ACKNOWLEDGEMENTS

To Dr. Andre DesMarais, my deepest thanks and sincerest appreciation for his most invaluable assistance in all I have undertaken. Without the inspiration and guidance which he has conveyed to me, my task in preparing this Thesis would have been all the greater.

I wish to thank Dr. L. P. Dugal, Chairman of the Department of Biology, for making available to me the facilities of the Department.

Many thanks to the members of the staff and graduate students for their aid and assistance during my tenure at Ottawa University.

I also wish to thank Miss L. Dugal and Miss C. Tetrault, whose technical assistance has been an important factor in enabling me to complete this Thesis.

To my wife Doreen, I wish to express my sincere thanks for her patient assistance in the stenographic preparation of this Thesis.

ABSTRACT

Weanling male albino rats were fed a vitamin A deficient diet. One group was given 1000 I.U. vitamin A per week; a second group was given 50,000 I.U. per week; and a third group had no supplement of vitamin A. Thyroid secretion rates, thyroid weights and ascorbic acid biosynthesis were determined for each group. In no instance did the level of dietary vitamin A or food intake influence any of these parameters.

Iodine deficiency did not cause any change in these results except to increase the trapping of I^{131} and its release from the thyroid.

Cold exposure at 2°C had no effect on these parameters except that it caused a general increase in thyroid activity in all animals after one week in the cold and thereupon the hyperfunction subsided and activity returned to normal values. The release of I^{131} also was notaffected by the cold. The ascorbic acid biosynthesis decreased after one week in all groups and then rose back to normal levels.

The vitamin A levels in the livers of the excess vitamin animals were found to be about fifty times that of the normal animals showing that the animals did indeed have different levels of vitamin A in the body.

Several parameters of thyroid function were examined. It was found that I^{131} uptake and release by the thyroid gland were subject to many misinterpretations if the complete condition of the animal was not known. It was also shown that the calculation of TSR by the injection method was not an absolute but a relative measurement. Sørensen's method of calculating TSR's was scrutinized and it was observed that our findings did not seem to be in agreement with his basic assumption. It was concluded that the TSR measurement, calculated by the injection method, was most suitable for use in rats, if one could be satisfied with relative levels of thyroid function.

STATEMENT OF THE PROBLEM

There has been much controversy about whether the level of dietary vitamin A has an effect on the thyroid gland and if vitamin A levels affect the biosynthesis of ascorbic acid. Because of this, experiments were done to see if vitamin A deficiency or vitamin A excess had any effect on thyroid activity or on the synthesis of ascorbic acid in vitro.

Since there are many ways in which thyroid activity can be measured, various parameters of thyroid activity were examined and compared so that the advantages and disadvantages of each would be known.

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LITERATURE REVIEW

Vitamin A and the Thyroid Gland

That a state of antagonism exists between vitamin A and the thyroid gland has been suggested and argued over for three decades. This subject has been reviewed by Moore (1) in 1955.

An antagonism or some form of interaction between the thyroid and vitamin A may be seen in the histological condition of the gland in animals under the influence of various vitamin A dietary levels. Sherwood et al (2) found that a decided change was produced in the thyroid glands of rats that received 1 to 5cc codliver oil per kilogram per day. The change was primarily a depletion of the colloidal content of the follicles. A control group was given corn oil, another was given viosterol, a third was given vitamin A-free codliver oil. It was seen that in the control groups the thyroid gland was not affected. There was however no change in the basal metabolism in any of the animals.

Carpenter and Sampson (3) gave rats 25,000 I.U. of vitamin A daily for one week and doubled or tripled the dose in succeeding weeks. When the rats showed loss of weight the thyroids were taken out and examined. The thyroids of the rats given the excess vitamin A had follicles which were small, highly irregular in shape and contained relatively

little colloid. The follicular cells were hypertrophied and frequently contained P.A.S. positive droplets in the cytoplasm. It was also found that the uptake of I^{131} by the gland was increased.

Coplan and Sampson (4) showed that vitamin A deficiency produced a definite hypertrophy in the thyroid gland of the female rat but consistent atrophy in the male during the period of experimentation. Other workers, Sampson et al (5) and Sure (6), found no differences in thyroid weights with vitamin A deficiency.

Metabolic changes also seem to show a relationship between the thyroid gland and vitamin A. Blaizot and Benac (7) discovered that the privation of vitamin A in the rat provoked an elevation of energy expenditure and that this elevation was an early sign of deficiency. It appeared before the weight plateau and the notable ocular signs and was seen before the mortal metabolic disorders were established.

Danowski et al (8) found that rats given 70,000 units of vitamin A per week had a slight but definite depression in blood protein iodine. This is in direct contrast to Sadhu and Truscott (9) who reported increased P B I 's in rats given excesses of vitamin A.

Frape et al (10) reported that both insufficient and excessive intakes of vitamin A lowered the rate of thyroid

secretion. They postulated that because of the lack of relationship between the secretion rate and growth rate in the pig, the effect of vitamin A on the thyroid gland is direct.

Baumann and Moore (11) found that an excess of vitamin A, an excess of thyroxine, or an excess of both caused animals to lose weight and could find no specific antagonism between thyroxine and vitamin A.

Lately some clinical work has been done on the relationship between vitamin A and goitre but this relationship is still obscure and controversial. Horvat and Mavor (12) consider goitre to be the result of a complex nutritional deficiency and that in addition to lack of iodine a deficiency in vitamin A plays an important role as well. This conclusion was drawn from the results of giving vitamin A treatment to school children and obtaining a reduction of 45% in the incidence of goitre as compared with a control group. In a later study, however, Horvat and her co-workers (13) found that I^{131} uptake by the thyroid gland did not change substantially when vitamin A was given. This finding does not support the suggestion that a low intake of vitamin A was a factor in the occurrence of goitre in this instance. Haubold (14), describing an outbreak of goitre in Bavaria, considered vitamin A and carotene deficiency in food was the cause. Eggenberger (15) noted on examination of pregnant women, that those with normal dark adaptation had only 9.5% of children with enlarged

thyroid glands; whereas women with pathological adaptations had 37% of children with goitre.

It has also been shown that thyroxine has an effect on ameliorating the deleterious effects of excess vitamin A and on enhancing the effects of an insufficiency of vitamin A. Since the work in this thesis does not cover this the reader is referred to the review by Moore (1) who concludes that there is little doubt of the ability of the thyroid to influence the metabolism of vitamin A. The degree of specificity of this influence, however, remains undefined.

Ascorbic Acid Status at Various Vitamin A Dietary Levels

Since vitamin A deficiency has been shown to cause a fall in the level of ascorbic acid in various tissues and leads to a lowering of ascorbic acid exretion in the urine, it has been suggested that the deficiency leads to an interference in the biosynthesis of ascorbic acid.

Sure et al (16) found that the ascorbic acid level in the heart, kidney, and liver decreased in vitamin A deficiency. The administration of ascorbic acid did not cause an increase in the tissue concentration. Mayer and Krehl (17) also showed that the liver and adrenal levels of ascorbic acid decreased in vitamin A deficient rats; however, if ascorbic acid was given, the levels of ascorbic acid in the liver and adrenal returned to normal. This result is in contrast with

that of Sure et al.

Sutton et al (18) observed that a suboptimum vitamin A intake resulted in a decreased concentration of blood plasma ascorbic acid and a decrease in urinary ascorbic acid. It has also been reported (19, 20) that in vitamin A deficiency the levels of plasma and cerebrospinal fluid ascorbic acid decreased. These decreased levels could be reversed by giving vitamin A. Eaton (21) noted that in vitamin A deficient cattle only administration of the vitamin could reverse the lowering of decreased plasma ascorbic acid and that administration of ascorbic acid itself did nothing. Meyer and Krehl (17) also showed that rats given a vitamin A deficient diet had lowered blood ascorbic acid levels.

Mapson and Walker (22) confirmed that in vitamin A deficiency the ascorbic acid levels in blood and liver were lowered. This lowering could not be accounted for by increased urinary excretion. They concluded that the lowered levels could be accounted for by a decreased food intake since, when normal rats were pair-fed with vitamin A deficient rats there was no difference in the ascorbic acid levels. They also believe that no specific relationship existed between the levels of vitamin A in the rat and its ability to synthesize ascorbic acid.

Hart et al (23) working with horses and later Booker et al (24) working with chickens found that plasma ascorbic

acid levels did not show any response to vitamin A levels.

There has been two reports examining the biosynthesis of ascorbic acid in the liver "in vitro". The first is by Chatterjee et al (25). They reported that the conversion of L-gulonolactone into L-ascorbic acid by liver microsomes from vitamin A deficient rats was negligible. The activity of the microsomes could be restored by feeding vitamin A to the deficient rats. It also was increased by the addition of the boiled supernatant to the incubation medium. The second report is by Sastry et al (26). They assert that the levels of ascorbic acid in the livers of deficient rats was only half that of the pair-fed control rats. When the "in vitro" synthesis of ascorbic acid from liver microsomes was examined it was discovered that it was reduced to 50% at the weight plateau stage and to 10-20% of the pair-fed controls at the acute stage.

Not only does a deficiency of vitamin A seem to affect the levels of ascorbic acid but there also seems to be an effect from an excess of vitamin A. It has been shown by Collett and Erickson (27) that an excess of vitamin A caused scurvy although a moderate excess dose of 2,400 I.U. per day had no injurious effect.

Moore and Wang (28) on the examination of the livers and adrenals of normal and excess vitamin A rats, found no differences in the ascorbic acid levels. They also noted that

there was no difference in the excretion of ascorbic acid in the urine of these rats. Morehouse et al (29) reported, however, that the level of ascorbic acid in the liver was lowered in the rats given an excess of vitamin A. This agreed with the results of Rodahl (30) who also found a decrease in blood ascorbic acid in guinea pigs. Eeg-Larsen and Pihl (31), however, considered that this decrease was due to a decrease in food consumption since they found the same decrease in rats showing the same weight loss during pair-feeding.

Effect of Cold Exposure on Thyroid Secretion

The role of the thyroid gland in cold acclimation has been quite extensively studied. Since this subject has been reviewed by Cottle (32), D'Angelo (33) and Hart (34) the effect of cold on thyroid activity will only be summarized below.

There is evidence that the thyroid gland reaches a maximum of activity early during cold exposure and gradually returns to normal or near normal conditions. Starr and Roshelley (35) observed that the height of the follicular cells in thyroids from rats kept at 12-17° reached a maximum in about 3 weeks and was near normal after about 6 weeks. Heroux et al (36) did not find that the thyroid glands of rats kept 3 months outdoors in winter conditions, showed any evidence of increased thyroid activity when examined histologically although the animals were cold acclimated.

Cottle and Carlson (37) observed that gland weight and I^{131} content 4 hours after injection increased immediately upon exposure but returned to about normal after 60 days at 5°C. When examining the biological decay of I^{131} and the 24 hour conversion ratio of PBI^{131}/Total serum I^{131} they found that these values showed a greater turnover of thyroid hormone during early exposure and after 180 days exposure at 5°C. Leblond et al (38) also examined the uptake of I^{131}. They found that the 2 hour uptake increased during a period of 1-3 weeks in the cold at 4°C but was similar to the controls after six weeks.

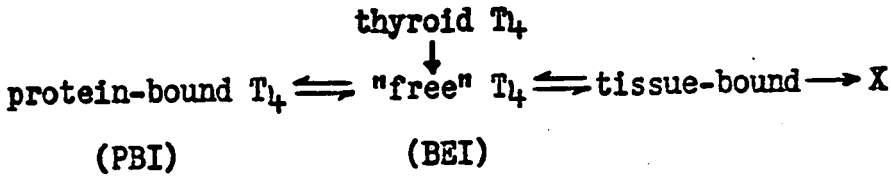
The measurements of I^{131} released by the gland and the attempts at measuring thyroid secretion show an increase in thyroid activity which is maintained during long exposures to cold. D'Angelo (33) found that after 6-8 weeks of cold exposure the release rate of I^{131} from the gland was significantly increased in both rats and guinea pigs. Woods and Carlson (39) observed that thyroxine secretion was greatly increased following 2 weeks in the cold and that thyroid activity remained at a high level throughout a 60 day exposure period.

In conclusion it seems that although there is agreement among investigators that there is an immediate increase in thyroid activity in the cold, there is no agreement as to the intensity and duration of the hyperactivity.

Measurement of Thyroid Function

a) Serum Protein-Bound Iodine (PBI)

PBI is a measurement of the amount of iodine which is mainly in the form of thyroxine and triiodothyronine bound to plasma proteins. The level of this iodine is a result of the amount of these hormones released by the thyroid and the rate at which these hormones are transferred from the blood to their site of action, the peripheral tissues.



This test is based on the premises that an increase in PBI denotes an increase in thyroid activity and a decrease in thyroid activity shows a decrease in PBI.

It is known, however, that PBI levels may be altered in circumstances other than changes in thyroid function. Danowski et al (40) found that PBI increased during pregnancy. This increase was not associated with any apparent thyrotoxicosis. Kydd and Man (41) noted that certain cases of hepatitis, especially those in early stages which are harder to detect, give high PBI values without corresponding increases in thyroid activity. Many patent medicines which contain iodine such as cough syrups, antiseptics and dentifrices, will also give erroneously high values.

There are also factors which will cause a low PBI without a decrease in thyroid activity. Most of these factors affect the levels of thyroxine binding protein (TBG) and thyroxine binding prealbumin (TBPA). If the levels of these proteins decrease PBI will decrease. Recant and Riggs (42) showed that in cases of nephrosis, PBI values are lowered. Kydd and Man (41) found that in certain cases of liver disease PBI values were lower without any apparent effect on the thyroid gland. Peters and Man (43) observed that in hypoalbuminemia the level of PBI was decreased and concluded that this was due to a lack of binding sites for the hormones. It has been shown by Hill et al (44) that treatment with ACTH or cortisone will cause a lowering of PBI values.

From the above it can be seen that when using PBI as an index of thyroid activity there has to be a clear appreciation for the numerous influences on the level.

b) Serum Butanol-Extractable Iodine (BEI)

Advantage has been taken of the fact that thyroxine and triiodothyronine are more soluble in butanol than various other iodinated compounds of the serum, after precipitation of the blood proteins. This method is believed to give a measure of the free-circulating hormones. In man, the following relationship has been found between PBI and BEI. Expressed as thyroxine in moles per liter, the PBI contains 1×10^{-7} and the BEI 6.2×10^{-11} (45). It has been most

helpful in correcting for the erroneous elevation of PBI caused by inorganic iodine administration and in cases of an abnormal noncalorigenic component in the serum. Unfortunately, however, it does not adequately eliminate the elevation in readings caused by compounds containing organic iodide (e.g. radiopaque mediums).

There does not seem to be too much work done on this aspect of thyroid measurement possibly because of technical difficulties and/or possibly because of the greater popularity of the PBI method; however, it is probable that in most of the cases mentioned in which PBI is changed BEI will be changed in a like manner.

c) Uptake of I^{131} by the Thyroid Gland

The uptake of I^{131} in a specific interval has been widely established as a good diagnosis of thyroid activity. The uptake is an overall resultant of various parameters which are; (a) the uptake of iodine, (b) its conversion to hormonal form, (c) release of hormone to the circulation and (d) its urinary excretion.

The use of the measurement of the uptake of I^{131} as an index of thyroid activity is based on the theory that the rate of net inward flux of iodine can be equated to the rate of hormone secretion under conditions of iodine equilibrium.

There are two ways in which this uptake can be measured and both have been used. The first of these ways is the amount of I^{131} uptake. The higher the percentage of administered I^{131} accumulated in the gland, the higher the activity of the thyroid is presumed to be. There are a few situations, however, in which there is little or no correlation between uptake and thyroid secretion. This can be shown by blocking the organic binding of iodine with thiouracils and other drugs. Wase and Foster (46) and Wollman and Reed (47) have demonstrated that in these cases the thyroid does not lose its iodine concentrating ability. Greer et al (48) noted that other goitrogenic compounds, e.g. vinyl thioorazalidone, prevent the trapping of iodine by the gland but do not block the hormone synthesis.

The second way of measuring I^{131} uptake is by the rate of accumulation in the gland. The faster the iodine is picked up the more active the gland is accepted to be. Soderberg (49) stated that little correlation can be found in cases where the rate of uptake is compared with the rate of secretion from the gland in animals which have a higher rate of iodine excretion than normal. This was demonstrated by the effects of antidiuretic hormone on renal iodide clearance. This alters the proportion of iodide that can be cleared from the blood stream by the thyroid.

The rate at which I^{131} is accumulated and the amount

of uptake do not necessarily express idential phenomona. There is a progressive decrease in the rate of accumulation of I^{131} with age (50) though the total I^{131} accumulation shows no such decline. Another discrepancy between both types of I^{131} uptake has been found in toxic goitre treated with adrenocorticotrophin (ACTH). Here the rate of uptake is altered by ACTH administration but total uptake remains unaffected (51).

This method of measuring the thyroid activity therefore depends on an understanding of normal thyroid physiology and on the various exogenous and endogenous factors that can modify the component steps.

d) Release of I^{131} from the Thyroid Gland

Another method of measuring thyroid activity is the release of I^{131} from the gland through its decrease in radioactivity (52,53). When plotted semilogarithmically the decrease in radioactivity forms a slope which represents the ratio of the amount of labelled hormone secreted per unit time to the total amount in the gland. An important point to be noted is that the thyroid gland is not uniform and has both small and large follicles. There is a more rapid turnover of I^{131} in the smaller follicles. This has been seen by Nadler (54) and Milcou et al (55). Thus there is a biphasic effect. The initial release would be from the smaller follicles and

as the larger follicles begin to secrete the labelled hormone the slope of I^{131} release will change and then become constant. This means that both parts of the curve have to be examined.

A straight line requires that the rate of uptake of the recirculating I^{131} is constant and that the hormones are uniformly labelled. The changes in the slope will be related to changes in the thyroid activity only if the total amount of hormone in the gland remains constant. Wahlberg (56) observed that the iodine content of the thyroid changes with variation of activity of the gland, thus a steady state is not necessarily present. The results, therefore, must be carefully evaluated.

The rate of hormone secretion is also related to the size of the stores of preformed hormone in the gland. This has been demonstrated by Solomon (57) who inhibited the synthesis of hormone in the gland by the use of drugs. If there were a constant secretion of hormone the slope of the release curve would gradually convex upwards instead of being a straight line when plotted semilogarithmically because, when synthesis is blocked, a steadily increasing proportion of the remaining thyroid hormone stores would be removed from the gland under constant rates of secretion. What is actually obtained is a straight line showing that the hormone secretion is not constant but varies with the content in the gland.

A careful handling of the experimental situation will usually give valuable information on the state of the thyroid. This method will show comparative values under exact conditions (size of thyroid, number and size of follicles, level of stored hormones) and these have to be similar in the group of animals studied or the results cannot be correctly interpreted.

e) Thyroid Secretion Rate Determined by the Injection of Thyroxine

This method stems from the attempt of Dempsey and Astwood (58) to measure the thyroid secretion rate. Their method involved the administration of thiouracil which causes a decrease in the hormone synthesis and thus a drop in blood thyroxine. Because of this, thyrotrophin is secreted from the hypophysis and the thyroid gland becomes hyperplastic and hypertrophic. If thyroxine is injected, the gland returns to normal because the blood thyroxine rises and the thyrotrophin secretion is thus lowered. The dose of injected thyroxine required to bring the gland back to normal as compared to controls is considered to be the thyroid secretion rate. One big drawback to this method is that the animals have to be sacrificed in order to determine the thyroid secretion.

Perry (53) found that graded doses of thyroxine would inhibit the release of I^{131} from the thyroid propor-

tionally to the amount of thyroxine given. Reineke and Singh (59), using this observation, designed a method (described elsewhere in this thesis) to determine the thyroid secretion rate without sacrificing the animals. They measure the amount of thyroxine required to block I^{131} release from the gland and they consider this to be the thyroid secretion rate.

Some of the objections to the usage of the measuring of I^{131} release from the gland, as mentioned previously, can be applied to this method. A steady state of thyroxine secretion may not necessarily be present during the period of measuring.

It has also been shown that the release curve gradually levels off and after some time becomes almost horizontal. If this occurs during the experiment period it would not be known if the stopping of the I^{131} release from the gland were due to this or to the injected dose of thyroxine.

In hyperthyroidism thyroid function is not suppressed by the administration of any thyroid hormone. Werner and Hamilton (60) discovered that massive doses of hormone did not affect the uptake of I^{131} and Johnson et al (61) found that it did not affect the rate of secretion of hormone from the gland either. If this is so then this method of measuring thyroid secretion is not valid in hyperthyroid animals.

Another more important objection may be that of Héroux (62). He found that the rate of thyroid secretion obtained depends on the initial dose of thyroxine given, the lesser the dose, the lower the secretion rate. It may be that the measurement is a relative and not an absolute one.

Thus it is apparent that this method has its fallacies also; however, many workers in the field of thyroidology are using it.

f) Thyroid Secretion Rate as Determined Without Injection.

Recently a new method of measuring the thyroid secretion rate has been devised by Sørensen (63).

In 1949 Oddie developed a general theory about rates of transfer of radioactive iodine between parts of the body. This was adapted by Brownell (64) to the estimation of fundamental rate constants for processes of thyroid function. The secretion rate is determined in the following way.

The apparent rate constant for thyroid I^{131} release, k_4', is estimated by the calculation of the half-life of the iodine release curve and applying it to the equation:

$$T \text{ (hrs.)} = \frac{0.693}{k_4'}$$

To measure the true rate constant for thyroid I^{131} release, k_4, the equation as supplied by Brownell is substituted into:

$$k_4' = k_4 \left(1 - \frac{U}{100}\right)$$

U, the maximum I^{131} content of the thyroid extrapolated to zero time, is calculated by extrpolating the release curve to time zero.

In order to bring in the kinetics of thyroxine I^{131} entering the blood the measurement of PBI and PBI^{131} is done at intervals. The maximum PBI^{131} extrapolated to time zero, $U_{PBI^{131}}$, is calculated by extrapolating the curve of PBI^{131} to time zero.

Assuming that the specific radioactivities of the secreted hormone and that of the circulating hormone in the blood are identical, the thyroid secretion rate is calculated as follows:

$$\frac{\text{secreted hormonal iodine/hr}}{\text{secreted hormonal } I^{131} \text{ \% dose/hr}} = \frac{\text{PBI/100ml}}{PBI^{131} \text{ \% dose/100ml}}$$

By multiplying k_4 by the I^{131} concentration in the gland the amount of secreted hormone I^{131} is determined. If the time for injection of I^{131} is selected as basis the equation becomes as follows:

$$\text{secreted hormonal iodine/hr} = \frac{k_4/\text{hr} \times U\% \text{ dose} \times \text{PBI}}{U_{PBI^{131}} \text{ \% dose/100ml}}$$

By multiplying the result obtained by the factor 36.7 the

result is converted to μgs thyroxine per day.

As calculated by Robertson and Falconer (65) the widest expected limits for the calculation of the secretion rate are likely to be within ± 25%.

Robertson and Falconer also found that these secretion rates have high significant correlations to I^{131} uptake and I^{131} release but not to PBI or PBI^{131} levels. On examination of Sørensen's results in which he finds that the thyroid secretion rate, as determined by this method, is positively correlated with daily butterfat production in cattle, it is seen that the secretion rate has no correlation with PBI, PBI^{131}, I^{131} uptake or I^{131} release from the thyroid. Since these parameters, used in other instances to determine thyroid activity, are also used in this case to calculate thyroid secretion rate, it may be fortuitous that the correlation between the thyroid secretion and butterfat was found or else these parameters are void when used alone.

GENERAL METHODS

Thyroid Secretion Rate Using Thyroxine Injections

The thyroid secretion rate (T.S.R.) was measured by the method of Reineke and Singh (59). Since the administration of exogenous thyroxine will reduce both thyroidal radio-iodine uptake and release, this suggests the possibility of measuring T.S.R. indirectly by determining the amount of thyroxine required to suppress the turnover of I^{131} by the thyroid.

Each rat was injected intraperitoneally with 10 µc of carrier-free I^{131}. 72 to 96 hours were allowed for fixation of the iodine by the thyroid and urinary elimination of the excess. The γ rays from the I^{131} were measured by the use of a scintillation detector (Nuclear Chicago, Model D'S-5-1) with the flat field collimator fitted with a half inch lead plate in which a hole of three-quarters of an inch in diameter had been bored. The counts were recorded on a count rate meter (Nuclear Chicago, Model 181A) with a 2% error level.

During counting, the rat was kept under light ether anaesthesia and the thyroid gland centered over the opening in the lead plate for two consecutive one minute counts. The average of these counts was taken as the gross

thyroid count per minute. Counts were then recorded over the epigastric region for one minute and the gross thyroid count was corrected by subtracting half the body count plus the room background. All readings were corrected for isotopic decay.

After the fixation of I^{131} by the thyroid, thyroxine was injected subcutaneously each day with progressive increases every 48 hours, and, unless stated otherwise, the dosage began at 0.5 micrograms per hundred grams of body weight. The thyroid counts were taken before each increase in dosage until the readings were between 80 and 100 percent of the previous 48 hour readings. The secretion rate was determined by fitting a regression line, obtained by the method of least squares, to the data to give a prediction equation, $Y = A + BX$, where Y is the percentage of the previous count and X is the thyroxine dose given. The thyroxine dosage is extrapolated to Y equals 100 percent and the secretion rate expressed in micrograms of L-thyroxine per day per 100 grams of body weight.

The measurement of the thyroid secretion rate in rabbits using this method was done in a slightly different manner than in the rats. Since the release of radioiodine from the rabbit thyroid is slow, thyroxine was injected subcutaneously with each progressive increase every 96 (instead of 48) hours.

Thyroid Secretion Rate Measured Without Injection

The method used was that of Sørensen (63). Each rabbit was injected intraperitoneally with carrier-free I^{131}. The activity of the radioiodine in the thyroid was measured with the scintillation counter. The rabbit was effectively immobilized in a box and the neck area in which the thyroid is located was placed over the counter. Two one-minute counts were measured and the average of these counts was taken as the gross thyroid count per minute. Counts were then taken at the femur region of the hind leg and subtracted from the gross thyroid count to give the net thyroid count.

To eliminate the need for decay corrections, the thyroid counts were expressed as a percentage of the count recorded from 170 μc injected into a "mock thyroid". The percentage uptake is

$$d\% = \frac{\text{net thyroid count}}{\text{"mock thyroid" count - background count}} \times 100$$

Blood was collected from the ear vein of the rabbits and the plasma was "counted" over the scintillation counter and compared to a similar volume of "mock serum" which had initially 170 μc of I^{131}. PBI^{131} counts were determined in a like manner and total PBI was measured by the HYCEL method (description below).

The thyroxine secretion rate was determined by using the following equation:

$$T.S.R.\ (\mu g/day) = \frac{K_4 \times U \times PBI \times 36.7}{U_{PBI^{131}}}$$

U is the maximum I^{131} content of the thyroid obtained by extrapolating the I^{131} release curve to time t=0 and is expressed in percent of the dose given. K_4' is the apparent rate constant for thyroid I^{131} release and K_4 is the true rate constant for thyroid I^{131} release, %/hr, and is calculated as follows:

Half life of the I^{131} release, $T_{\frac{1}{2}} = \frac{0.693}{K_4'}$

$$K_4' = K_4 \left(1 - \frac{U}{100}\right)$$

$U_{PBI^{131}}$ is the maximum protein bound I^{131} extrapolated to time t=0 and PBI is the average protein bound iodine. 36.7 is the conversion factor used to transform the quantity of iodine into thyroxine.

Protein Bound Iodine (PBI)

0.3 ml of plasma was used in order to determine PBI levels according to the HYCEL semi-micro PBI determination method (66). To the plasma was added 4 ml of Tungstic acid. This was allowed to stand for ten minutes and then the mixture was centrifuged for ten minutes. The supernatant was decanted and to the precipitate procelain chips were added.

The precipitate was then swirled in order to give a fine even film on the flask. 4 ml of the digestion reagent was added and when the precipitate had dissolved 2 ml of the chromic reagent was added and the mixture was swirled. After this it was boiled on the HYCEL heater. 1 ml of the arsenious reagent was put in the micro-still. To the boiled mixture was added phosphorous reagent and this was distilled for six minutes. The distillate was collected in a graduated distillate tube containing 0.5 ml arsenious solution. The distillate was made up to six ml with distilled water, 1.0 ml of ceric reagent was added and the mixture was incubated at 37°C for 30 minutes. The colour was read at 440 mμ on the junior Coleman spectrophotometer. This was compared to prepared blanks. The result was expressed in γ%.

Butanol Extractable Iodine (BEI)

The method used was that of the HYCEL semi-micro BEI determination (66). First 0.2 ml of BEI acid, then 2 ml butanol was added to 0.3 ml of plasma and after mixing it was centrifuged for five minutes. The butanol extract was put in a semi-micro digestion flask and the precipitate washed with 1 ml butanol. The combined butanol extract was mixed with 3 ml alkali wash and centrifuged for five minutes. The alkali layer was siphoned off and the butanol extract washed with alkali two more times. The butanol extract was evaporated to dryness over the flask heater, then 4 ml of digestion reagent and porcelain chips were added to the residue.

This was then treated as in the PBI method.

Vitamin A Determination

The determination of liver vitamin A was done by the method of Gade and Kadlec (67). Weighed liver samples (3 to 5 grams) were blended with a surfactant in a semi-micro Waring blendor to solubilize the vitamin. This mixture was then stirred with a mixed solvent (petroleum ether and ether) to extract the vitamin. When the solvent layer had separated, duplicate aliquots of 5 ml were put into test tubes and evaporated to dryness under vacuum while the tube was swirled in a + 45°C water bath. This was then stored under dry nitrogen in the deep freeze. Later (in about six weeks) the residue was dissolved in a known amount of dry chloroform. A 2 ml aliquot was taken and to it was added one drop of acetic anhydride and 10 ml. of Carr Price Reagent. The blue solution was read immediately at 620 mμ, using a Coleman Jr. Model 6A Spectrophotometer, as the colour fades rapidly.

Ascorbic Acid Synthesis

Enzyme III activity was determined in the liver by the method of Grollman and Lehninger (68). It was assayed indirectly by measuring the ability of the liver, which is the site of ascorbic acid synthesis in the rat, to carry out the overall conversion of L-gulonolactone to L-ascorbate.

L-gulonolactone was used instead of L gulonate as in the original method, since it saves one step in the ascorbate synthesis (69).

Livers, were excised from rats, immediately frozen on dry ice and stored in the deep freeze. When assayed the livers were homogenized in 2.5 volumes of 0.15 M KCl. Three aliquots were then taken. One aliquot was incubated with gulonolactone for two hours at 37°C, the second was treated similarly but with no galonolactone added and the third, also without gulonolactone, was used for the measurement of ascorbate at zero time. In all cases the net accumulation of "total ascorbic acid" (i.e. the sum of diketogulonate, ascorbate and dehydroascorbate) was determined colorimetrically by the method of Roe and Kuether (70) using the Coleman Jr. Model 6A Spectrophotometer.

The amount of ascorbate formed was considered to be that amount in aliquot one minus the amount in the second aliquot. To determine the rate of destruction the amount in the second aliquot was subtracted from the amount obtained at zero time. No correction was made for the destruction rate when the biosynthesis of ascorbate was calculated as the kinetics of the reaction have not been determined.

Statistical Analysis

A method of determining if the difference between two values extrapolated from regression lines, as calculated in the TSR measurements is significant or not was formulated by Dr. N. T. Grideman, statistician at the National Research Council of Canada. The calculated of the standard error of the difference between two x values is based on the variance of both the observed and calculated Y values. From this, the significance is obtained through the t test.

Other experimental data has been subjected to the analysis of variance as outlined in Chapter 13 of "Principles and Procedures of Statistics" (71). Whenever the F values were found to be significant the multiple range test was used to locate the significant differences if more than two factors were involved otherwise the t test was used.

PART A

Protocol

All animals used in the vitamin A experiments were weanling male albino rats of the Wistar strain except for those in experiment 2 which were of the Sprague-Dawley strain. These were put immediately on a vitamin A deficient diet as described by Lachance (72).

Experiment 1 Pair-Feeding.

In experiment one the animals were divided into three groups. One group was without vitamin A, the second was given 1000 I.U. of vitamin A per week, and the third was given a dose at 25,000 I.U. twice a week. The vitamin A was given by a feeding tube directly introduced into the stomach. The animals on 1000 I.U. of vitamin A per week were considered to be normal animals and the animals on the higher dose were considered to be in an excess vitamin A condition. The normal and excess vitamin A rats were pair-fed with vitamin A deficient animals as it was previously noted by Lachance (72) that vitamin A deficient animals eat less than animals on an adequate vitamin A diet. When the vitamin A deficient animals stopped gaining and began to lose weight they were considered to be vitamin A deficient. At this stage the rats were used to determine the thyroid secretion rate. After this part of the experiment was

finished half the rats were sacrificed and the remaining rats were again injected with 5 μc of I^{131} and the iodine release from the thyroid was determined. Three or four days after injection the animals were put in the cold at 2°C, and the iodine release curve followed. When the animals were sacrificed the thyroid glands were taken and weighed.

Experiment 2 Ad Libitum Feeding.

The same procedure was followed in experiment two except that the animals were fed ad libitum. This change was done because of the observation of Pipes et al (73) that animals kept on a lower food consumption had lower thyroid activities. Some of the vitamin A deficient rats were force-fed so that their food consumption could be on the same level as that of normal rats; however, these animals died leaving us with the opinion that either the lowering of food intake may be a defense mechanism or that the force feeding put too much stress on an already weakened rat. The livers of the rats in this experiment were taken for the assay of ascorbic acid biosynthesis.

Experiment 3 Iodine Deficiency.

Since it is known that an iodine deficient diet will increase thyrotrophin (TSH) secretion, it was decided to feed the rats on an iodine deficient diet to find out whether iodine deficiency would affect the response of the thyroid to various

vitamin A levels. Thus the same diet as in experiment two was used except that it was deficient in iodine.

Experiment 4 Vitamin A and Iodine Effects.

Since it took longer for the animals in experiment three to become vitamin A deficient and since the thyroxine secretion rates showed a difference, it was decided to repeat experiments two and three simultaneously using Wistar rats in both cases, as the Sprague-Dawley rats may show a strain difference.

Experiment 5 Long Range Effect of Vitamin A Excess

In experiment five there were only two groups of rats, the normal vitamin A and the excess vitamin A rats. Since this experiment was designed to study the long range effect of vitamin A, vitamin A deficient animals could not be used as they could not survive until the end of the experiment. The rats were examined at six weeks and at sixteen weeks and TSR's and ascorbic acid biosynthesis in the liver as well as the effect of cold (2°C) on the iodine release from the thyroid gland were measured.

Experiment 6 Vitamin A and Duration of Cold Exposure

Cold causes an increase in TSR, at least in the first week, hence it was thought that maybe the effect of cold may be changed in different vitamin A states. In this experiment,

as in experiment five, only two groups of rats were used as vitamin A deficient animals do not survive for long in the cold. All of the animals were kept on the diet for sixteen weeks so that they would be at similar vitamin A levels. Ten animals of the normal vitamin A group were put in the cold (2°C) after six weeks on the diet; another ten were put in after eleven weeks, a third ten at thirteen weeks, a fourth at fourteen weeks, and a fifth ten at fifteen weeks. Thus animals were in the cold for ten weeks, five weeks, three weeks, two weeks and one week respectively. The same procedure was followed for the vitamin A excess rats. TSR's and ascorbic acid biosynthesis in the liver were determined.

Since the assumption that the rats were in normal and excess vitamin A states is very important in these experiments, the livers of the rats in experiment six were examined for vitamin A content.

To help in the recording of results and in the discussion various abbreviations and symbols were used. These are listed in tabular form below.

LD	animals on a vitamin A-deficient diet.
LDN	animals on a vitamin A-deficient diet given a supplement of 1000 I.U. vitamin A per week.
LDS	animals on a vitamin A-deficient diet given a supplement of 50,000 I.U. vitamin A per week.
NIA	animals on a vitamin A-deficient, iodine-deficient diet.

NINA animals on a vitamin A-deficient, iodine-deficient diet given a supplement of 1000 I.U. per week.

NISA animals on a vitamin A-deficient, iodine-deficient diet given a supplement of 50,000 I.U. of vitamin A per week.

RESULTS

I Thyroid Secretion Rate

The data obtained from the experiments is listed in tables 1, 2, 3, 4, 5 and 6. Since the values are listed in the tables, the individual regression lines for the calculation of TSR are not needed and therefore they are grouped at the end of this section. (Pages 37 and 38).

Experiment 1 Pair-Feeding.

After six weeks on the vitamin A deficient diet the rats reached a weight plateau and began losing weight, thus showing vitamin A deficiency. As shown by Table 1 there is no significant difference in the thyroid secretion rates of the three groups.

TABLE 1 - The Effect of Various Vitamin A Dietary Levels on the T.S.R. of Animals which Have Been Pair Fed.

	LD	LDN	LDS
T.S.R. µg/100g BW	1.05 (7)	0.89 (8)	1.05 (8)
Comparison with	LDN	LDS	LD
Difference ± S.E.	0.16±0.22	0.16±0.61	0.00±0.21
t	0.74	0.10	0.00
p	ns	ns	ns

Experiment 2 Ad Libitum Feeding

The time taken to reach a vitamin A deficient state was the same as in experiment 1. There is no significant difference between the TSR's of the three groups as seen in table 2 showing that the food intake makes no difference in this case.

TABLE 2 - The Effect of Various Vitamin A Dietary Levels on the T.S.R. of Animals which Have Been Fed Ad Libitum.

	LD	LDN	LDS
T.S.R. µg/100g BW	1.27 (11)	0.81 (9)	1.18 (9)
Comparison with	LDN	LDS	LD
Difference ± S.E.	0.37±0.19	0.46±0.27	0.09±0.27
t	1.95	1.69	0.33
p	ns	ns	ns

Experiment 3 Iodine Deficiency

The iodine deficient rats reached a vitamin A deficient state after seven weeks which was a little longer than in the previous experiment. Since, as shown in table 3, the TSR's are not significantly different it was not known whether this delay was caused by previous treatment of the animals or whether iodine deficiency had an effect on vitamin A depletion. Thus experiment 4 was undertaken.

TABLE 3 - The Effect of Various Vitamin A Dietary Levels on the T.S.R. of Animals which Are Iodine Deficient.

	NIA	NINA	NISA
T.S.R. µg/100g BW	1.54 (11)	1.98 (9)	1.87 (9)
Comparison with	NINA	NISA	NIA
Difference ± S.E.	0.44±0.37	0.11±0.40	0.33±0.30
t	1.19	0.28	1.11
p	ns	ns	ns

Experiment 4 Vitamin A and Iodine Effects.

It took eight weeks for the iodine deficient animals to become vitamin A deficient and nine weeks for the rats on normal iodine diet to become vitamin A deficient. This difference, however, is not significant. There is, also, no significant difference between the TSR's of any of the groups showing that iodine deficiency has no effect on the TSR measurement of the rats.

TABLE 4 - The Effect of Iodine Deficiency and Vitamin A Levels on T.S.R.

	T.S.R. µg/100g BW	Comparison with	Difference ± S.E.	t	p
LD	1.39 (7)	LDN	0.37±0.34	1.10	ns
		NIA	0.20±0.33	0.60	ns
LDN	1.76 (10)	LDS	0.10±0.32	0.31	ns
		NINA	0.29±0.28	1.04	ns
LDS	1.86 (10)	LD	0.47±0.35	1.33	ns
		NISA	0.23±0.31	0.74	ns
NIA	1.59 (13)	NINA	0.11±0.26	0.42	ns
NINA	1.48 (9)	NISA	0.15±0.25	0.67	ns
NISA	1.63 (10)	NIA	0.04±0.28	0.14	ns

Experiment 5 Long Range Effect of Vitamin A Excess

As seen in table 5 the length of time a rat is on a vitamin A excess diet has no effect on the TSR measurement, and, also, the age of the animals has no effect on the measurement.

TABLE 5 - Comparison of the T.S.R.'s of Rats Kept on Various Dietary Vitamin A Levels With Respect to Duration of Treatment.

	T.S.R. µg/100g BW	Comparison with	Difference ± S.E.	t	p
LDN (6 weeks)	1.40 (9)	LDS (6 weeks)	0.11±0.24	0.45	ns
		LDN (16 weeks)	0.11±0.31	0.36	ns
LDN (16 weeks)	1.29 (6)	LDS (16 weeks)	0.03±0.23	0.13	ns
LDS (6 weeks)	1.51 (11)	LDS (16 weeks)	0.19±0.20	0.94	ns
LDS (16 weeks)	1.32 (9)	-	-	-	-

Experiment 6 Vitamin A and Duration of Cold Exposure

This experiment shows that cold has an effect on the TSR's of the animals. It is seen that the secretion rate is significantly different (table 6) in the first week in the cold and gradually decreases to normal levels. There is however no difference in the responses of the two groups of rats. Thus cold stimulus does not differentiate the response of the thyroid between rats on a normal vitamin A diet and those on an excess vitamin A diet.

TABLE 6 - Effect of Duration of Cold Exposure on the T.S.R. of Animals at Different Vitamin A Dietary Levels.

Weeks in cold	LDR	LDS	Difference ± S.E.	t	p
0	1.29 (6)	1.32 (9)	0.03±0.23	0.13	ns
1	2.25 (7)*	2.37 (7)*	0.12±0.36	0.33	ns
2	1.50 (9)	2.22 (3)	0.72±0.55	1.30	ns
3	2.25 (4)	1.40 (10)	0.35±0.94	0.90	ns
5	1.16 (7)	1.61 (9)	0.45±0.22	2.05	<.05
10	1.57 (7)	1.05 (9)	0.52±0.24	2.20	<.05

* Significantly different from 0 week only.

Fig. 1 TSR Regression Lines

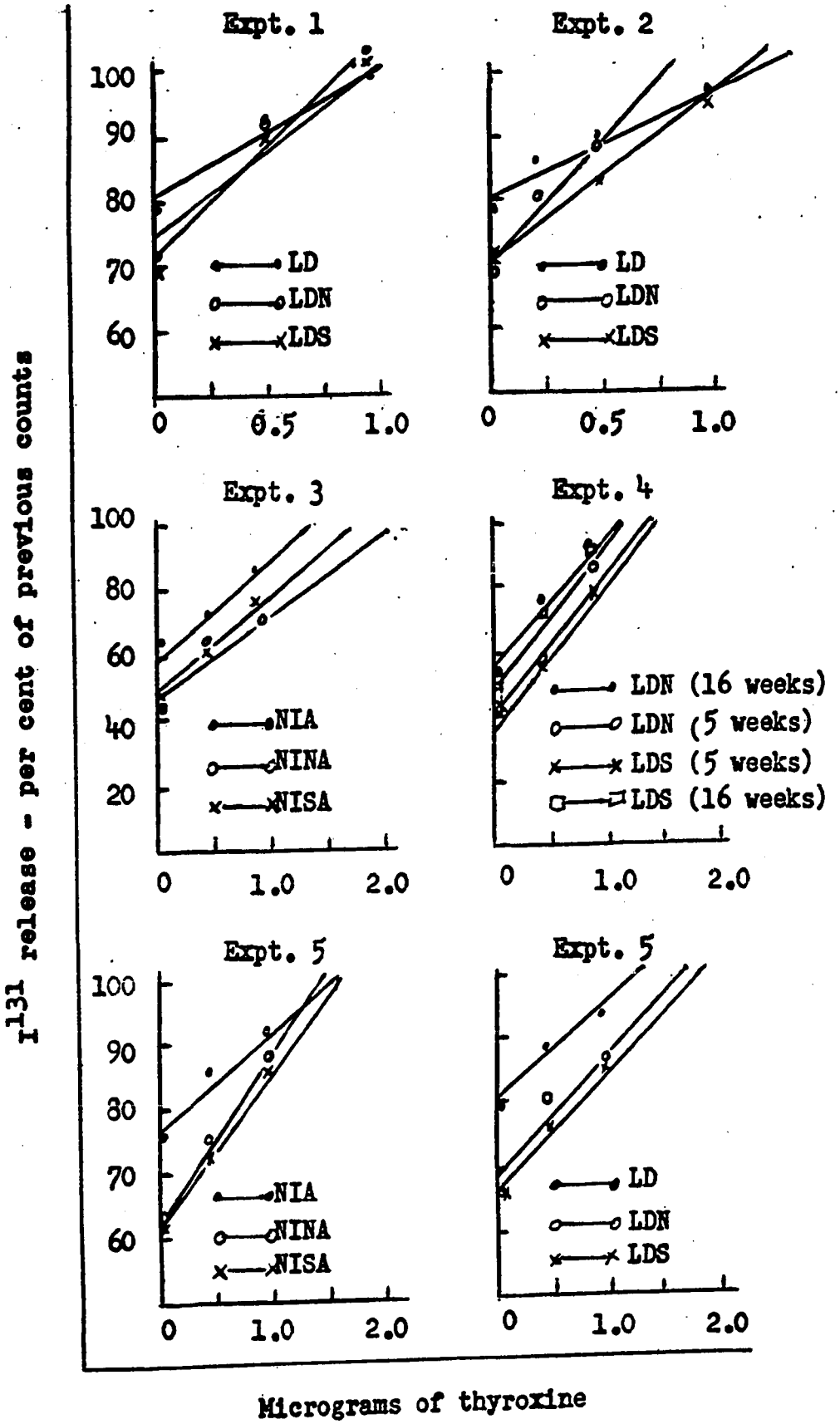
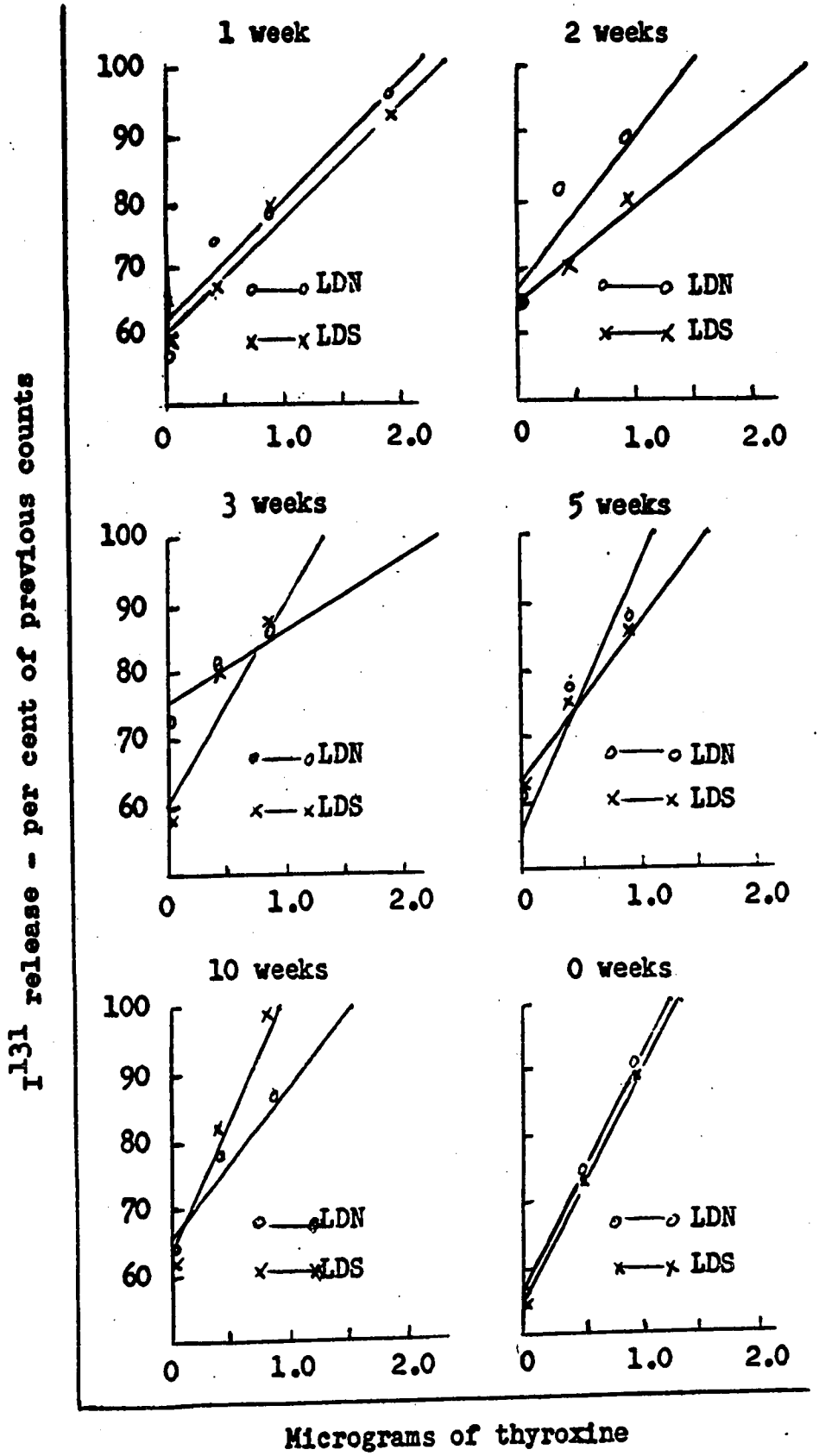


Fig. 2 Expt. 6 TSR Regression Lines

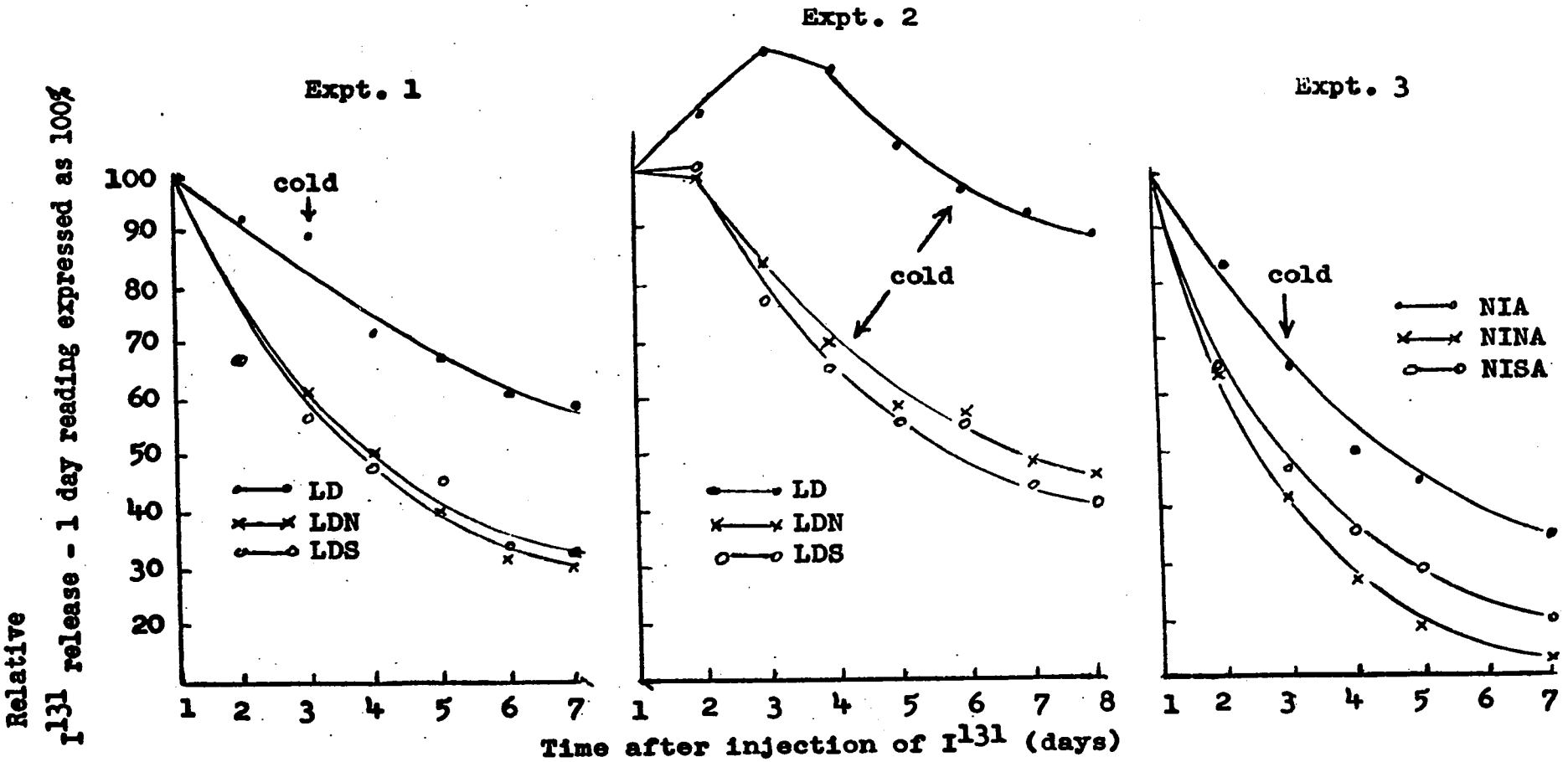


II Effect of Cold Exposure on I^{131} Release from the Thyroid Gland

As seen in figs. 3 and 4 the I^{131} release from the thyroid gland does not alter appreciably in any of the experiments done, when the animals are put in the cold. Thus cold does not alter the I^{131} release nor do dietary levels of vitamin A affect the lack of response of I^{131} release to cold exposure. It also does not show a different effect on the various groups of the iodine deficient animals.

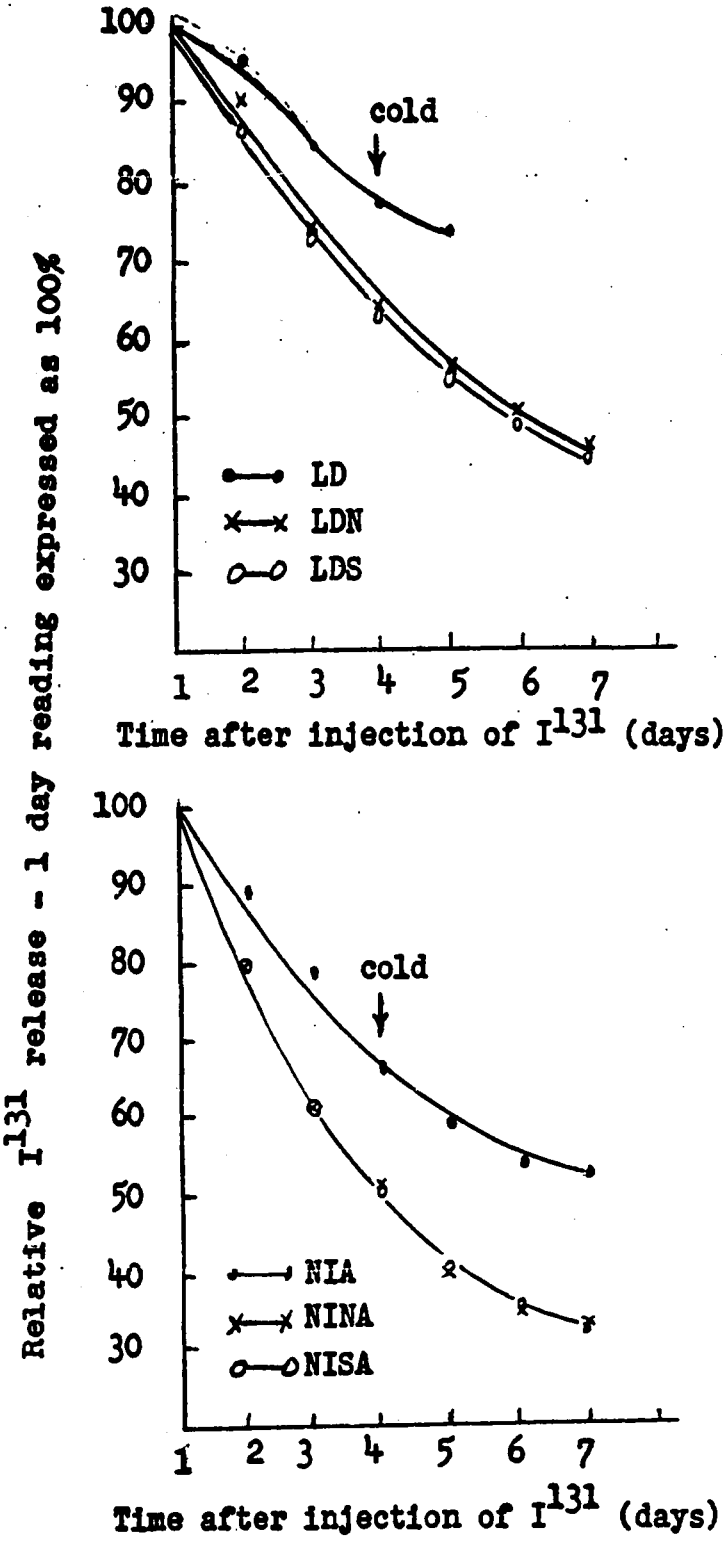
The vitamin A deficient groups have a decreased rate of release of I^{131}. This observation will be discussed in more detail in Part B.

Fig. 3 Effect of a cold stimulus on I^{131} release



Expt. 4

Fig. 4 Effect of a cold stimulus on I^{131} release



III Thyroid Gland Weights

The results of the experiments are listed below. In order to clarify the results the statistical values are placed below their corresponding tables. All weights are expressed in mgs.

Experiment 1 Pair-Feeding.

The results are shown in table 7. From them it can be seen that neither vitamin A nor cold exposure significantly affects thyroid weight.

TABLE 7 - The Effect of Various Vitamin A Dietary Levels on the Thyroid Weights Which Have Been Pair Fed.

Results	LD	LDN	LDS
Room temp.	24.7±4.5(4)	19.5±1.7(6)	20.6±1.0(5)
2°C	39.0(1)	38.6±3.7(3)	39.3±2.2(3)

Analysis of Variance

Source	df	SS	MS	f	p
Vitamin A	2	18.59	9.29	0.24	ns
Temperature	1	1466.06	1466.06	38.51	<.01
Interaction	2	14.88	7.44	0.20	ns
Error	15	571.00	38.07		

Temperature	t	p
t-test	0.704	ns

Experiment 2 Ad Libitum Feeding

At initial analysis it seemed as though vitamin A and temperature affect thyroid weight (table 8). The apparent significant differences observed when the data were processed by the analysis and shown to be not significant with the multiple range test can be explained by the fact that there was no data available for the vitamin A deficient animals at 2°C. Since this was not taken into account for the analysis of variance, a false test of significance was obtained. The significant interaction may also result from this.

TABLE 8 - The Effect of Various Vitamin A Dietary Levels on the Thyroid Weights of Animals Which Have Been Pair Fed.

Results	LD	LDN	LDS
Room temp.	20.6±3.1(5)	21.7±0.9(4)	20.6±1.3(4)
2°C	-	29.1±1.0(4)	24.4±1.3(5)

Analysis of Variance

Source	df	SS	MS	f	p
Vitamin A	2	1723.89	861.95	52.91	<.01
Temperature	1	108.11	108.11	6.64	<.05
Interaction	2	1589.62	794.81	48.79	<.01
Error	16	260.57	16.29		

a) Vitamin A

One-Way Analysis of Variance	f	p
	1.82	ns

b) Temperature

t test	t	p
	0.52	ns

Experiment 3 Iodine Deficiency

Neither iodine nor temperature affect the weight of the thyroid gland. This is seen in table 9.

TABLE 9 - The Effect of Various Vitamin A Dietary Levels on the Thyroid Weights of Animals Which Are Iodine Deficient.

Results	NIA	NINA	NISA
Room temp.	18.8±2.4(4)	20.4±3.8(4)	18.8±5.9(4)
2°C	23.9±2.4(3)	30.3±2.8(4)	30.8±1.4(5)

Analysis of Variance

Source	df	SS	MS	f	p
Vitamin A	2	68.68	34.34	0.73	ns
Temperature	1	476.78	238.39	5.04	<.05
Interaction	2	47.32	23.41	0.49	ns
Error	18	851.53	47.31		

Temperature	t	p
t test	0.85	ns

Experiment 4 Vitamin A and Iodine Effects

The results are listed in table 10. It can be seen that neither vitamin A, iodine, or temperature has an effect on thyroid weight.

TABLE 10 - The Effect of Iodine Deficiency and Vitamin A Levels on the Thyroid Weights.

Results

Groups	Room Temp.	2°C
LD	23.0±4.4(3)	16.8(1)
LDN	18.7±3.8(5)	17.3±1.7(4)
LDS	19.1±1.8(5)	20.6±1.1(5)
NIA	12.5±0.6(6)	15.4±1.5(2)
NINA	19.5±2.5(4)	16.4±2.5(4)
NISA	20.6±2.2(5)	16.7±2.0(5)

Analysis of Variance

Source	df	SS	MS	f	p
Iodine	1	85.50	85.50	4.55	<.05
Temperature	1	8.39	8.39	0.45	ns
Interaction	1	0.10	0.10	0.005	ns
Vitamin A	2	71.30	35.65	1.90	ns
Temperature	1	14.36	14.36	0.76	ns
Interaction	2	6.28	3.14	0.17	ns
Vitamin A	2	7.30	3.65	0.19	ns
Iodine	1	114.29	114.29	6.08	<.05
Interaction	2	117.70	58.85	3.13	ns
Error	36	676.74	18.80		

a) Iodine

	t	p
t test	0.36	ns

Experiment 6 Vitamin A and Duration of Cold Exposure

As is seen in table 11, the thyroid weights are not significantly different.

TABLE 11 - Effect of Duration of Cold Exposure on the Thyroid Weights of Animals at Different Vitamin A Dietary Levels.

Results

Weeks in cold	LDN	LDS
0	25.5±4.4(3)	18.9±1.7(5)
1	26.2±1.1(7)	27.9±2.0(7)
2	27.4±2.6(9)	23.1±1.4(8)
3	24.9±2.9(3)	21.0±1.5(10)
5	19.4±1.0(7)	19.7±1.9(8)
10	16.2±1.8(7)	20.3±1.1(8)

Analysis of Variance

Source	df	SS	MS	f	p
Vitamin A	1	38.42	38.42	1.52	ns
Time	5	822.87	164.58	6.52	<.01
Interaction	5	254.35	50.87	2.01	ns
Error	70	1768.22	25.26		

Time

	f	p
I) One-Way Analysis of Variance	12.40	<.01

2) Duncan's test (P=.05)

Weeks in the Cold	10	5	0	3	2	1
Weights	18.4	19.6	21.4	21.9	25.4	27.1

IV Ascorbic Acid Biosynthesis

The results, expressed in micromoles of total ascorbic acid formed per gram of wet liver per hour at 37°C, and statistical analysis are listed in the same manner as those of the thyroid gland weights.

Experiment 2 Ad Libitum Feeding

The results for the experiment are listed in table 12.

TABLE 12 - The Effect of Various Vitamin A Dietary Levels on Ascorbic Acid Biosynthesis in Ad Libitum Fed Rats.

Results	LD	LDN	LDS
Room Temp.	9.3±1.0(5)	15.2±0.4(4)	13.8±0.9(3)
2°C	-	9.5±0.9(4)	11.7±0.7(5)

Analysis of Variance

Source	df	SS	MS	f	p
Vitamin A	2	547.34	273.67	87.71	<.01
Temperature	1	243.19	243.19	77.95	<.01
Interaction	2	68.50	34.25	10.98	<.01
Error	14	43.69	3.12		

a) Vitamin A

One-Way Analysis of Variance	f	p
	2.74	ns

b) Temperature

t test	t	p
	0.312	ns

At initial analysis it seemed as though vitamin A and temperature affect the biosynthesis. Because of the same reasons described in experiment 2 of thyroid weights there is, on final analysis, no significant effect on the biosynthesis of either vitamin or temperature.

Experiment 3 Iodine Deficiency

From the results and statistical values of table 13 it can be observed that iodine deficiency does not alter the response of the biosynthesis to vitamin A and temperature.

TABLE 13 - The Effect of Various Vitamin A Dietary Levels on Ascorbic Acid Biosynthesis in Iodine Deficient Rats.

Results	NIA	NINA	NISA
Room temp.	8.0±2.4(4)	13.4±2.0(4)	12.9±1.3(4)
2°C	12.5±2.6(3)	13.6±1.1(5)	16.5±1.5(5)

Analysis of Variance

Source	df	SS	MS	f	p
Vitamin A	2	81.87	40.93	3.22	ns
Temperature	1	46.23	46.23	3.64	ns
Interaction	2	21.28	10.64	0.84	ns
Error	19	241.22	12.70		

Experiment 4 Vitamin A and Iodine Effects.

From these results (table 14) it seemed as though there may be a change in the biosynthesis with different levels of vitamin A, however, Duncan's test gave no significant differences.

TABLE 14 - The Effect of Iodine Deficiency and Vitamin A Levels on Ascorbic Acid Biosynthesis.

Results	Room Temp.	2°C
LD	4.5±1.5(3)	4.1(1)
LDN	6.1±0.7(5)	7.2±0.6(4)
LDS	8.0±1.1(5)	9.5±1.5(5)
NIA	5.6±0.3(6)	5.2±1.7(2)
NINA	8.0±1.4(4)	12.6±0.7(3)
NISA	11.1±1.3(5)	5.9±0.7(5)

Analysis of Variance

Source	df	SS	MS	f	p
Vitamin A	2	85.24	42.62	8.53	<.01
Temperature	1	0.18	0.18	0.04	ns
Interaction	2	43.59	21.80	4.36	<.05
Iodine	1	5.09	5.09	1.02	ns
Temperature	1	5.04	5.04	1.01	ns
Interaction	1	56.68	56.68	5.63	<.05
Vitamin A	2	92.06	46.03	9.22	<.01
Iodine	1	21.26	21.26	4.26	ns
Interaction	2	46.57	23.28	4.66	<.05
Error	35	174.79	4.99		

Vitamin A

				f	p
1) One-Way Analysis of Variance				31.69	<.001
2) Duncan's test (p=.05)	Deficient		Normal		Excess
	5.1		8.0		8.6

Experiment 6 Vitamin A and Duration of Cold Exposure

The results of this experiment, as seen in table 15, show that the level of vitamin A has no effect on the biosynthesis, however, after one week in the cold the biosynthesis is significantly lowered and returns to normal at two weeks.

TABLE 15 - Effect of Duration of Cold Exposure on the Ascorbic Acid Biosynthesis in Animals at Different Vitamin A Dietary Levels.

Results

Weeks in cold	LDN	LDS
0	11.5±1.4(3)	12.0±1.7(5)
1	3.9±1.0(7)	5.5±0.9(7)
2	9.7±1.1(7)	9.0±0.8(8)
3	10.2±0.5(3)	10.4±0.7(10)
5	7.5±1.2(7)	8.0±0.6(4)
10	10.8±0.7(7)	10.1±0.6(7)

Analysis of Variance

Source	df	SS	MS	f	p
Vitamin A	1	0	0	0	ns
Time	5	374.61	74.92	12.22	< .01
Interaction	5	12.82	2.56	0.42	ns
Error	63	386.04	6.13		

Time

1) One-Way Analysis of Variance — f = 14.59, p < .001

2) Duncan's test (p=.01)

Weeks in cold	1	5	2	3	10	0
Averages	4.7	7.7	9.4	10.3	10.5	11.8

V Vitamin A Levels in the Livers

The results are expressed in table 16. It is seen that the excess vitamin A rats had a significantly larger quantity of vitamin A in their livers that the normal rats.

TABLE 16 - Level of Vitamin A in the Livers of Rats Kept at Various Time-Exposures to Cold.

Results

Weeks in cold	LDN	LDS
1	1931±313(7)	45,905±1042(7)
2	1668±109(9)	59,149±5418(8)
3	1432±136(3)	69,632±4335(10)
5	1553±102(7)	63,271±5715(9)
10	1187± 69(6)	70,431±4105(8)

Analysis of Variance

Source	df	SS	MS	f	p
Vitamin A	1	60.2×10^9	60.2×10^9	56.79	<.01
Time	4	12.4×10^8	3.1×10^8	0.29	ns
Interaction	4	1.4×10^9	3.5×10^8	0.33	ns
Error	63	6.74×10^9	1.06×10^9		

Vitamin A

	t	p
t test	5.217	<.001

It can also be seen that exposure to cold did not significantly alter vitamin A storage in the liver.

DISCUSSION

In our experiments we have fed our animals on a vitamin A free diet with various supplements of vitamin A. The animals which were considered to be on a normal diet of vitamin A were fed 1000 I.U. per week. This was in accordance with Moore (1) who states that in order to allow experimental rats to accumulate reserves equal to those of wild animals doses of at least 100 I.U. daily are necessary. Since we gave the vitamin only once a week the dose was set at 1000 I.U. per week to allow for some of the vitamin which would not be absorbed.

Most of the studies on the effects of excess vitamin A have been done on animals which have been given such a great amount of the vitamin as to create a pathological condition. Such studies can hardly be interpreted in terms of physiological effects of the vitamin. In order to avoid this serious criticism our animals given "excess vitamin A" received only fifty times as much vitamin as the controls, a dose insufficient to cause any pathological disturbance.

In none of our experiments was there any difference in TSR which could be attributed to a change in dietary level of vitamin A. Since this is the first time, to our knowledge, that this type of measurement has been done on rats subjected to different levels of vitamin A we can only compare our results to other types of thyroid measurements obtained in

similar experiments.

Our results agree with those of Oliverea and Serfaty (74) and Blaizot and Benac (75) who were unable to find a definite effect of vitamin A deficiency on the BMR of rats. There is a lack of agreement however among those who have studied the histological picture of the gland in vitamin A deficient rats. McCarrison (76) and Spence (77) both noted that the gland showed a hypertrophy indicating a more active gland, whereas, De Ruytter (78) observed that the gland showed a decrease in activity. However, when it is considered that the histological picture of the gland is influenced greatly by the hypophysis, the changes seen may not be indicative of a difference in thyroid secretion but of a change in thyrotrophin secretion.

Lipsett and Winzler (79) up to now are the sole investigators who have studied the effects of vitamin A deficiency on the thyroid gland with I^{131}. They found that while the total iodine picked up by the thyroid was not different from controls the uptake per mg of thyroid was lower in the deficient animals since their thyroids were heavier. This is in contrast to our results. As will be shown in Part B, Expt. 8, the uptake of I^{131} by the gland of the deficient animals is greater than in the normal and vitamin A excess animals, although the thyroid weights are the same. The vitamin A deficient diet used by Lipsett and Winzler was low in iodine and this may

have modified the I^{131} uptake so as to make it more like that seen in our iodine deficient animals which had no differences in iodine uptake between the different vitamin A groups. However we found no differences in thyroid weights caused by different vitamin A levels. It would thus seem that the results of Lipsett and Winzler are explainable in terms of dietary levels of iodine rather than changes in vitamin A intake.

We also found that a deficiency of iodine had no effect on the TSR, whatever the level of vitamin A. Halmi (80) has shown that thyrotrophin secretion increases in an iodine deficient state and Moore (1) quotes Schneider (31) as reporting that large doses of vitamin A oppose the action of thyrotrophin. Although our observations agree with the former author they do not agree with the latter. On the iodine deficient diet there is a definite increase in I^{131} uptake which denotes increased activity of TSH but no difference in iodine uptake can be attributed to excess vitamin A in the diet. (Part B)

It seems reasonable to us that the TSR's of normal animals would not be different from those of iodine deficient animals since it seems likely that the requirement for thyroxine would not be changed. Although various authors (10,82) have found that the thyroid gland increases in weight during iodine deficiency we only found a tendency in this direction; however, since we gave thyroxine to these rats for about a

week it may be that this caused a decrease in thyroid weight.

Since vitamin A did not affect the responses of TSR to the cold environment we have further evidence that excess vitamin A does not oppose the effects of TSH as it has been shown that cold causes an increase in TSH secretion (83). Our results in experiment 6 agree with those of Starr and Roshelley (35), Leblond et al (84) and Cottle and Carlson (85) who all noted that in the cold thyroid activity initially increased and then returned to values approaching normal. Not only are the TSR's significantly increased at the first week but we also have a trend towards an increase in weight of the thyroid glands. The lack of significance in the weights may be due to the experimental procedure used for TSR's since the injection of thyroxine could have caused a reversal of thyroid hyperplasia as observed in the case of iodine deficiency.

It can also be seen that initial exposure does not seem to have an immediate action on all parameters of thyroid activity. In rats in which the release of I^{131} was followed, there has been no change in release rate when the animals were put in the cold, suggesting a failure of the thyroid to modify this phase of its activity. The thyroid weights however show a trend towards an increase, which might have been significant if more animals had been examined.

A confirmation of our work is seen in the findings of Brown-Grant (86) who studied the effect of exposure to

varying degrees of cold on the rate of release of I^{131} in male rats. It was found that the rate did not change at exposures of 16°C and 0°C, but increased at 11.5°C and 6.5°C and slightly decreased at 2°C. In this article it is also mentioned that similar results were found in rabbits. It is suggested that the more severe cold exposure acted as a non specific stress (increase in gluco-corticoid secretion) and that this reduced the secretion of TSH; thus, the rate of I^{131} release was decreased or remained the same. These arguments could also apply to our results. After some time, about 4-5 days, the effect of cold on the thyroid probably gained ascendancy over that of the non specific stress and the thyroid became more active as seen in our TSR measurements.

Before the effects of vitamin A on ascorbic acid synthesis is discussed, it should be said that these results were obtained from tissue homogenates. Although they may not be the same in the intact animals, it is generally believed that they indicate what may be expected in vivo.

From our results it is seen that there is little effect of dietary vitamin A on ascorbic acid synthesis. This is in direct contrast to the reports by Chatterjee et al (25) and Sastry et al (26) who both found that vitamin A deficiency decreased ascorbic acid synthesis. There is however a difference between our methods and theirs. We used the complete tissue homogentate whereas they used only the microsome fraction.

Since the whole tissue homogenate seems to be closer to actual conditions in the animal we believe that our results are more indicative of the in vivo state. An observation by Chatterjee et al. seems to confirm this. They found that they could restore some of the ascorbic acid synthesis by adding the boiled supernatant. This fraction, since it was boiled, would in all probability have no enzymative activity but may have an important factor involved in the synthesis. If this is true then the microsome fraction should not be studied alone. This however does not explain the greater synthesis obtained from the microsomes of livers of animals on normal vitamin A intake.

One result of Sastry et al (20), used to show that ascorbic acid synthesis is lowered in deficient animals, is the lowered ascorbic acid excretion in the urine during chloretone treatment in the severe stage of deficiency. It has been noted by Moore (1) that the amount of urine excreted during this stage is very much lower than normal; thus it would be expected that ascorbic acid excretion would also be lowered.

Mapson and Walker (22) noted that all differences in ascorbic acid content of the liver and blood and in urinary excretion could be accounted for by differences in food intake. Lachance (72) also observed that pair-feeding could account for the decrease in liver ascorbic acid which occurred in terminal vitamin A deficiency. Our results show that the food intake played no part in the ascorbic acid synthesis since

vitamin A had no effect whether the animals were fed ad libitum or pair-fed. Although synthesis does not seem to be modified, it would seem that food intake would modify the storage of ascorbic acid through various levels of dietary intake of this vitamin.

Iodine deficiency had no effect on ascorbic acid synthesis, nor did it modify the effect of vitamin A dietary levels on the synthesis. This, however, is not surprising since the thyroid activity did not seem to change and the general metabolism of the animals appeared normal except for a lowered iodine intake.

The various dietary levels of vitamin A did not evoke a difference in the response of the animals to cold exposure. In experiment 6 the level of synthesis of ascorbic acid decreased after one week in the cold but vitamin A had no effect on this response. Since in the other experiments cold did not seem to affect the synthesis it may be that the exposure had not been long enough. They were only exposed about three days whereas the animals in experiment six were exposed to cold for over a week. The synthesis returned to normal in the animals exposed for over two weeks. This confirms the work of Akpabio (87); however unlike him we found no increase in synthesis at a later time. We cannot at this time explain this discrepancy.

The vitamin A assay of the livers of the animals of experiment 6 showed that the animals had significantly different vitamin A levels. According to Moore (1) adult female rats have the capacity to store 100,000 I.U. of vitamin A for the total liver. Considering that our values are expressed in I.U. per gram of liver the livers have about as much vitamin A as can be stored short of being toxic. The results for the normal vitamin A animals are what is expected in wild rats. Our assay was done only on rats in the cold; however according to Porter and Masoro (88) cold exposure has no influence on vitamin A storage. In their experiments rats fed ad libitum on a normal diet had more vitamin A in their livers when kept at 2°C than when kept at 25°C. They concluded that since the animals ate more in the cold the greater vitamin A storage resulted from an increased intake. This conclusion was verified because when they gave equal amounts of vitamin A to the animals at 2°C and 25°C the levels in the liver were the same.

In conclusion it can be stated that within the limited of our techniques, vitamin A has no effect on the TSR, on TSH secretion, on thyroid weights or on ascorbic acid synthesis.

PART B

Experiment 7 TSR (Variable Doses)

Protocol

Because of Heroux's observation that TSR's, calculated according to the method of Reineke and Singh, differed with the initial dose of thyroxine, being lower with lower starting doses, the following experiment was done. Rats were divided into four groups; two groups were put in the cold at 2°C and two at 30°C. They were fed fox chow pellets. After four weeks in these conditions their TSR's were calculated. To one group at 2°C and one at 30°C the starting dose of thyroxine was 0.5 μg per 100 gm body weight per day with 0.5 increments after 43 hours. The other groups were given a starting dose of 0.1 μg of thyroxine per 100 gm body weight per day which was doubled after 48 hours. Even though the release of I^{131} from the gland was almost stopped, injections of 0.5 μg of thyroxine per 100 gm body weight per day were given to the latter groups for two days, following the last dose of 0.2 μg.

For easier notation the following symbols were used:

LT (30) rats which were at 30°C and given a starting dose of 0.1 μg thyroxine per 100 gm body weight.

LT (2) similar to LT (30) except that the rats were at 2°C

HT (30) similar to LT (30) except that the rats were given a starting dose of 0.5 µg thyroxine per 100 gm body weight.

HT (2) similar to HT (30) except that the rats were at 2°C

Results

From the results obtained (Table 17), it can be seen that Heroux's observations have been confirmed.

TABLE 17 - Comparison of the Effect of the Starting Injection Dose on T.S.R. Results.

	T.S.R. µg/100g BW	Comparison with	Difference ± S.E.	t	p
LT(30)	0.28 (10)	LT(2)	0.04±0.05	0.80	ns
		HT(30)	0.97±0.13	7.46	<.001
LT(2)	0.32 (8)	HT(2)	0.97±0.13	7.46	<.001
HT(30)	1.25 (10)	LT(30)	0.04±0.17	0.24	ns
HT(2)	1.29 (10)	-	-	-	-

It is apparent from this table that the animals given low doses have significantly lower T.S.R's than those given higher starting doses of thyroxine.

One very interesting result which is observed in Fig. 5 is that when the rats on the low starting dose were given 0.5 µg thyroxine per 100 g body weight per day, which

is more than their calculated TSR, there is a greater release of iodine from the thyroid. The readings obtained are quite close to those of the rats on the higher dose when they were given 0.5 μg of thyroxine. It seems as though the thyroids are reacting similarly to an identical dose of thyroxine.

It is also seen that the TSR's of the rats in the cold is not different from that in the warm room. This has also been shown in a previous experiment in which the animals have been acclimatized for four weeks. (Page 36, experiment six).

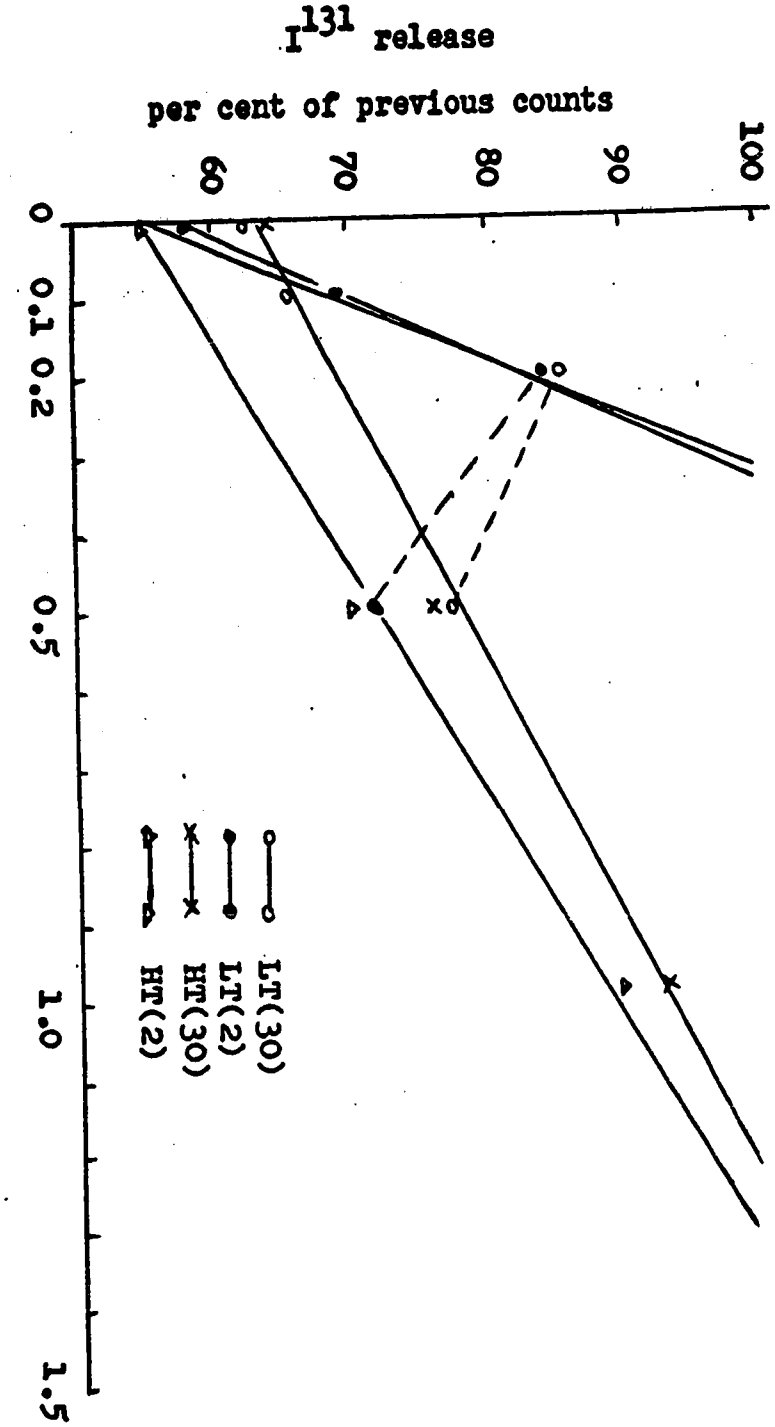


Fig. 5 Regression lines showing different TSR's with different starting injection doses of thyroxine

Experiment 8 Comparison of Various Parameters of Thyroid Function

Protocol

This experiment was done to compare the methods of measuring thyroid function using I^{131}. The data used was that of experiment 5 in part A. Not only was the TSR measured but so was both the uptake and release of I^{131} from the thyroid.

Results

Figs. 6, 7 and 8 show that not only do iodine deficient rats pick up more I^{131}, they also release it more quickly. When Fig. 6 is examined it can be seen that, if the data are calculated according to total thyroid I^{131} counts, the vitamin A deficient rats have more active thyroids than the other two groups as more I^{131} is picked up. However if the counts are calculated as percentages of the 24 hour count the vitamin A deficient animals have less active thyroids than the other groups as they take longer to reach a peak. There is not such a striking discrepancy in the iodine deficient animals, as the iodine deficiency seems to mask the vitamin effects if there are any.

On examining the release curves of I^{131} it can be seen that both iodine deficient rats and normal rats show that vitamin A deficient rats release I^{131} slower than do the other vitamin A groups; however, in all cases the iodine deficient rats release I^{131} more quickly than the normal iodine rats.

It is also to be remembered that the TSR's calculated previously showed no difference between any of the groups.

Fig. 6 Uptake of I^{131}

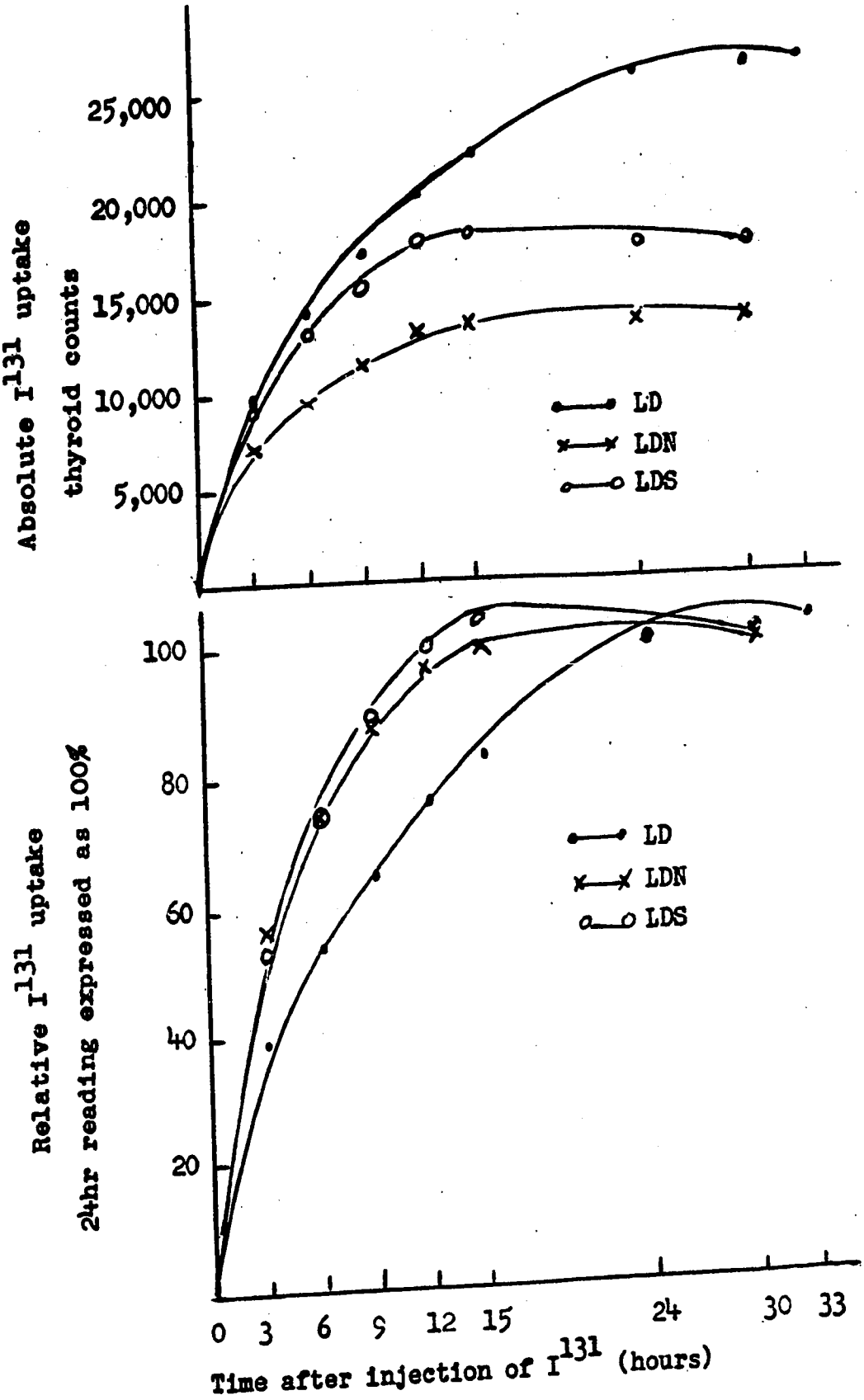


Fig. 7 Uptake of I^{131}

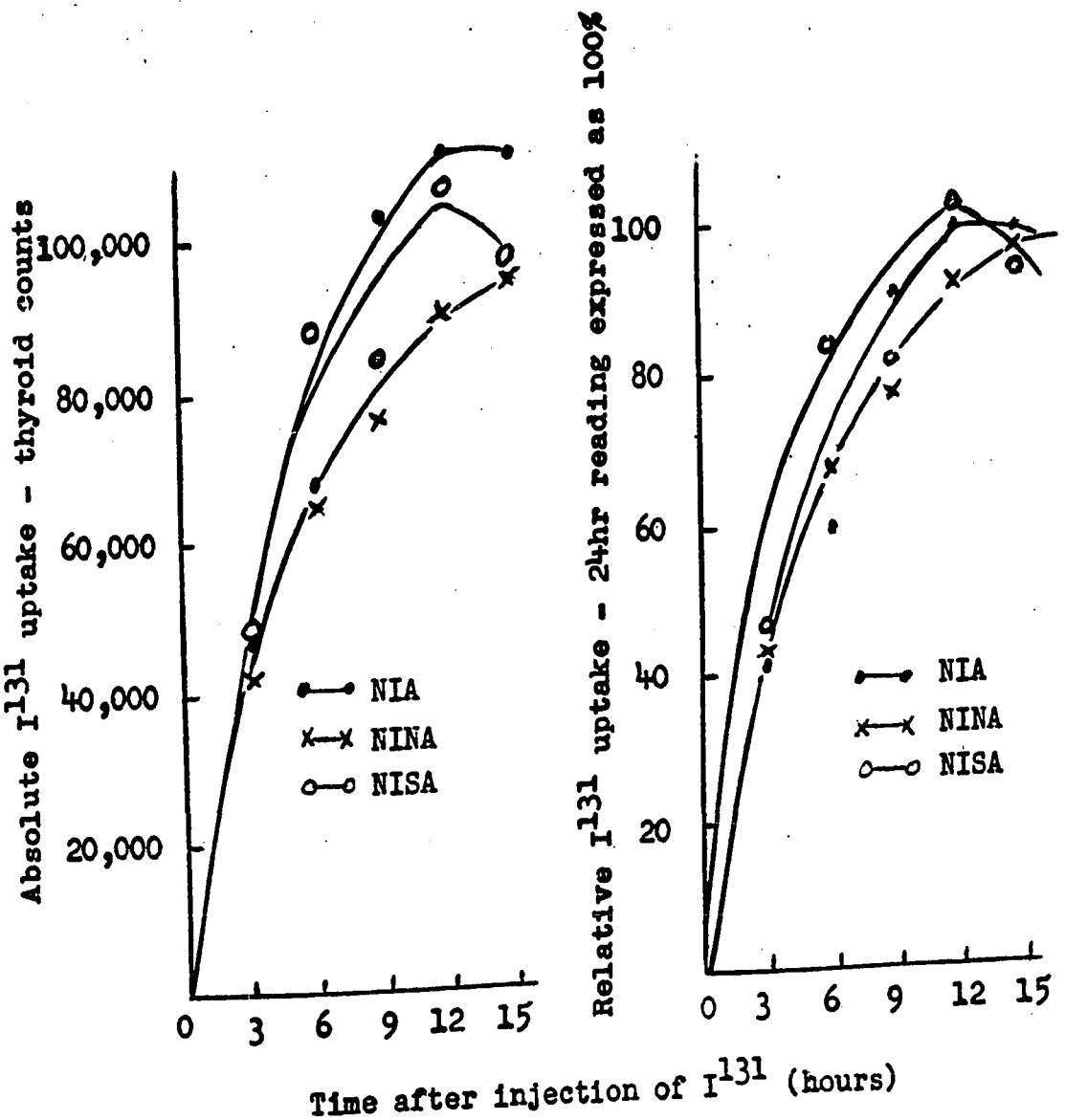
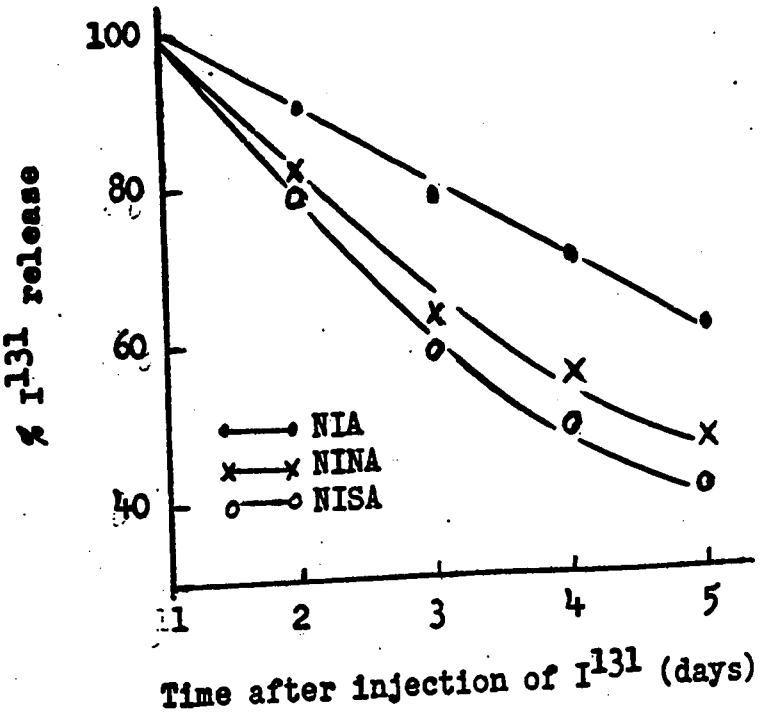
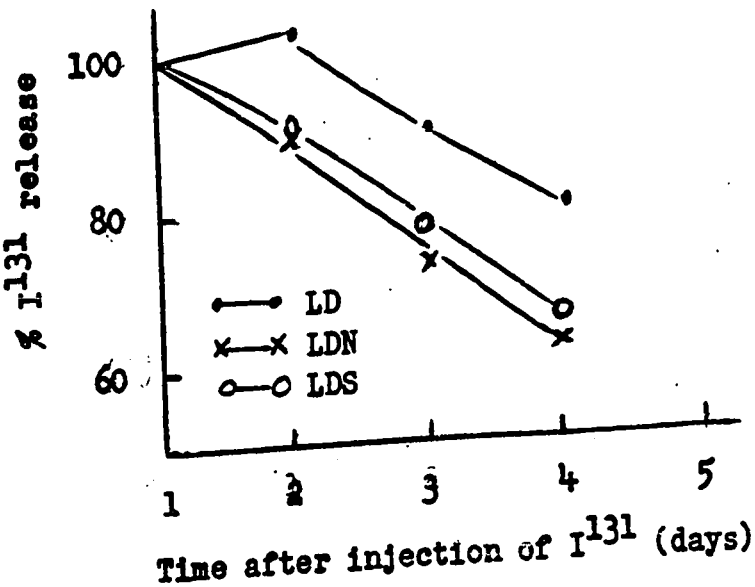


Fig. 8 Release of I^{131}
1 day reading expressed as 100%





Experiment 9 Comparison of Two Different Methods of TSR Measurement

12 male albino rabbits (average weight: 2.02 kg) were divided into 2 groups of six. One group was put in a constant temperature room at 30°C and the other group at 2°C. After four weeks each rabbit was injected with 170 μc of carrier free I^{131} and both the uptake and release of I^{131} by the thyroid gland were measured. PBI and PBI^{131} were measured every 24 hours for four days and the TSR was calculated using Sørensen's Method (63).

On the seventh day after I^{131} injection each group of six rabbits was divided into two groups of three. To one group at 2°C and to one group at 30°C a dose of 0.5 μg of thyroxine per kilogram per day was given to each animal subcutaneously. Four days later this was increased to 1.0 μg per kilogram per day. Even though this dose almost stopped the release of I^{131} from the gland the dosage was increased to 1.5 μg per kilogram per day four days later.

A dose of 1.5 μg of thyroxine per kilogram per day was given to the remaining groups at 2°C and 30°C. Four days later the dose was doubled to 3 μg per kilogram per day. PBI's and BEI's were determined before each increase in injection and four days after the last increase. The TSR's were calculated according to the method of Reineke and Singh (59).

For purposes of brevity and easier wording the following symbols were used for the groups.

LDK rabbits which were kept at 2°C and were given an initial dose of thyroxine of 0.5 µg per kg body weight.

LDH rabbits treated similarly to LDK but kept at 30°C.

HDK rabbits treated similarly to LDK but given an initial dose of 1.5 µg of thyroxine per kg body weight.

HDH rabbits treated as those of HDK but kept at 30°C.

Results

After four weeks of acclimation the weights of the rabbits were 3.06 kg for those in the cold and 2.31 kg for those at 30°C. Thyroid I^{131} uptake and release are shown in Fig. 9. PBI^{131} results can be seen in Fig. 10. TSR's were calculated according to the Method of Sørensen and the various parameters are listed in Table 18.

Because of the inaccuracy of the individual values only the average of the group values were used in the calculations.

As is seen the rabbits in the cold have a higher thyroid secretion rate. This corresponds quite well with the I^{131} uptake and release; however, the values of PBI do not

Fig. 9 Uptake and release of I^{131} with measurement of U

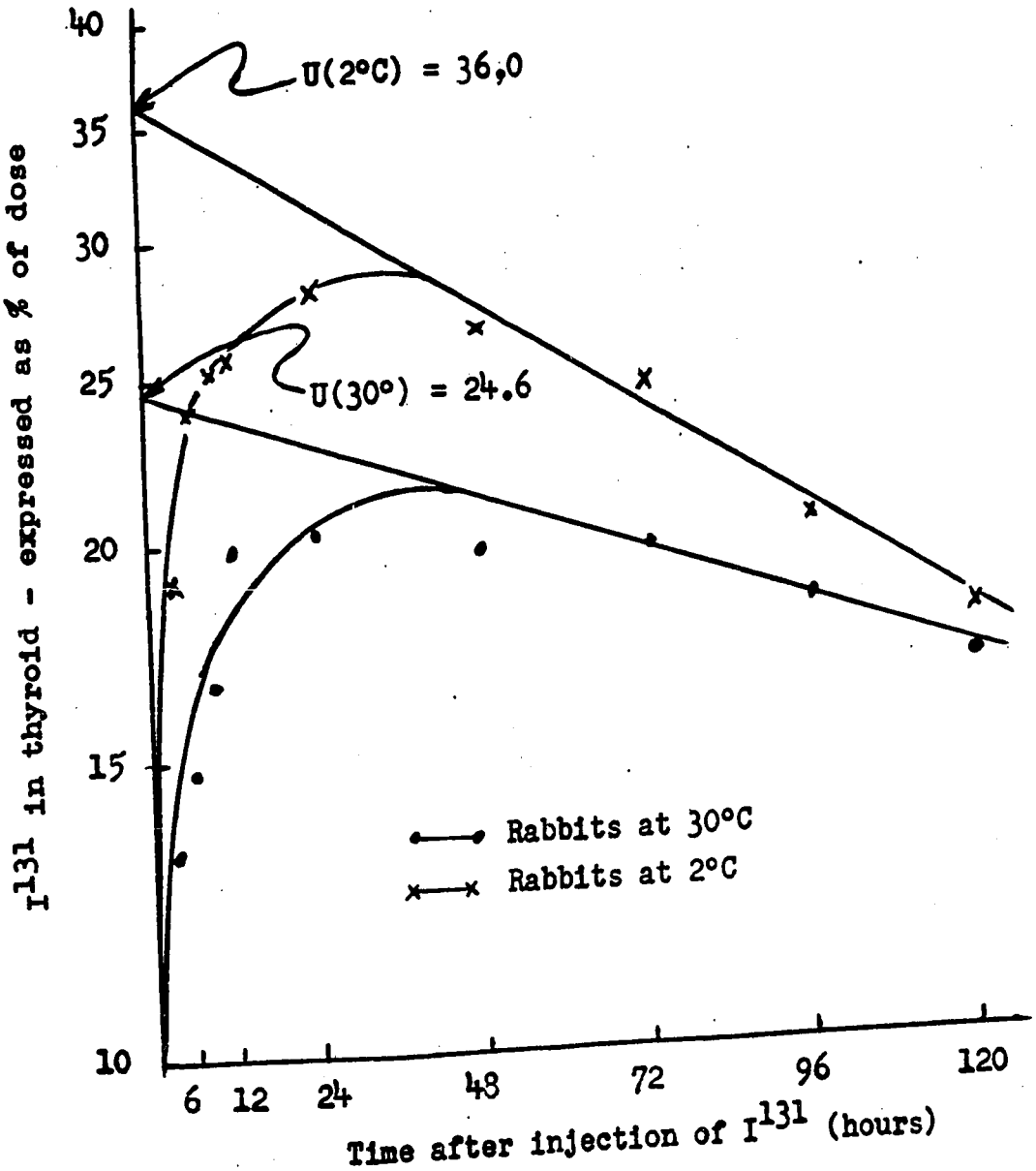
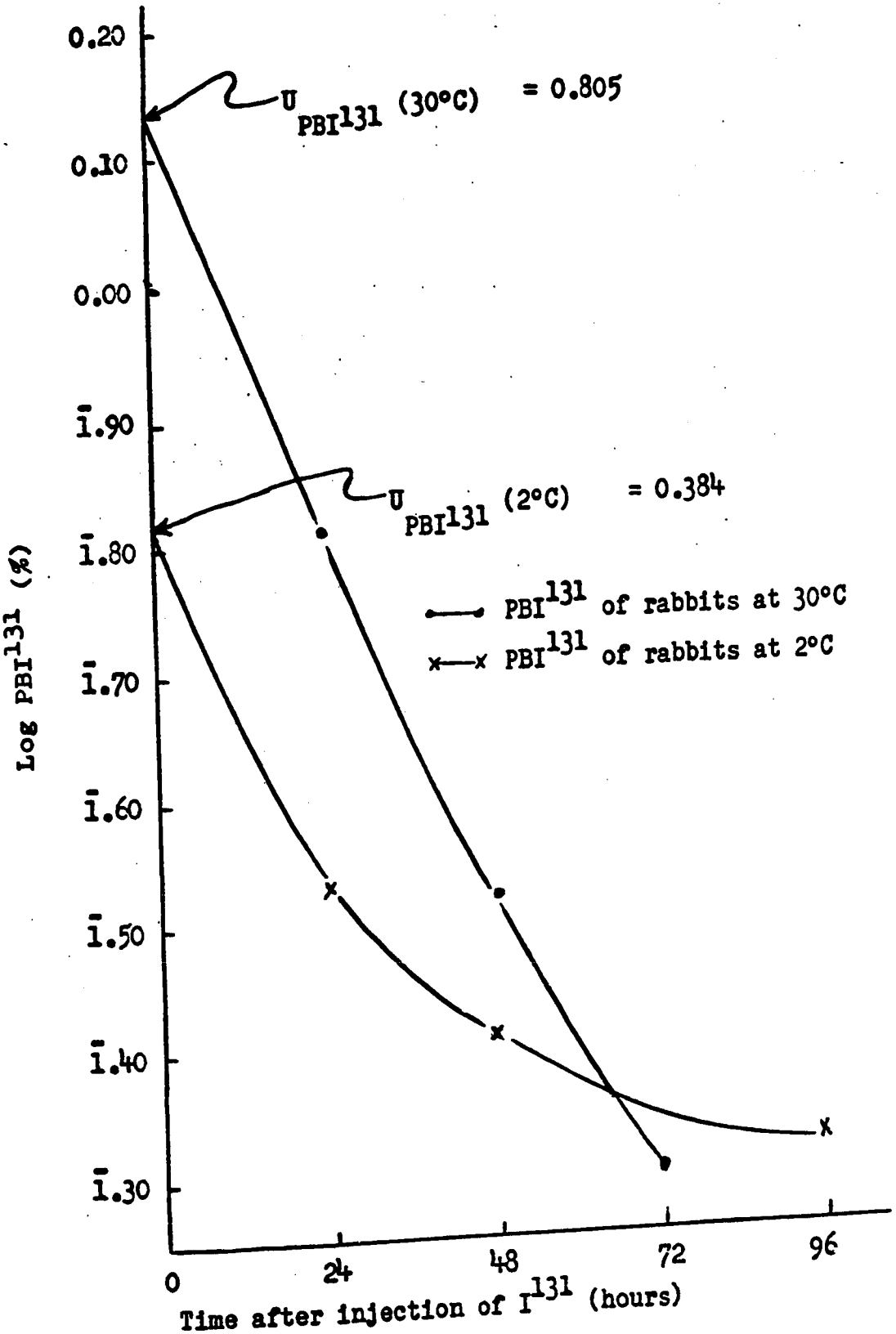


Fig.10 Measurement of $U_{PBI^{131}}$



give any indication one way or another. The PBI^{131} values seem to be more disposed towards a higher thyroid activity for the 30°C rabbits.

TABLE 18 - Various Parameters of Thyroid Activity as Used to Calculate T.S.R. by Sørensen's Method.

	30°C	2°C
U	24.6%	36.0%
$U_{PBI^{131}}$	0.805%	0.384%
$T_{\frac{1}{2}}$	222 hours	123 hours
PBI (μg)	3.17±0.22	4.01±0.34
K_4	0.00417	0.00330
TSR	14.3μg	120.5μg
TSR/kg	6.2μg	39.5μg

When TSR's are calculated using the injection method the results (Table 19) do not agree with those of Sørensen.

When the TSR's of the high dose rabbits are compared with one another and those of the low dose rabbits compared with one another, an interesting fact is observed. The ratio of TSR's of $\frac{HDE}{HDK}$ is 0.71 and the ratio of $\frac{LDE}{LDK}$ is 0.70.

As is shown in Fig. 11, when the low dose rabbits are given an excess of thyroxine (1.5μg/kg) a curious result is obtained. The release of iodine is accelerated but it is released in parallel fashion for the high and low temperature acclimated animals.

Fig.11 Regression lines showing different TSR's with different starting doses of thyroxine

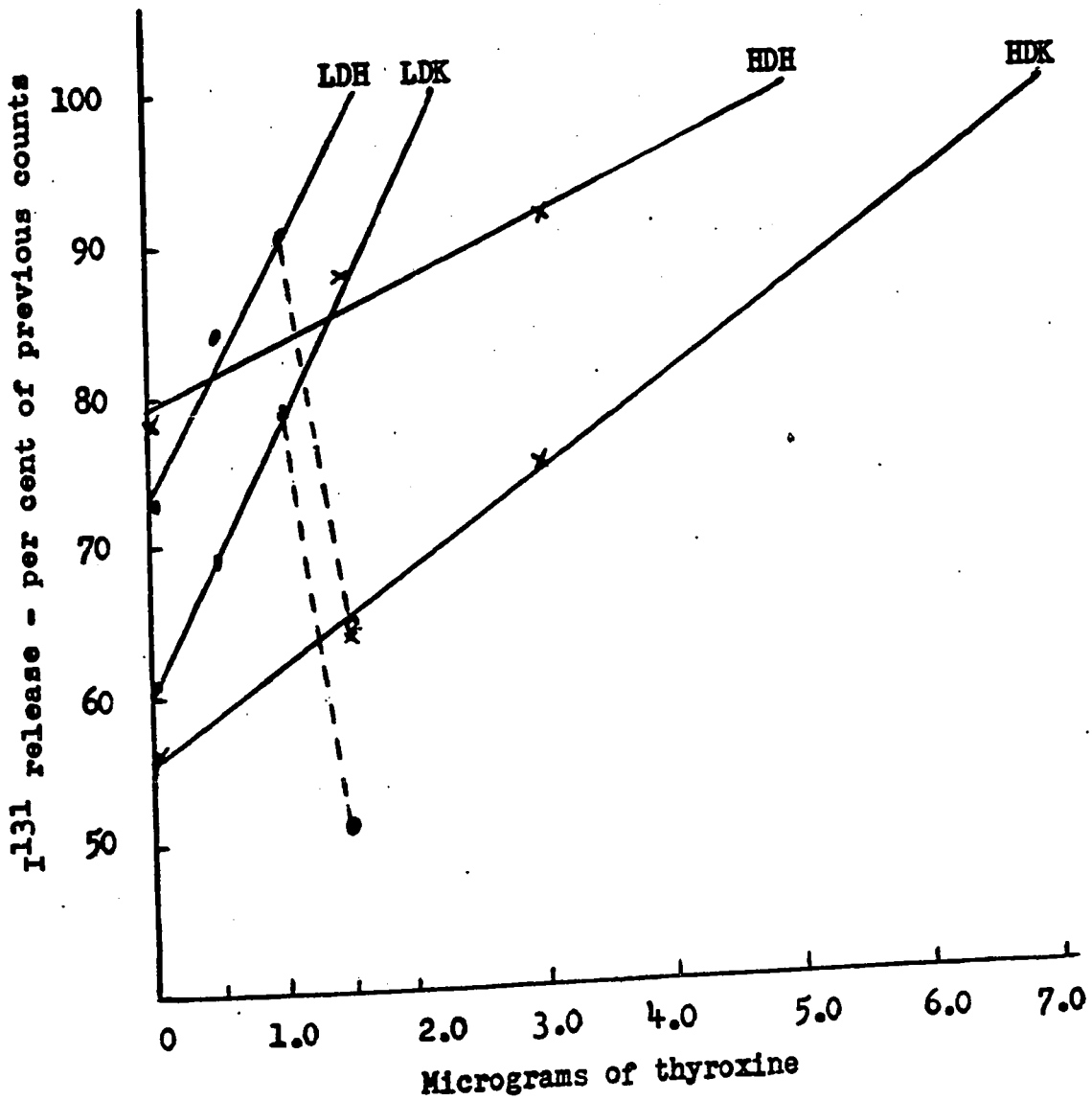


TABLE 19 - Comparison of the Effect of the Starting Injection Dose on T.S.R. Results.

	T.S.R. µg/kg BW	Comparison with	Difference ± S.E.	t	P
LDH	1.51(3)	LDK	0.66±0.73	0.90	0.40
		HDH	3.38±1.60	2.11	0.05
LDK	2.17(3)	HDK	4.73±2.43	1.94	0.10
HDH	4.39(3)	HDK	2.01±2.95	0.68	0.50
HDK	6.90 (3)	-	-	-	-

The results of the BEI and PBI measurements as well as their statistical analysis can be seen in Tables 20 and 21 respectively. All the results are expressed in γ/100ml serum. The values are similar in both the high and low starting doses. It also is seen that in both PBI and BEI the levels are lowered with increase in the injected dose of thyroxine whatever the starting dose. Though this is not significant it might have been if more samples had been obtained.

TABLE 20 - D.E.I. measurements

	Injection µgs/kg	30°C	2°C
High Dose	0	2.7±0.7 (3)	2.3±0.2(3)
	1.5	1.2±0.7(3)	1.3±0.7(3)
	3.0	1.3±0.2(2)	1.7±0.6(3)
Low Dose	0	2.8±0.8(3)	3.6±0.9(3)
	0.5	1.7±0.4(3)	1.7±0.2(3)
	1.0	0.6±0.4(2)	1.3±0.3(3)

Analysis of Variance

Source	df	SS	MS	f	p
Concentration	2	18.30	9.15	6.53	<.01
Temperature	1	0.71	0.71	0.51	ns
Interaction	2	0.28	0.23	0.20	ns
Concentration	2	8.07	4.03	2.38	ns
Dose	1	0.19	0.19	0.13	ns
Interaction	2	0	0	0	ns
Temperature	1	0.09	0.09	0.07	ns
Dose	1	0.93	0.93	0.67	ns
Interaction	1	0.15	0.15	0.11	ns
Error	16	22.38	1.40		

Concentration

1) One-Way Analysis of Variance — f = 18.56, p < .01

2) Duncan's Test (p=.05)

Dose 0	Dose 1	Dose 2
2.86	1.45	1.24

TABLE 21 - P. B. I. Measurements.

	Injection gs/kg	30°C	2°C
High Dose	0	2.5±0.8(3)	4.0±0.6(3)
	1.5	1.3±0.3(3)	1.9±0.3(2)
	3.0	0.6±0.4(2)	1.4±1.3(2)
Low Dose	0	3.2±0.5(3)	3.6±0.9(3)
	0.5	0.8±0.3(3)	2.3±0.7(3)
	1.0	1.3±0.2(2)	1.2±0.7(3)

Analysis of Variance

Source	df	SS	MS	f	p
Concentration	2	28.04	14.02	7.75	<.01
Temperature	1	21.12	21.12	11.67	<.01
Interaction	2	0.80	0.40	0.22	ns
Concentration	2	30.28	15.14	8.36	<.01
Dose	1	0.15	0.15	0.08	ns
Interaction	2	0.04	0.02	0.01	ns
Temperature	1	4.96	4.96	2.74	ns
Dose	1	0	0	0	ns
Interaction	1	0	0	0	ns
Error	14	25.35	1.81		

a) Concentration

1) One-Way Analysis of Variance — f = 12.78, p < .01

2) Duncan's Test (p=.05)

Dose 0	Dose 1	Dose 2
3.33	1.56	1.10

b) Temperature

	t	p
t test	0.86	ns

DISCUSSION

In our studies on the effect of vitamin A on the thyroid gland we observed several contradictions in the various parameters used to measure thyroid function. The more obvious of the contradictions was seen on comparison of the relative and absolute I^{131} uptakes of the thyroids and the I^{131} release from the thyroids (Expt. 3).

In the rats with normal dietary levels of iodine, vitamin A seems to affect both the rate of uptake and the total accumulation of I^{131} in the gland. According to the rate of uptake it would seem as though the vitamin A deficient animals have a lower thyroid activity than the rats on the normal and excess vitamin A diets; however, total accumulation of I^{131} gives a different picture. Here we see that the deficient rats have the greatest uptake and hence seem to have a greater thyroid activity. This apparent contradiction might be explained by a pathological effect of a deficiency of vitamin A. Moore (1) says that the "main complications of vitamin A deficiency in the urinary system, as seen in the rat, are prevention of micturition through blockage of the urethra, nephrosis, infection of the ureters and kidneys with pyogenic organisms and stone formation." Since this was not examined in our rats we can only assume that these complications occurred; however these symptoms have always been shown to be present in vitamin A deficiency. This

would explain why more iodine was picked up in the deficient rats. If there is decreased micurition there would be more iodine remaining in the body and this would eventually be picked up by the thyroid. It can also explain the decreased rate of uptake as is seen when relative amounts are examined. If, in all three groups the I^{131} was picked up at the same rate, the time taken for the maximum I^{131} uptake would be greater in the deficient animals because of the relatively greater amount of iodine to be trapped in the gland.

In the iodine deficient animals it can be seen that neither the absolute nor relative uptakes by the thyroid glands give any indication that vitamin A affects the thyroid activity. Because the iodine trapping ability of the thyroid in these animals is so great they do not excrete much I^{131}; thus, the lack of micturition in the vitamin A and iodine deficient animals seems to have little effect.

When the release curves are examined it can be observed that the vitamin A deficient animals, both normal and iodine deficient, show a slower amount of I^{131} release from the gland. This may also be explained by a lack of micturition so that there is less iodine being excreted and more retrapped by the gland; thus it would seem that less I^{131} was released from the gland.

Comparing the iodine deficient rats with the nor-

mal iodine rats it can be seen that more iodine is picked up in a shorter time, and this iodine is released more quickly by the iodine deficient rats; therefore, if the previous history of the animals were not known it would seem as though the iodine deficient rats had a higher thyroid secretion rate.

Thus it can be seen that the parameters described have little value unless all the circumstances of the experiment and the condition of the animal is known.

As can be seen the experiments of Heroux have been confirmed; however unlike him (62) we did not find a difference in the secretory rate of the cold and warm acclimated rats in Expt. 7 when either the low or the high starting dose of thyroxine was used. We did, however, see an indication of a difference between the warm and cold acclimated rabbits when either starting dose was used. There were no significant differences probably because there were only three rabbits in each group. One important observation is that the ratios of the TSR's between the cold and warm acclimated animals are approximately the same. This gives an indication that similar starting doses of thyroxine should be given to animals when TSR's are to be determined or the difference in the results obtained may not be due to differences in thyroid secretion. It thus seems as though this method of calculating TSR's measures the relative and not the absolute rate of secretion.

It was observed in fig. 5 that the rats on the low starting dose of thyroxine when given an excess dose over the calculated TSR had an increased release of I^{131}. This type of observation has been seen by both Akpabio (87) and Lodge et al (88). This is probably due to an action of thyroxine on the thyroid gland itself causing more thyroxine to be released. This is supported by the findings of Ingbar and Freinkel, (89) who found that in rats, which had previously been given radioiodine, there was an increase in the specific activity of plasma thyroxine after administration of an excess of non-labelled thyroxine. The important thing about this finding is that the percentage of thyroid counts over the previous 48 hour ones was approximately the same as that found in the rats given an identical dose and which had been given the high starting dose. This seems to show that in rats, at least, no matter when the starting dose is, there is a similar reaction to identical doses of thyroxine.

In the rabbits the so-called excess dose of thyroxine given to the animals on the low dose turned out to be about 100% of the calculated TSR for the warm-acclimated ones and under 100% for the cold-acclimated animals, however the dose still caused an increase in the release of radioiodine from the gland. This increased release rate did not show the same characteristics as in the rat. Identical doses did not give similar reactions. The accelerated release however was in a parallel fashion. Thus there seems to be a

species difference in the respose of iodine release to the administration of an excess of thyroxine.

It can be seen in Expt. 9 that the Sørensen method for measuring TSR's gave different results from the injection method. The results for the warm acclimated rabbits were quite close but the results for the cold acclimated rabbits were completely different. The results obtained by the injection method with the high starting dose (4.39 μg thyroxine/kg/day) are quite similar to those obtained by Pipes et al (73) and Grosvenor (90) on rats and Ray et al (91) on guinea pigs which were equal to 4.8 to 10.0 μg thyroxine/kg/day. Sørensen using his own method found TSR's of 7.4 μg thyroxine/kg/day in cows (92) and 11.7 μg/kg/day in pigs (93); thus we are in approximately the same range in the warm acclimated rabbits with a secretion rate of 6.2 μg thyroxine/kg/day. The cold acclimated rabbits, however, show a very high secretion rate of 39.5 μg thyroxine/kg/day when calculated; whereas the injection method gave a maximum of 6.90 μg thyroxine/kg/day.

Our results also confirm those of Falconer and Robertson (65) that the secretion rates agree with I^{131} uptake and release but not with PBI^{131} levels. The PBI's obtained, although slightly higher in the cold acclimated rabbits, are not significantly different. The results for PBI^{131} seem peculiar. If the thyroid secretion were greater in the cold

acclimated animal it would seem as though more thyroxine I^{131} would appear in the plasma; however this does not seem so. Since the basic assumption is the following equation

$$\frac{\text{secreted hormonal iodine/hr}}{\text{secreted hormonal } I^{131} \text{ \% dose/hr}} = \frac{\text{PBI/100ml}}{PBI^{131} \text{ \% dose/100ml}}$$

the stores of thyroxine in the thyroid would have to be much greater in the cold acclimated rabbit so that the ratio of $\frac{\text{secreted hormonal iodine/hr}}{\text{secreted hormonal } I^{131} \text{ \% dose/hr}}$ would be very large; hence the proportion of $\frac{PBI}{PBI^{131}}$ would be large. Since PBI is not changed PBI^{131} would have to be less so that this proportion is increased. Since the release of I^{131} from the gland is greater in the cold acclimated rabbit there is a contradiction. Thus there seems to be an important doubt thrown on the basic assumption.

Johnson et al (61) found that the injection of thyroxine did not affect the rate of release of I^{131} from the gland in hyperthyroid animals. Thus if the cold acclimated animals had such a large secretion rate they would be approaching or be at a hyperthyroid condition and injections of thyroxine would have no effect on the release rate of hormone which, as seen, is not the case.

The method of obtaining U and $U_{PBI^{131}}$ is not too exact as the extrapolation of points on a curve is not always correct; thus these values could be quite erroneous. It was

also found that detecting radioactivity in the PBI samples was quite difficult and therefore the PBI^{131} results are very poor.

Another difficulty in this method is that the amount of blood required is quite large. This means that only larger animals can be used for this type of measurement. Rats cannot be used.

One of the most puzzling observations is the decrease in PBI and BEI values when thyroxine is injected. This decrease though not significant probably would be if more animals were used. Hamolski and Freedberg (94) have noted that T_3 in sufficient dosage usually results in a decrease in PBI; however T_4 in excess results in an increase in PBI. Since we did not give an excess of T_4 we would not expect an increase but neither would we expect a decrease.

This discussion shows that there is still no good method of measuring the absolute thyroid secretion rate. There are many aspects in the methods described which should require more work for clarification.

In conclusion these experiments show that as far as measuring the TSR in rats, the injection method is one of the better methods although it seems to measure relative rather than absolute amounts.

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