroid compounds" was suggested (33); goitrin or L-5-vinyl-2-thiooxazolidone belongs to this group (17).

The finding of Chesney _et al_. (16). and Marine _et al_. (45) that cabbage feeding produced thyroid hyperplasia in rabbits must be considered as a milestone towards progress in this field. Subsequently, a large number of foodstuffs like cauliflower, rape, mustard and cabbage seeds, turnip and rutabaga were found to possess goitrogenic properties (28, 38). Most of these workers have shown that among these various vegetables, those belonging to the genus _Brassica_ possessed the greatest goitrogenic effect.

Altamura _et al_. (1) reported the isolation from fresh cabbage leaves of goitrin, a strongly antithyroid compound previously found in seeds. The isolation of L-5-vinyl, 2-thiooxazolidone by Astwood _et al_. (7) from ground rutabaga and white turnip was of considerable significance and gave a greater impetus to the search for similar goitrogenic agents in foodstuffs more commonly used in various diets.

Bachelard and Trikojus (8) compared milk from cows fed hay, chou-moellier or pasture highly contaminated with cruciferous weeds. They found that the cruciferous weeds contained thioglycosides, cheirolin and isothiocyanate and suspected that they were responsible for goiter in the Tasmania area. Bell (10) found that rape seed oil meal contains L-5-vinyl, 2-thiooxazolidone which is goitrogenic.

Clements (17) and Clements and Wishart (18) indicated that the endemic goiter in Tasmania and Australia was due to the action of goitrogens. They speculated that the goitrogens come from the milk of cows fed certain Brassica species. Greene <u>et al</u>. (24) reported that the milk of cows fed kale contained substances which temporarily suppressed the uptake of I^{131} by human thyroid. Bachelard <u>et al</u>. (9) concluded that extensive investigations did not support the idea that milk from cows grazing on pastures heavily contaminated with cruciferous weeds is a principal causative factor in iodine-resistant endemic goiter affecting young children, though there was much evidence in its favor.

Greer (27) studied various foodstuffs and reported that rutabaga and turnips are the only edible plants from which an active goitrogen has so far been isolated. The active antithyroid agent in these vegetables has been identified as goitrin. It is present in the seeds of most Brassica as progoitrin, an inactive compound. Goitrin is apparently liberated from progoitrin only through specific enzymatic hydrolysis by a thioglycosidase contained in the plant or seed itself. Cooking destroys this enzyme and inhibited its goitrogenic potency by preventing the liberation of goitrin from progoitrin.

Greer (25, 26) reported that the goitrogenic effect in turnips and rutabaga seemed to be contained in a compound which is liberated from a precursor in the plant by enzymatic hydrolysis. The liberated antithyroid compound was later isolated and proved to be L-5-vinyl, 2-thiooxazolidone hence forth called goitrin. Greer and Astwood (28) tested sixty-one different foods in one hundred different subjects by using radioactive iodine. Of these rutabaga was found to possess the greatest antithyroid activity. Raw turnip was definitely inhibitory in a dose of 441 gm but had no effect when half of that amount was taken. Goitrin of rutabaga could be extracted with ether and was contained in the water soluble portion of this dried ether extract.

The naturally occurring goitrogen of two carbamide series in turnip and rutabaga roots was proposed to be L-5-vinyl, 2-thiooxazolidone (30). Langer (39) studied the antithyroid effect of fresh plants on guinea pigs. The radioactivity in thyroid was measured. It was found that kale which belongs to the genus Brassica was the most goitrogenic. Thiocyanate was considered to be a cause of the antithyroid effect of these plants but it was thought that some other substances might also be present.

Marine *et al*. (45) noticed that the active factor in cabbage was an organic cyanide. The finding of McCarrison (47) and Kennedy and Purves (38) of the incomplete control of "cabbage goiter" by iodide might be due to vinyl thiooxazolidone formed in the cabbage used in their animal experiments. Mildred and Curtis (49) have confirmed that of the edible portion of the Brassicae; only rutabaga and turnip have been shown to contain the goitrin, L-5-vinyl, 2-thiooxazolidone.

Peltola and Krusius (54) investigated the goitrogenic factor contained in milk collected in the goiter and nongoiter area of Finland. Rats were fed with milk from such areas. This study indicated that the milk collected in the goiter area contain a goitrogen, probably of the thiouracil or thiooxazolidone type, the effect of which could not be eliminated by iodine ingestion in excess. The goitrogenic factor of milk was due to the pasture fed to the cattle.

The goitrogenic effect of groundnut was studied using albino rats. The goitrogenic factor appeared to be water soluble and could not be identified with any of the known dietary goitrogens (52, 65).

The foods which are more goitrogenic are certain members of the genus Brassica, such as cabbage, turnip, rape and mustard etc. (45, 46, 72). The goitrogens mostly found in these foods are thiocyanate, thiourea, thiouracil,, sulfonamides and L-5-vinyl, 2-thiooxazolidone (15). The goitrogenic action of these are similar to that of the blockage of the organic binding of iodine by tyrosine (46, 48).

Ways of Testing for Goitrogenicity

Thyroid enlargement and the rate and degree of thyroid absorption of tracer doses of radioactive iodine have been the bases of various clinical test for estimating the degree of activity of the thyroid gland (17). Two criteria were postulated by Brownell (14) as essential for a radioactive diagnostic test for the determination of thyroid function. The test should be indicative of thyroid status and the test should measure a fundamental parameter of thyroid. In support of the second criterion the metabolism of labelled iodine and the release of labelled hormone by the gland were analyzed on the basis of a simple technique.

Brassica seeds, like the thiourea and sulfonamides, inhibited the thyroid hormone (4). Sulfonamides and thioureas produced symptoms in rats similar to those of hypothyroidism (5). Astwood et al (6) have made an extensive studies on the effect of various articles of diet on the induction of goiter in animals. Certain plants such as cabbage, turnip, rape seed, soybean and peanut have been reported to cause thyroid enlargement.

In Tasmania, Clements (17) has noticed that milk from cows fed upon chou-moellier might, upon ingestion, inhibit the accumulation of radioactive iodine in the thyroid gland. Clements and Wishart (18) have observed that ethanolic extracts of freeze-dried skim milk from cows fed chou-moellier and grazing on pastures heavily contaminated with wild turnips prevented the uptake of I^{131} in rats in the same manner as neomercazole. Chou-moellier, used to a great extent in a fodder for cows, contained a substance which caused epithelial hyperplasia in thyroid of rabbit and also interfered with the uptake of I^{131} by thyroid (24).

Franklin et al. (21, 22) noticed that thiouracil depressed thyroxine synthesis from DIT by rat thyroid both in vivo and vitro. The thyroids of rats with goiter produced by administration of thiouracil collected less radioiodine (57). It was concluded that this antithyroid drug interfered in some way with hormone synthesis. The pioneer work of Greer and his associates (30) on the effect of certain foods on thyroid function has indicated the possibility that in certain cases endemic goiter might be the result of a specific goitrogen in the diet. These substances inhibited the uptake of iodine by the glands. Greer _et al_. (29) concluded that antithyroid compounds of the thianamide series interfered with the synthesis of the thyroid hormone, principally by inhibiting the oxidation of inorganic iodide and its subsequent incorporation into tyrosyl residue.

Hercus and Purves (34) reported that turnip roots have a goitrogenic activity comparable with that found for the most active sample of cabbage on rabbit. It was also found that this goiter could be controlled only with thyroxine, rather than with iodine, and thus it differed from cabbage goiter. Highley (35) established that the synthetic goitrogen, thiouracil, exerted a maximum effect on thyroid glands of rats when the iodine level in diet was at a minimum. Levine _et al_. (42) observed the following effects of goitrogenic rations on the thyroid gland of rats: marked hyperplasia with little or no iodine containing colloid, increased average fresh weight, and a low concentration of iodine in the gland. The goitrogenic ration included cabbage and other members of the genus Brassica. Enlargement of the thyroid gland has been produced in rats and mice by the administration of sulfonamides and thiourea (44). Peltola and Krusius (54) reported that inhibition or partial blocking of thyroid hormone was produced by the action of a goitrogenous factor. In consequence of this effect, the iodine uptake per unit weight of thyroid was reduced and TSH secretion of adeno-hypophysis increased, causing enlargement of the thyroid. Pitt-Rivers (55) classified the antithyroid compounds into three groups according to their mode of action: 1) inhibition of iodide trapping from the blood, 2) inhibition of hormone synthesis and 3) inhibition by tissue destruction. Thiocyanate is an example of the first group. This was confirmed after studying that thiocyanate-promoted goiter could be prevented by iodine administration (3).

Socolow and Susuzuki (64) investigated the possible goitrogenic effect of Japanese food. Groups of albino rats were fed a low iodine Remington ration supplemented with chinese cabbage, turnip, buck wheat and soybean or seaweed. Goiter developed in the rats of all groups except those fed seaweed. This investigation indicated goitrogenicity of the Remington ration but failed to demonstrate positively the goitrogenicity of supplemental food.

Taurog _et_ _al_. (68) and Vanderlaan and Vanderlaan (71) observed that the organic binding of iodine was blocked by propylthiouracil (PTU), the T/S ratio was increased greatly and all iodine in the gland was in the form of iodide.

One of the principal effects of the goitrogens on entering the organism is a lowered production of thyroid hormone bringing about a hypothyroid condition in the whole organism, a lower over all metabolic rate and lower oxygen utilization by the tissue (74).

Cabbage-Sulfur Levels

Since the discovery of the goitrogenicity of cabbage (16), much attention has been devoted to study of the effect exerted on its activity by climatic factors. Greer (27) found that the progoitrin content of the seeds of Brassica varied from batch to batch and season to season even for the same variety. The goitrogenic activity of cabbage was confirmed in India by McCarrison (47), and it was also noticed that there was geographical and seasonal variation in its activity. Webster (73) pointed out the positive correlation between number of sunny days and cabbage goitrogenicity.

Rats were given a goitrogenous and non-goitrogenous variant of cabbage grown in sand culture with nutrient solutions (60). The nutrient solution for the non-goitrogenous variant of cabbage contained 1 mgm equivalent of sulfur and that used for the goitrogenous variant 25 mgm. The other nutrient substances were present in equal amounts in both solutions. Goitrogenicity of cabbage thus cultivated was tested experimentally on white female rats. The results showed that rats fed with goitrogenous variant of cabbage store in the liver twice as much total sulphydryl compounds, expressed in terms of glutathione, as do these fed on non-goitrogenous cabbage. Sedlak et al. (61) reported that an increase in the intake and utilization of sulfates from the nutrient environment by cabbage necessarily provokes an elevated level of sulfur compounds and among these the naturally occurring sulfur goitrogenic agents.

MATERIALS AND METHODS

The preliminary work for this research problem was conducted to determine the levels of sulfur in nutrient solution required for successful production of low and high level sulfur turnips using sand as a growth medium.

Turnip Growing

The sand used as the plant growth media and the local tap water were analyzed for total sulfur determination by the turbidimetric method (37).

A completely randomized design with three replications and five levels of sulfur (table 1) was used in an outdoor pot trial. Five levels of sulfur were as follows:

1. Hoagland-Arnon nutrient solution lacking sulfur in distilled water (indicated as Dw).
2. Hoagland-Arnon nutrient solution lacking sulfur in tap water (indicated as Tw).
3. Hoagland-Arnon nutrient solution with only 1/9th of the sulfur contained in the complete solution; made up in distilled water (indicated as 1/9 S).

4. Hoagland-Arnon nutrient solution with only 1/3rd of the sulfur contained in the complete solution; made up in distilled water (indicated as 1/3 S).
5. Complete Hoagland-Arnon nutrient solution made up in distilled water (indicated as 1S), (36).

Table 1. Composition of Hoagland-Arnon nutrient solution.

Complete		Lacking sulfur	
Salt	ml. in a litre of nutrient solution	Salt	ml. in a litre of nutrient solution
M KH_2PO_4	1	M $Ca(H_3C_2O_2)_2$	2
M KNO_3	5	M $Ca(NO_3)_2$	3
M $Ca(NO_3)_2$	5	M KNO_3	5
M $MgSO_4$	2	M KH_2PO_4	1
		M $Mg(NO_3)_2$	2

The micronutrients, Cu, Fe, Cl, Mn, Zn, Mo and B, were added.

Clay pots 25 x 30 cm. were marked with respective treatments and lined with plastic bags to avoid sulfur contamination from the pots. One hole at the bottom was left for drainage.

The pots were filled with equal amounts of sand and irrigated with distilled water. Five seeds of a local variety of turnip[2] were planted in each pot at a depth of 1 to 2 cm. on May 29, 1964. Daily irrigation with distilled water was applied till the emergence of seedlings. When the germination was completed, irrigation was done once daily with assigned nutrient solution, and sometimes twice when it was necessary.

Two weeks after planting the plants were thinned to two plants in each pot. The pots were rotated weekly to minimize the light effect and were leached weekly with distilled water to remove the accumulated salts.

After a seven week period, when the crop matured, it was harvested on July 22, 1964. Yields of fresh roots were recorded.

Tops and roots were analyzed for total sulfur determination. Plant tissue was predigested in HNO_3 prior to addition of $HClO_4$. The digestion was carried at an elevated temperature according to the procedure described by Jackson (37).

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[2] A local variety of turnip was obtained from the Division of Horticulture, School of Agriculture, AUB.

Statistical analyses as applied to the completely randomized design and Duncan's range test were made according to LeClerg _et al._ (41).

In the next section of the study, turnips of the same variety were raised in 180 pots under conditions similar to the previous work. The purpose was to produce turnips with a low content of sulfur so Hoagland-Arnon solution without sulfur was made up in tap water (Tw). The sulfur in the sand and in the tap water was sufficient for reasonable plant growth at a relatively low sulfur content. When the crop matured, it was harvested and stored in a refrigerator.

For normal level of sulfur content three samples of 10 kg each of fresh turnip roots were obtained in a local market and stored in a refrigerator. Sulfur was determined on the three samples and the one with the highest content along with the low sulfur turnips raised in the greenhouse were used for the diet preparation in an animal experiment.

Animal Experiment

The experimental animals used were weanling, female albino rats of the Sprague-Dawly[3] strain. The

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[3] Obtained from Animal Suppliers (London) Ltd.

rats were received by air and were kept on a laboratory stock diet[4] for two days before being placed on experiment.

A total of 124 rats weighing 38 to 69 grams each were divided into 31 groups of 4 each, and matched closely for body weight. The animals of each group were housed two to a cage in mesh-bottomed cages in an air-conditioned room held at 70 ± 2°F with a relative humidity of 60 percent. The animals of each group were assigned to the diets at random and the groups were completely randomized. Animals of all groups were offered 10 grams experimental diet per day per rat, for the first week, and 15 grams per day per rat for the successive experimental period. Distilled water was supplied *ad libitum*. The spillage of individual groups was collected and weighed very carefully every three days. Food consumption and weight gains were determined weekly. Each group of rats was maintained with the designated experimental diet for 18 days.

The basal diet had the following composition on an air-dry basis:

Ingredients	Percentage
Casein	40
Corn starch	27

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[4] Obtained from Vitasni Feed Company, Beirut, Lebanon.

Ingredients	Percentage
Corn oil	20
Iodine free salt mixture[5]	5
Vitamin mixture[5]	2
Alphacel (non-nutritive cellulose)[5]	6

A central composite, rotatable, incomplete factorial design, plan 8A-1 (19) was chosen to study the main effects and the interaction of turnip sulfur, Ca, I, and nitrate each at five levels on thyroid weight, weight gain, T/S and thyroid uptake of I^{131} in the albino rat.

The rates of I and nitrate were varied according to the logarithmic scale to the base 2 in order to cover a wide range of application and to straighten the response curves. The amount of dietary variables were coded as -2, -1, 0, +1, +2 to simplify the calculation of the regression equation (table 2). The coded 0 level was set to supply a medium quantity of each variable. The coded -2 level was assumed to be a possible deficiency rate and +2 a possible excess rate. There were 25 diets, one of which was repeated six times in order to estimate the experimental error. One group of 4 rats was maintained

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[5] Obtained from Nutritional Biochemicals Corporation, Cleveland, Ohio, U.S.A.

Table 2. Amount of variables added to basal diet.

Level of addition	Coded level	Amount of added variables				
		Turnip (g/Kg) Low S	Turnip (g/Kg) High S	Ca(g/Kg) as $CaCO_3$	KIO_3 (ug/Kg)	KNO_3 (g/Kg)
1	-2	500	0	0	0	0
2	-1	375	125	7.0	5.0	2.5
3	0	250	250	14.0	10.0	5.0
4	+1	125	375	21.0	20.0	10.0
5	+2	0	500	28.0	40.0	20.0

on the control diet. The diets were prepared in bulk and kept refrigerated to assure a uniform freshness.

Biochemical Methods

At the end of the experiment, all animals were injected subcutaneously with 5 uc of radioactive iodine[6] in the form of carrier-free NaI^{131}. The rats were anaesthetized with chloroform at the end of 24 hours. At that time, the final body weight was recorded, blood was drawn from the inferior vanacava and collected in centrifuge tubes. The blood was centrifuged at 3500 rpm for 10 minutes and the serum collected for radioactivity

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[6] Obtained from the Radiochemical Center, Amersham, England.

measurement. Thyroid glands were removed, weighed immediately on an ultra-matic precision torsion balance and fixed in 10 percent formalin. These preserved glands were used for measurement of I^{131} uptake. Serum total I^{131} and 24 hours I^{131} uptake were determined by counting[7] for one minute the serum and the formalin fixed thyroid gland, respectively, after allowing a suitable decay period.

Parameters Studied

The parameters studied in this experiment to evaluate the thyroid activity were:

a) Thyroid I^{131} uptake: The rate and degree of thyroid absorption of tracer doses of radioactive iodine have been the basis of various clinical tests for estimating the degree of activity of thyroid gland (17). The thyroid glands of individuals in a goiter area usually have a low iodine concentration and increased avidity for iodine. The 24 hours I^{131} uptake was expressed as the percentage of dose I^{131} trapped by the gland.

b) The T/S ratio: It is well established that thyroid possesses a mechanism whereby it concentrates

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[7] The instrument used to measure radioactivity was a well-type, crystal scintillation counter; counting efficiency was 60 percent. The instrument was manufactured by "Baird Atomic, Inc.", Cambridge, Mass. U.S.A. Counts were measured at 1750 Volts; all counts were corrected for background.

("traps") inorganic iodide (71). Studies using radioactive iodine have disclosed a thyroid/serum gradient. This term is defined as the ratio of the radioactivity of 100 mg of thyroid tissue (calculated after measuring the absolute activity of two lobes) to the radio activity of 100 mg of serum (calculated as the radioactivity of 0.1 ml of serum diluted to 2 ml with distilled water). Thyroid/serum radioiodide concentration ratios were examined in the experimental rats which received I^{131}. This T/S ratio permits the evaluation of the ability of thyroid gland to concentrate iodide from plasma (20, 31, 32).

c) Thyroid weight: Though iodine deficiency has been confirmed for goiter production (65), certain low iodine diets do not produce goiter and those that do produce goiter may be effective for reason other than their low iodine content. This fact was supported by the discovery of a number of goitrogenic substances and particularly the finding in natural foods of such a substance as vinyl thiooxazolidone (4) that inhibited thyroid function and consequently produced enlarged thyroid.

Statistical Analysis

The statistical analysis was done according to the method described by Cochran and Cox (19). Regression equations of cubic form for weight gain, T/S and thyroid uptake of I^{131} were computed from the collected data. The following equation was used:

$$Y = b_0 + b_1x_1 + b_2x_2 + b_3x_3 + b_4x_4 + b_{11}x_1^2 + b_{22}x_2^2 + b_{33}x_3^2 + b_{44}x_4^2 + b_{12}x_1x_2 + b_{13}x_1x_3 + b_{14}x_1x_4 + b_{23}x_2x_3 + b_{24}x_2x_4 + b_{34}x_3x_4 + b_{123}x_1x_2x_3 + b_{124}x_1x_2x_4 + b_{134}x_1x_3x_4 + b_{234}x_2x_3x_4 .$$

Where b = Regression coefficient for treatment effect.

x_1 = Coded level of turnip sulfur

x_2 = Coded level of Ca

x_3 = Coded level of I

x_4 = Coded level of nitrate.

Analysis of variance of the collected data was performed and the "F" test was used to find the significance of first order, quadratic, cubic and lack of fit terms. The percentage of equation sufficiency and the coefficient of variance were calculated. The "t" test was used to evaluate the significance of the individual regression coefficients. An IBM 1620 computer was used for the computation.

RESULTS AND DISCUSSION

Effect of Sulfur Levels on Yield and Sulfur Content of Turnips

A pot experiment was laid out with five levels of sulfur in nutrient solution to determine the levels of sulfur necessary for successful production of turnips with low to high sulfur concentration. The analysis of the locally available tap water and the sand used for turnip growing indicated 5.1 and 1.4 ppm total sulfur content, respectively.

The yield of fresh roots ranged between 69.7 and 135.0 gm per pot (table 3). Although only the lowest and the highest values differed significantly, there was a definite trend of increasing yield of roots up to the 1/9 S level and progressive decreases at the 1/3 S and 1 S levels. This indicated that turnips are sensitive to the relative supply of nutrient sulfur and that the proportion of sulfur in the standard Hoagland-Arnon solution is probably too high for optimum growth of turnips. The total sulfur concentration of turnip roots (table 3) increased significantly and progressively from 0.23 to 0.95 percent with increasing sulfur in the nutrient solution. The sulfur content of the tops closely paralleled that of the roots and the relatively wide

Table 3. Yield (gm/pot) of fresh turnip roots and percent sulfur content (dry weight basis), of roots and tops treated with 5 levels of sulfur.

	Treatment	Yield (gm/pot)	Total sulfur content (percent) Roots	Tops
1	DW	69.7	0.23	0.23
2	TW	94.3	0.27	0.28
3	1/9 S	135.0	0.50	0.38
4	1/3 S	119.3	0.63	0.70
5	1S	101.7	0.95	0.96

Analysis of Variance

Source	d.f.	S.S.	M.S
Roots - Between Treatment	4	1.024	0.256^{xx}
Error	10	0.001	0.0001
Tops - Between Treatment	4	1.155	0.289^{xx}
Error	10	0.011	0.001
Fresh root yield - Between Treatment	4	7421.331	1855.332
Error	10	5814.670	581.467

[x] Statistically significant at the 5% level.

[xx] Statistically significant at the 1% level.

Treatments	DW	TW	1S	1/3S	1/9S
Yield^{+} (Mean)	69.7	94.3	101.7	119.3	135.0
Treatments	DW	TW	1/9S	1/3S	1S
Roots (Mean %)	0.23	0.27	0.50	0.63	0.95
Treatments	DW	TW	1/9S	1/3S	1S
Tops (Mean %)	0.23	0.28	0.38	0.70	0.96

[+] Underlined means do not differ significantly at 5% level.

spread of values indicated a probable sensitive "critical level" of about 0.4 to 0.6 percent in the tissues at the point of maximum yield. More work on this is needed because of the limited size of the experiment. Turnips obtained in the local market contained 0.49 percent sulfur indicating that the supply of nutrient sulfur was about optimum under the local conditions of growth. Turnips raised subsequently in 180 pots using the Tw treatment had a sulfur content of 0.28 percent corresponding closely to the average of 0.27 percent found in the first study. These turnips along with the ones purchased locally were used in a study with rats to assess the goitrogenicity of turnips containing different levels of sulfur.

Animal Experiment with Albino Rats

An animal experiment was designed to evaluate the goitrogenicity of turnips grown on high and low levels of sulfur. The interrelationships and direct effects of turnip sulfur, Ca, I and nitrate each at 5 levels on thyroid weight, weight gain, T/S and thyroid uptake of I^{131} in albino rats were studied. The applicability of a central composite, rotatable, incomplete factorial design to animal experimentation was also evaluated. This design allows the computation of regression equations in the quadratic or partial cubic form for use in studying the nature of the response surfaces. A positive sign of a regression coefficient of a first order term for a variable indicates an overall increasing effect of that variable on the properties studied, while a negative sign denotes an overall depressing effect of that variable. The sign of a regression coefficient for a squared term indicates whether the response curve is concave upward, positive, or concave downward, negative. The relative magnitude indicates the degree of curvature. A positive sign for the interaction quadratic term of two variables denotes that the positive response of the property under study to one of the varied variables increases (or becomes less negative) as the second variable is increased, while a negative sign for the coefficient shows that an increase in the level of one variable decreases the positive effect of the other. The "t" test was used to evaluate the significance of the individual regression coefficients. In this discussion, the term "highly significant" will be used for an effect with a probability of 0.99 or more of being true and "significant" will be used for a probability of 0.95 to 0.99 of being true.

The cubic terms for the three factor interactions were added to the regression equation because they were found to be of considerable magnitude in a number of cases.

Effect of Variables Studied on Uptake of I^{131}

The 24 hour uptake of I^{131} by thyroid gland ranged between 2.51 and 14.00 percent of the administered dose (table 4). The analysis of variance (table 5) indicated that the proportion of the total treatment sum of squares accounted for by the cubic regression equation (equation sufficiency) was 88.4 percent, which indicated a close fit of the regression equation to the observed uptake data. The percent uptake of I^{131} per 100 mg thyroid indicated a similar but more definite trend of results than given by percent uptake of I^{131} by the thyroid. Therefore, the following discussion will concern I^{131} uptake per 100 mg of thyroid. The T-Ca interaction (table 6) was too small to be significant but at a low level of I (figure 1) there was a definite effect. At a low Ca level, turnip sulfur generally increased I^{131} uptake while at high Ca, turnip sulfur had a strong decreasing effect. At high I level the T-Ca interaction tended to disappear. This illustrated the effect of the significantly positive T-Ca-I three way interaction (table 6). This may also explain the variation in effect of Ca found by a number of other workers. Braham (13) did not find any effect of high calcium intake on either thyroid weight or its iodine content. Sharpless _et al._ (62) also reported that when other factors were kept

Table 4. Observed I^{131} uptake, I^{131} uptake /100 mg thyroid, T/S, total gain and thyroid weight as affected by various combinations of turnip sulfur, Ca, I and nitrate in albino rats (Average of 4 rats per group).

Treatment levels				I^{131} uptake, %	I^{131} uptake /100 mg thyroid, %	T/S^{+} $\times 10^{-2}$	Total gain gm	Thyroid weight, mg/100 gm body weight
T^{x}	Ca	I	Nitrate					
2	2	2	2	4.50	66	32.4	55.4	6.81
4	2	2	2	7.92	91	38.2	49.8	8.47
2	4	2	2	9.60	154	43.8	25.8	7.82
4	4	2	2	7.78	110	33.7	26.9	8.87
2	2	4	2	5.27	86	41.6	42.5	6.49
4	2	4	2	2.51	29	16.6	47.3	8.48
2	4	4	2	3.19	59	24.5	30.0	6.61
4	4	4	2	5.05	67	22.9	37.5	8.42
2	2	2	4	7.95	104	48.3	33.8	8.83
4	2	2	4	7.96	85	30.9	55.7	8.81
2	4	2	4	12.90	150	33.6	29.3	10.10
4	4	2	4	5.66	56	24.9	37.8	11.00
2	2	4	4	4.12	50	33.6	49.1	7.96
4	2	4	4	3.30	36	18.6	52.0	8.74
2	4	4	4	5.87	77	28.5	37.3	8.28
4	4	4	4	3.75	45	21.7	33.0	9.53
5	3	3	3	6.18	78	24.9	46.2	8.00
1	3	3	3	4.82	61	26.0	40.0	8.34
3	5	3	3	6.75	105	52.8	20.5	8.59
3	1	3	3	6.05	75	38.8	60.0	7.07
3	3	5	3	5.03	83	30.9	38.3	6.48
3	3	1	3	13.30	134	24.0	49.3	9.59
3	3	3	5	11.10	117	38.4	33.8	10.70
3	3	3	1	5.71	72	44.5	35.0	8.97
3	3	3	3	9.79	111	40.5	39.0	9.25
3	3	3	3	14.00	150	58.4	37.8	10.20
3	3	3	3	10.90	117	38.4	44.7	9.33
3	3	3	3	7.71	101	47.7	33.0	8.75
3	3	3	3	7.96	91	44.8	37.0	9.53
3	3	3	3	7.99	108	49.6	34.3	8.06
3	3	3	3	8.30	97	34.8	37.5	9.47

[x] Turnip sulfur.

[+] Thyroid/serum radio-iodide concentration ratio.

Table 5. Analysis of variance for thyroidal uptake of I^{131} (%), percent uptake of I^{131} per 100 mg of thyroid, T/S, total gain, and thyroid weight (mg per 100 gm body weight), in albino rats.

Source	Total	First order	Second order	Third order	Lack of fit	Error	C.V. %	Equation sufficiency %+
Percent uptake of I^{131}								
d.f.	30	4	10	4	6	6	24.09	88.4
S.S.	271.58	113.88	81.77	21.89	44.35	31.59		
M.S.		28.47^{x}	8.17	5.47	7.39	5.26		
Percent uptake of I^{131} per 100 mg of thyroid								
d.f.	30	4	10	4	6	6	17.53	92.9
S.S.	31704.84	12980.60	7575.22	4697.83	8887.58	2261.43		
M.S.		3245.15^{x}	757.52	1174.46	1481.26	376.90		
T/S								
d.f.	30	4	10	4	6	6	17.63	88.7
S.S.	3336.10	471.86	1577.96	683.82	910.47	375.81		
M.S.		117.97	157.80	170.95	151.74	62.63		
Total gain								
d.f.	30	4	10	4	6	6	10.00	96.8
S.S.	2652.29	1893.29	270.37	106.50	403.85	84.83		
M.S.		473.32^{xx}	27.04	26.63	67.30^{x}	14.14		
Thyroid weight (mg per 100 gm body weight)								
d.f.	30	4	10	4	6	6	7.27	93.3
S.S.	40.08	22.10	11.20	3.72	4.08	2.70		
M.S.		5.53^{xx}	1.12	0.93	0.68	0.45		

+ Percentage of total treatment sum of squares accounted for by the partial cubic regression equation. ^{x} Significant at 5% level. ^{xx} Significant at 1% level.

Table 6. Regression coefficients (b) and their standard errors (s_b) for the percent uptake of I^{131}, percent uptake I^{131}/100 mg thyroid and T/S in albino rats.

Term	I^{131} uptake %		I^{131} uptake/100mg thyroid %		T/S[+]	
	b	s_b	b	s_b	b	s_b
Mean	9.52		110.72		44.89	
T[ø]	-0.28	0.47	-8.04	3.96	-3.38	1.61
Ca	+0.49	"	+9.63	"	+0.06	"
I	-1.99[xx]	"	-19.54[xx]	"	-2.67	"
Nitrate	+0.69	"	+1.29	"	-1.08	"
T^2	-1.22[x]	0.42	-12.23[x]	3.59	-5.56[xx]	1.46
Ca^2	-0.99	"	-7.11	"	-0.47	"
I^2	-0.30	"	-2.48	"	-5.06[x]	"
$Nitrate^2$	-0.49	"	-5.98	"	-1.56	"
T-Ca	-0.57	0.57	-6.06	4.85	+1.53	1.98
T-I	+0.11	"	+2.31	"	-1.13	"
T-Nit.	-0.68	"	-5.69	"	-1.06	"
Ca-I	-0.31	"	-4.81	"	+0.06	"
Ca-Nit.	-0.04	"	-4.06	"	-1.18	"
I-Nit.	-0.23	"	-0.44	"	+0.45	"
T-Ca-I	+0.99	"	+11.94[x]	"	+2.43	"
T-Ca-Nit.	-0.50	"	-5.56	"	+0.59	"
T-I-Nit.	+0.42	"	+6.06	"	-1.66	"
Ca-I-Nit.	+0.25	"	+7.19	"	+2.28	"

[+] Thyroid/serum radio-iodide concentration ratio.

[ø] Turnip Sulfur.

[x] Statistically significant at the 5% level.

[xx] Statistically significant at the 1% level.

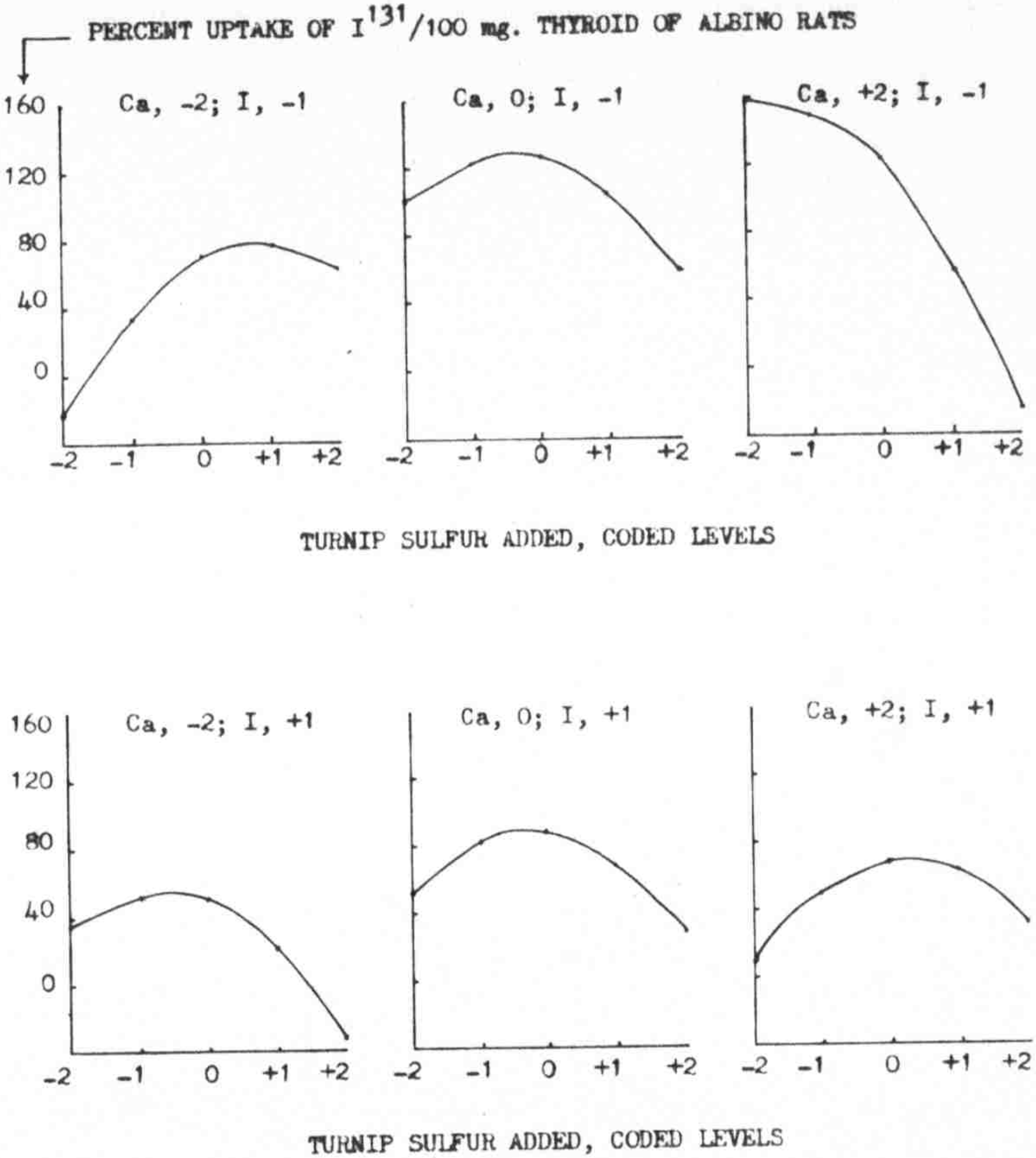

Figure 1. Uptake of I^{131} (per cent per 100 mg. thyroid) of albino rats as affected by levels of turnip sulfur at constant levels of added calcium. I was held at -1 (above) and +1 (below) and nitrate was held at the 0 coded level.

constant, all of the evidence indicated that calcium was not a goitrogenic substance.

The general first order effect of turnip sulfur was negative although not quite significant (table 6) and this is also seen in figure 1. However, under certain conditions, low Ca and low I, the positive trend predominated. The negative effect of turnip was in agreement with those obtained by Socolow and Susuzuki (64) who reported lower uptake of I^{131} by thyroid, while feeding a turnip supplemented diet to rats. The depressed uptake of radioactive iodine (I^{131}) by the thyroid was also reported by Storaasli <u>et al</u>. (67) when water and Remington diet (42) were given to the animals.

The general first order effect of Ca on I^{131} uptake was positive (almost significant, table 6). This result is in agreement with those obtained by Taylor (70) who found that the addition of 2 percent calcium carbonate to a low iodine diet greatly enhanced the radioactive iodine (I^{131}) uptake by thyroid. At high turnip sulfur and low I levels the effect of Ca on percent uptake of I^{131} per 100 mg thyroid was negative (figure 2). Thus, the effect of both Ca and turnip sulfur on I^{131} uptake are variable and need further study with regard to reasons. The relatively high degree of downward curvature of the lines in figures 1 and 2 are the result of the

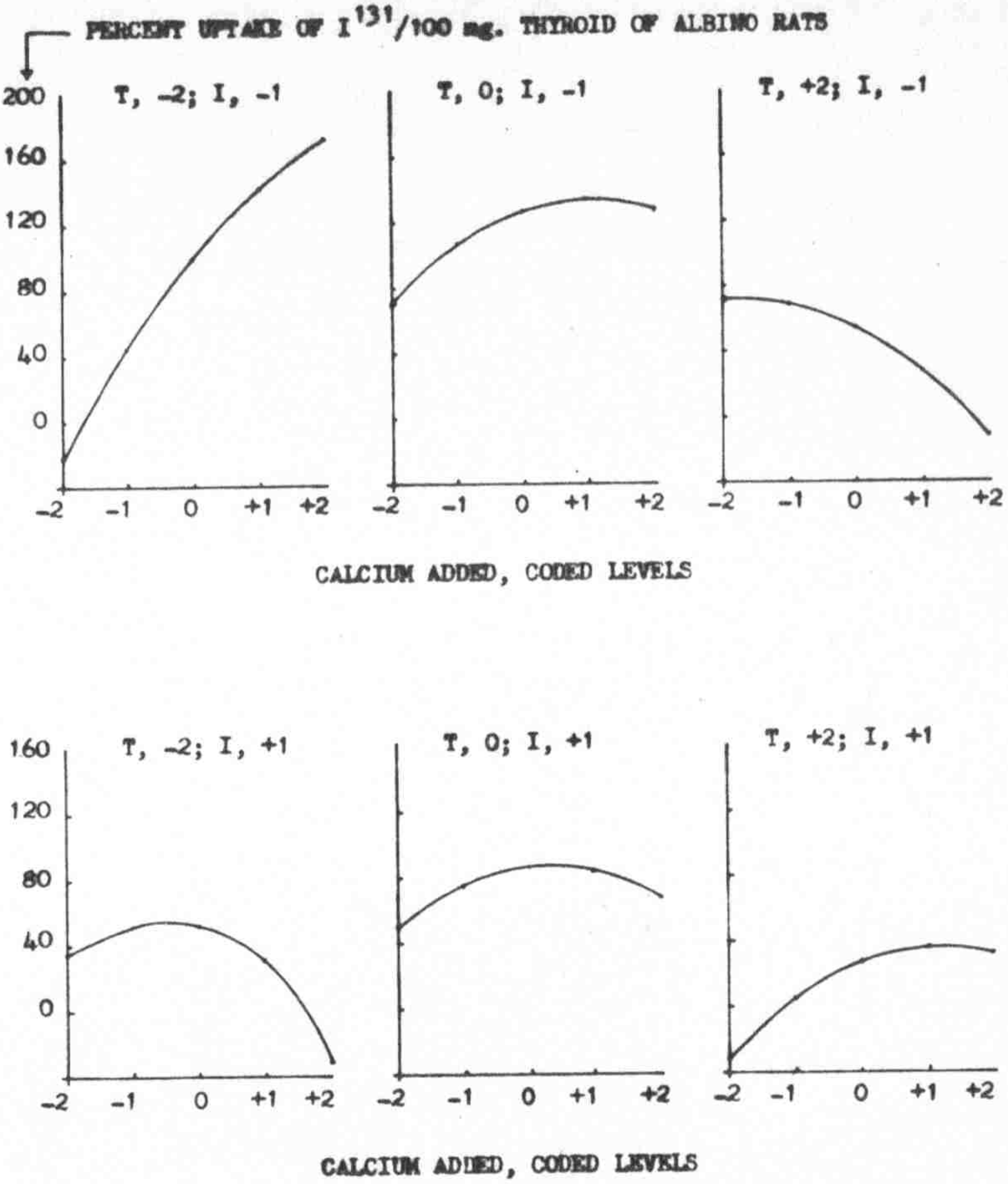

Figure 2. Uptake of I^{131} (per cent per 100 mg. thyroid) of albino rats as affected by calcium at constant levels of added turnip sulfur. I was held at -1 (above) +1 (below) and nitrate was held at the 0 coded level.

large negative squared terms for T (significant) and Ca (almost significant) shown in table 6.

The effect of increasing I levels in the diet was negative to subsequent uptake of I^{131} as shown by the highly significant first order term (table 6). The probable reason is that thyroid well supplied with I would not have the avidity to take up I at a high rate as compared to a thyroid that was iodine-deficient. Other workers (51, 53) explained that an excess of I lead to the inactivation of oxidative enzyme responsible for the conversion of iodide to iodine, thus, an excess of iodide present in thyroid might disturb the optimal condition for iodination and uptake of I^{131}.

Effect of Variables Studied on T/S Ratio

The data for T/S ratio (table 4) indicated a range of 16.6×10^2 to 58.4×10^2. A thyroid-iodide/serum-iodide concentration ratio of 25:1 has been reported in normal rat thyroid after administration of propylthiouracil (PTU). The ratio increased to 200-300:1 during chronic administration of an antithyroid agent (23, 32, 63). In the present work, the T/S values were much higher than those reported by the above workers because no PTU treatment was used. The regression coefficient for the first order effect of increasing the level of turnip sulfur was negative (almost significant, table 6) and the squared term effect of turnip sulfur was highly significantly negative. This indicated that turnip sulfur prevented movement of I from the blood into the thyroid and the effect was greatest at high level of turnip sulfur. The significantly negative squared term for applied I indicated that the first order effect of I became more negative at high I level.

Effect of Variables Studied on Body Weight

The observed total gain in body weight varied from 20.5 to 60.0 gm (table 4). The T-Nitrate interaction (table 7) was positive although too small to be significant. However, the total gain in body weight was influenced by the significantly negative T-I-Nitrate interaction (figure 3). When the iodine level was low, the predicted effect of turnip sulfur was to decrease body weight at a low level of nitrate and to increase it substantially at a high level of nitrate. Thus, the positive T-Nitrate interaction was enhanced at low I levels but was reversed and became somewhat negative at high I levels. The significant first order positive effect of turnip sulfur can be seen in figure 3.

The response to nitrate (figure 4) was negative at a low level of turnip sulfur and iodine and at high

Table 7. Regression coefficient (b) and their standard errors (s_b) for the total gain and thyroid, mg/100 gm body weight, in albino rats.

No.	Term	Total gain in body weight		Thyroid mg/100 gm body weight	
		b	s_b	b	s_b
	Mean	37.62		9.23	
1	T^{+}	+2.05^{x}	0.77	+0.36^{x}	0.14
2	Ca	-8.63^{xx}	"	+0.38^{x}	"
3	I	-0.33	"	-0.52^{xx}	"
4	Nitrate	+0.43	"	+0.61^{xx}	"
5	T^2	+1.34	0.70	-0.27	0.12
6	Ca^2	+0.63	"	-0.35^{x}	"
7	I^2	+1.51	"	-0.30	"
8	$Nitrate^2$	-0.84	"	+0.15	"
9	T-Ca	-0.70	0.94	+0.04	0.17
10	T-I	-0.94	"	+0.14	"
11	T-Nit.	+1.33	"	-0.22	"
12	Ca-I	+1.36	"	-0.23	"
13	Ca-Nit.	+1.35	"	+0.19	"
14	I-Nit.	+0.96	"	-0.14	"
15	T-Ca-I	+0.14	"	-0.00	"
16	T-Ca-Nit.	-1.88	"	+0.14	"
17	T-I-Nit.	-3.04^{x}	"	+0.00	"
18	Ca-I-Nit.	-2.41^{x}	"	-0.06	"

[+] Turnip sulfur.

[x] Statistically significant at the 5% level.

[xx] Statistically significant at the 1% level.

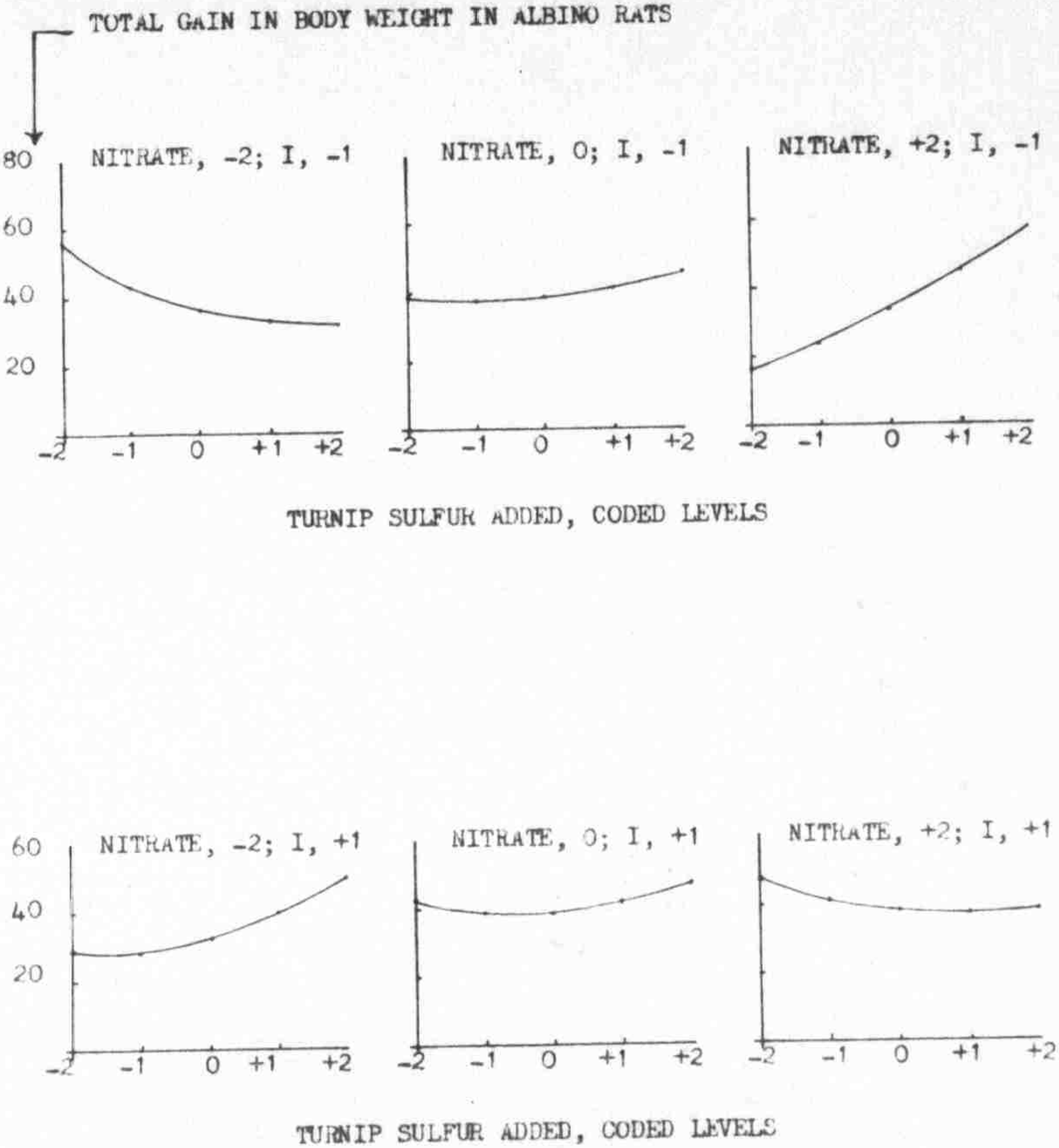

Figure 3. Total gain in body weight in albino rats as affected by levels of turnip sulfur at constant levels of added nitrate. I was held at -1 (above) and +1 (below) and calcium was held at the 0 coded level.

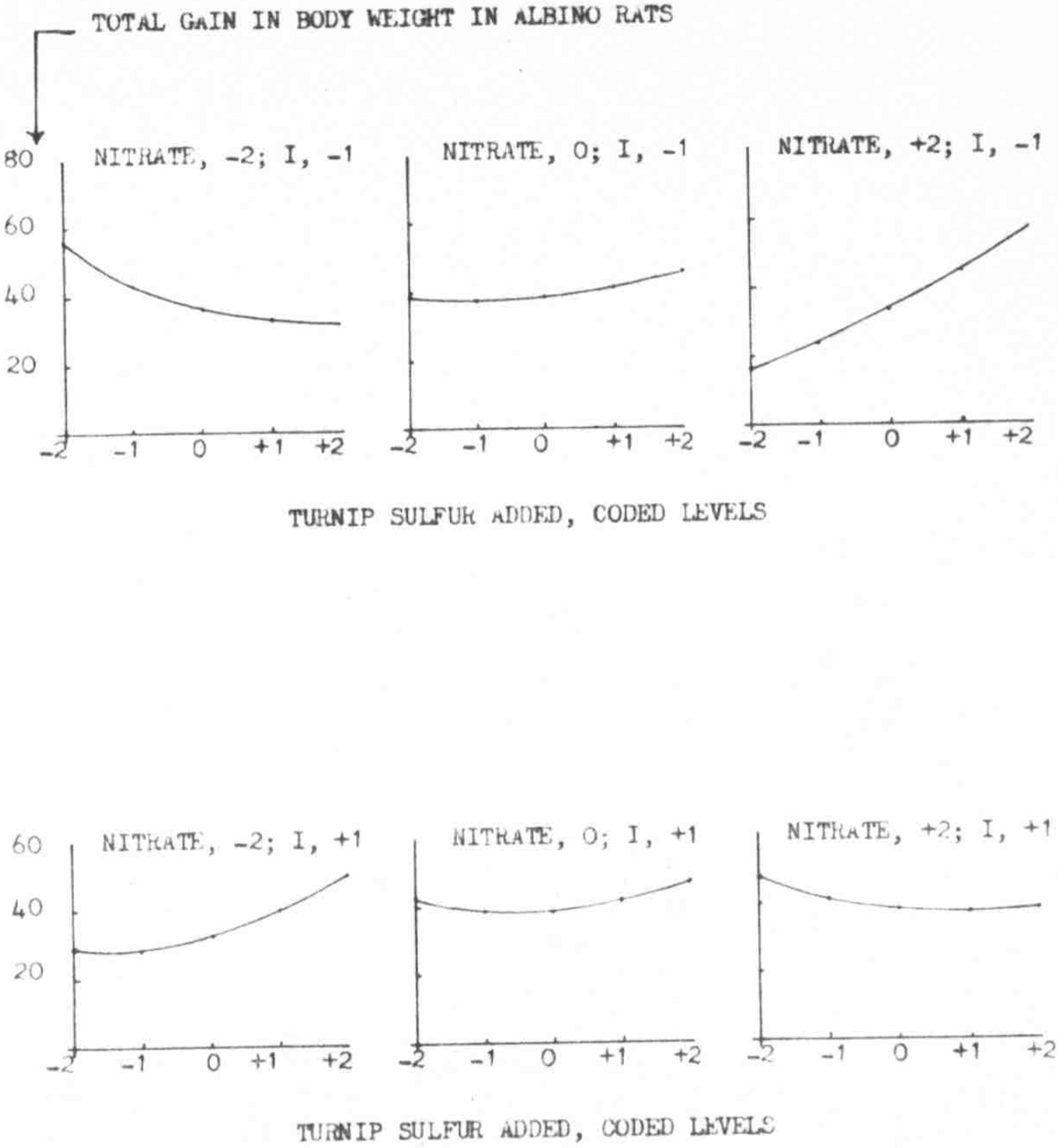

Figure 3. Total gain in body weight in albino rats as affected by levels of turnip sulfur at constant levels of added nitrate. I was held at -1 (above) and +1 (below) and calcium was held at the 0 coded level.

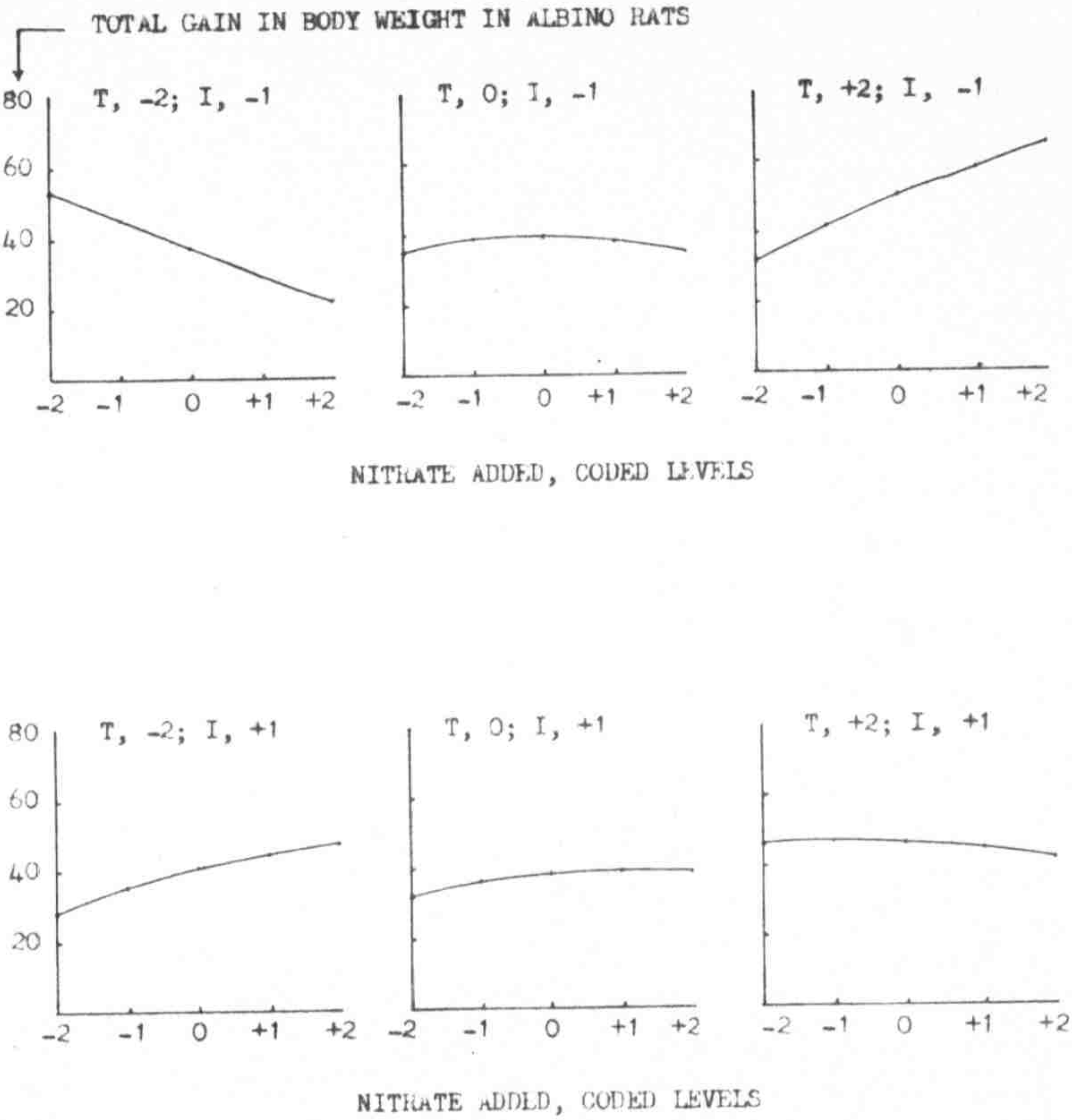

Figure 4. Total gain in body weight in albino rats as affected by nitrate at constant levels of added turnip sulfur. I was held at -1 (above) and +1 (below) and calcium was held at the 0 coded level.

level of turnip sulfur and at low level of iodine the effect was positive (figure 4). At a high I level the T-Nitrate interaction tended to disappear.

It was concluded from figures 3 and 4 that the effect of nitrate and turnip sulfur levels and the interaction between them were important only at a low level of I in the ration.

The interaction of Ca and nitrate in the diet was influenced by the level of I as indicated the significantly negative interaction of Ca-I-Nitrate (figures 5-6). At all constant levels of added nitrate (figure 5) the total gain in body weight was decreased with increasing Ca level (highly significantly negative first order effect, table 7). The decrease in total gain was most severe at low levels of nitrate and I (figure 5). The negative effect of nitrate was greatest when Ca and iodine were present at low levels (figure 6). At a high level of I the Ca-Nitrate interaction was small.

It was concluded that Ca was the most depressing on weight gain at low levels of nitrate and I.

Effect of Variables Studied on Thyroid Weight

The weight of the thyroid (table 4) ranged from 6.48 to 11.00 mg per 100 gm body weight. The first order effect of increasing the level of turnip sulfur on the

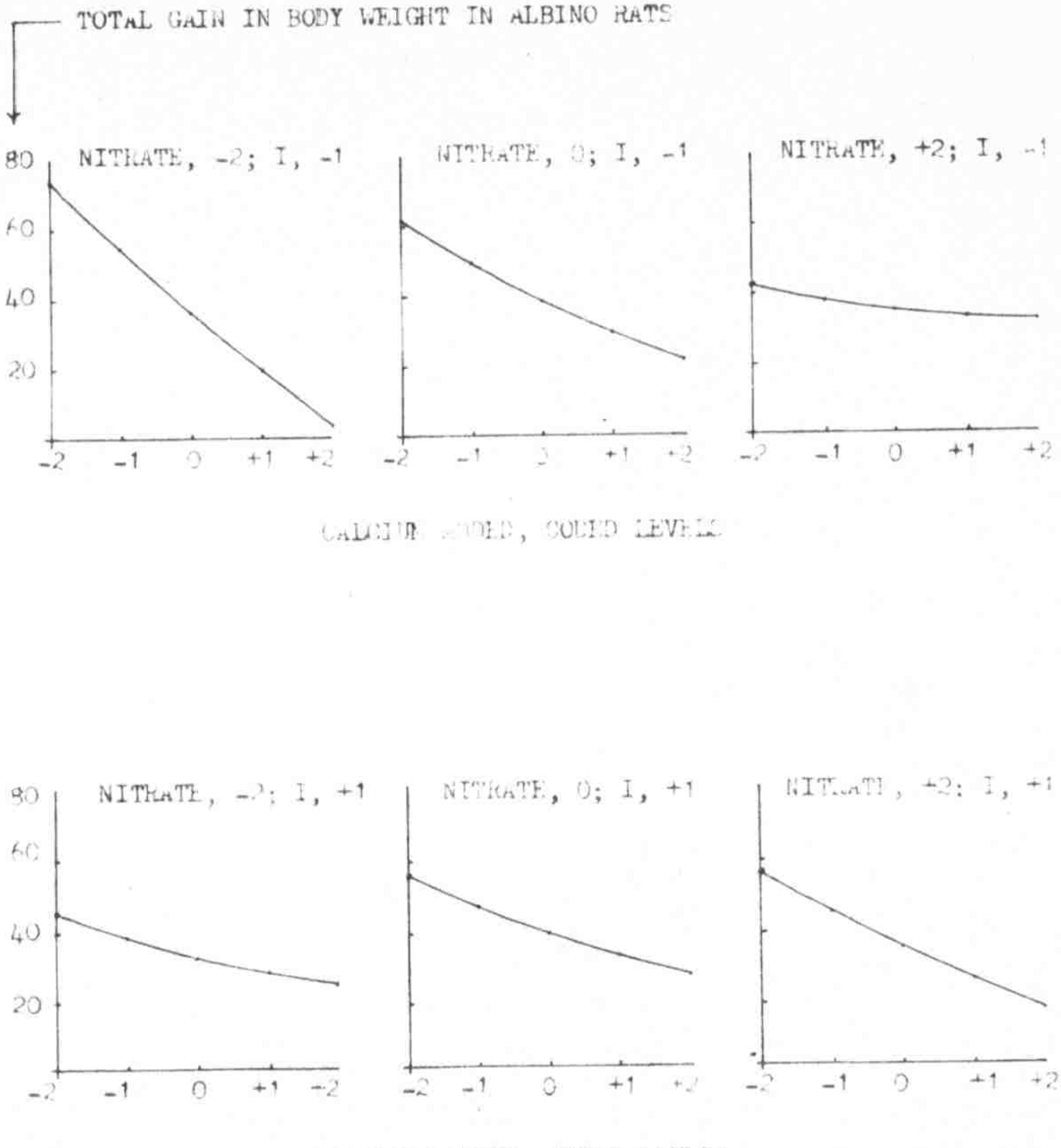

Figure 5. Total gain in body weight in albino rats as affected by calcium at constant levels of added nitrate. I was held at -1 (above) and +1 (below) and turnip sulfur was held at the 0 coded level.

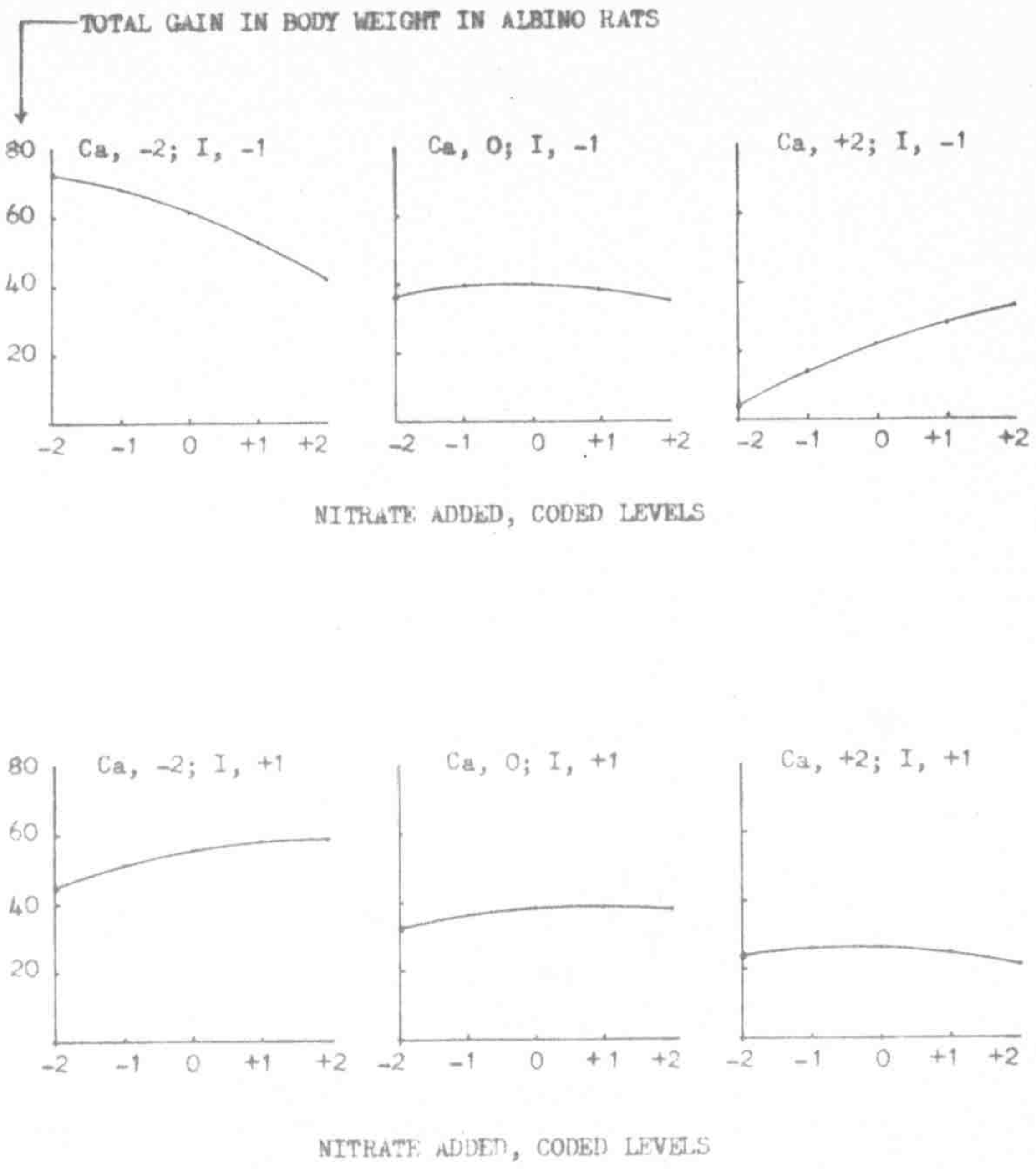

Figure 6. Total gain in body weight in albino rats as affected by nitrate at constant levels of added calcium. I was held at -1 (above) and +1 (below) and turnip sulfur was held at the 0 coded level.

thyroid enlargement was significantly positive (table 7). The explanation might be that turnip roots containing goitrogen produced thyroid enlargement by inhibiting the uptake of iodide or obstructing the oxidation of iodide to iodine and consequently interfering with the production of thyroxine. This, in turn, stimulated the pituitary to increase thyrotrophin (TSH) secretion. The TSH acted upon the thyroid cells causing thyroid enlargement.

As was found by Taylor (70), Ca addition to the diet increased the thyroid weight (mg per 100 gm body weight) significantly (table 7). The Ca squared term was significantly negative. The fact that I^{131} was getting into the thyroid would tend to discount Taylor's theory that Ca might be precipitated with iodine as insoluble compounds in the gut or iodine might form unavailable complex with Ca thus preventing iodization of tyrosine in the thyroid gland. Such lack of active iodine (I_2) in the thyroid gland would cause endemic goiter.

The first order effect of I on thyroid enlargement (mg per 100 gm body weight) was highly significantly negative (table 7). The regression coefficient (table 7) for the direct effect of nitrate was highly significantly positive. These results are in agreement with those of other workers (12, 75). They found that high levels of dietary nitrate adversely affected the normal functioning of thyroid gland and subsequently increased thyroid weight.

In table 6 it was shown that the Ca effect on I^{131} uptake per 100 mg thyroid was positive (almost significant) and the nitrate was inconclusive although it tended to be positive in the percent I^{131} uptake. Turnip sulfur tended to have a negative effect on I^{131} uptake at the same time it has been shown that all the three goitrogens increased the thyroid weight significantly. This indicated that the function of turnip sulfur was different from that of Ca and nitrate in goiter production. Goitrogens can be classified in three categories (56, 72). Firstly, there are substances such as thiocyanate and perchlorate which inhibit the uptake of iodide from the blood into the thyroid. Secondly, a number of sulfur-containing substances, for example, thiourea and thiouracil, obstruct oxidation of iodide to the iodine that iodizes tyrosine within the thyroid cells. Thirdly, several other substances, such as sulfonamides and resorcin, inhibit unspecifically the iodization of tyrosine in the thyroid gland. All the three groups of substances enumerated above result in production of goiter. The turnips containing goitrin inhibited the uptake of I^{131} by the thyroid and may belong to the first or second category. The Ca and nitrate differ in their function from that of turnip and are probably included in the third group above.

Since the results of the present exploratory investigation, in some cases, are inconclusive and also do not always agree with the results obtained by others, further research is needed. The disagreement of the results with those of other workers may be due to different conditions under which the animal experiment was conducted. In order the results to be more conclusive, a similar experiment should be run with a larger number of rats per diet, so that weekly observations on thyroid enlargement, its histological examination and thyroid function as correlated with radioactive iodine would be possible over a period of time. Analysis should be made for determination of the concentration of soluble and calcium precipitated thyroidal iodine for each group of rats. Calcium and nitrate should be assayed for their ability to discharge iodide accumulated within the thyroid, or to block iodide uptake in the rat. Also, lengthening of the feeding period for the experimental animals would allow an intensification of the effects.

Effectiveness of Experimental Design

The design under study was used for exploratory work where the object was to determine quickly the effect of each of a number of variables over a wide range of conditions. The design permitted the coverage of a wider range of variables, levels and combinations than otherwise would have been possible with the time and the facilities available. The analysis of variance (table 5) indicated that the proportion of total treatment sum of squares accounted for by the cubic regression equation (equation sufficiency) was not less than 88.4 percent, in any case, which indicated a close fit of the regression equations to the actual data. It was concluded from the limited data of this study that the central composite, rotatable design was suitable for small animal experimentation where exploratory work is necessary.

SUMMARY AND CONCLUSIONS

Nutrient solutions containing five levels of sulfur were used to determine the possibility of production of turnips with low to high sulfur concentration in sand culture pots. The total sulfur concentration of turnip roots increased significantly and progressively from 0.23 to 0.95 percent with increasing sulfur in the nutrient solution. The sulfur content of the tops closely coincide with that of roots. The highest yield was obtained with the sulfur level maintained at 1/9 the standard Hoagland-Arnon solution indicating that turnips are sensitive to the supply of sulfur.

Turnips containing 0.28 percent sulfur were produced in the green house in sand culture with a nutrient solution deficient in sulfur. These turnips were used in a goitrogenicity study with rats along with normal, locally obtained turnips containing 0.49 percent sulfur. Female weanling albino rats were used. Four variables, turnips with low and high sulfur levels, Ca, I and nitrate, each at five levels were added to the basal diet and fed the rats in various combination to determine the effect of each variable individually and in combination with others on thyroid uptake of radioactive iodine (I^{131}), T/S, weight gain and thyroid weight.

At a low levels of I and Ca, turnip sulfur generally increased I^{131} uptake per 100 mg thyroid while at high Ca level, turnip sulfur had a strongly depressing effect. At high I level the T-Ca interaction tended to disappear. Ca increased I^{131} uptake particularly at low levels of turnip sulfur and I. The effect of nitrate and turnip sulfur levels and the interaction between them on total gain in body weight tended to be effective only at a low level of I in the diet. The Ca effect on weight gain was depressing, especially at low levels of I and nitrate. The first order effects of all the three goitrogens studied were significantly positive in goiter production but the function of turnip sulfur was different from that of Ca and nitrate as shown by the I^{131} uptake.

Increasing level of turnip sulfur resulted in an increase in thyroid weight of rats, depressed I^{131} uptake by the thyroid and lowered the T/S ratio indicating that this vegetable contained a high level of goitrogen. Much experimental work and effort will be needed to solve satisfactorily the question of the goitrogenicity of turnips and its interaction with other variables.

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