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DIETARY PROTEIN UTILIZATION AND THYROID ACTIVITY IN TWO INBRED LINES OF RATS AND THEIR RECIPROCAL CROSSBREDS

A THESIS
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by

FREDERICK MICHAEL CONNELLY

EDMONTON, ALBERTA

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled "Dietary Protein Utilization and Thyroid Activity in Two Lines of Rats and Their Reciprocal Crossbreds" submitted by Frederick Michael Connelly, B. Sc., in partial fulfilment of the requirements for the degree of Master of Science.

ABSTRACT

Two series of experiments were conducted on the Wistar and Sprague-Dawley lines of rats and their reciprocal crossbreds. One series dealt with dietary protein utilization and the other with thyroid activity. Both inbred lines had 4 generations of full sib matings in their immediate past.

Consumption of nitrogen varied directly with the protein level of the diet and was positively related to gain and food conversion ratio. Apparent nitrogen digestibility increased on high protein diets whereas the efficiency of protein utilization decreased. Between diets, gain and per cent ADN were highly and negatively correlated.

Gain difference between sexes existed and was probably dependent upon greater food consumption by males. There was no sex difference for apparent food or nitrogen digestibility and each sex was equally efficient in the utilization of dietary protein. During the early post-weaning period each sex had a characteristic thyroid activity which appeared to bear a positive relationship to gain. At later stages of maturity sex difference in thyroid activity diminished.

Gain and food conversion differed between inbred lines and a heterotic effect was noted in the crossbreds. The fastest growing inbred line consumed the least food. Crossbreds, however, consumed more food than inbreds and were, nevertheless, more efficient in food conversion. A relatively large difference in efficiency of protein utilization existed

between the inbreds and a somewhat smaller difference existed in favour of crossbreds over inbreds. Between genotypes the correlation of gain with per cent ADN and with thyroid activity was high and on this basis it is suggested that there may have been a similar relationship between per cent ADN and thyroid activity.

Differential genetic response to dietary protein levels was found for consumption and efficiency of nitrogen utilization. A genotype x age interaction for thyroid activity was also evident but no other measures displayed this characteristic.

Apparent nitrogen digestibility but not apparent food digestibility was influenced by genotype. However, it was deduced that true protein digestibility was probably unaffected by genotype. Reciprocal crossbreds consistently differed from one another for most measures made. The cause of these differences was not determined.

Deleterious effects from inbreeding occurred and were manifest by impaired reproductive potential and lowered resistance to bronchial disease.

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TABLE OF CONTENTS

TABLE OF CONTENTS (Continued)

TABLE OF CONTENTS (Continued)

LIST OF TABLES

LIST OF FIGURES

Page

Genotype x Diet Interactions from the Main Metabolism Experiment

Equipment and Method for Determining Thyroid I^{131} Decay

Thyroid I^{131} Decay During the Preliminary Thyroid Experiment

Thyroid I^{131} Decay During "Period 1" of the Main Thyroid Experiment

LIST OF FIGURES (Continued)

Page

Thyroid I^{131} Decay During "Period 2" of the Main Thyroid Experiment

Thyroid I^{131} Decay During "Period 3" of the Main Thyroid Experiment

Influence of Age on Thyroid I^{131} Decay

Interactions With Age

INTRODUCTION

The importance of protein in a production-type ration and the relationship of protein to other nutrients in the diet has been a field of active investigation in recent years. Genetic influences on protein and other dietary requirements have not been elucidated. The fact that different strains have different minimum requirements is well established but the concept of optimum ratios of dietary nutrients would seem to necessitate a new research approach into the realm of genotype x nutrition interactions, or more specifically, into differential genotypic responses to dietary modifications.

It is also a possibility that heterosis or the expression of hybrid vigor may lead to less precise nutrient requirements or to different minimum and/or optimum requirements from those established for the pure lines entering a particular cross.

The thyroid gland is known to mediate overall body metabolism and thus will influence specific nutrient utilization. The heritable nature of thyroid activity and its possible relationship to the metabolism of nutrients, particularly protein, should be of great interest to both geneticists and nutritionists.

This thesis covers a study of protein metabolism in two lines of rats and their reciprocal crossbreds fed approximately isocaloric diets varying in protein level. Estimates have been made of thyroid I^{131} decay and an effort

has been made to relate thyroid activity to gain and metabolic measures.

The metabolism studies were conducted between July, 1958, and September, 1959, and the thyroid work was done between September, 1959, and April, 1960.

LITERATURE REVIEW

I. Influence of Dietary Protein Variation on Gain, Food Consumption, Digestibility, and Nitrogen Retention.

Studies in recent years have emphasized the importance of the ratio of energy to protein in the diet. Sibbald (1957), at this institution, using Sprague-Dawley rats demonstrated a curvilinear relationship between the ratio ADE/ADN(1) and efficiency of nitrogen utilization measured as per cent ADN retained. A similar tendency has been reported with 50 and 110 pound pigs (Likuski, 1959), although 15 pound pigs increased rather than decreased the per cent ADN retained when dietary protein levels were raised.

Both Allison and Anderson (1945), with dogs, and Armstrong and Mitchell (1955), with swine, have reported a rectilinear relationship between truly absorbed nitrogen and nitrogen retention when low protein rations were fed. However, at higher dietary protein levels this relationship became curvilinear which meant that the efficiency of protein utilization was decreased. A similar depressing effect of high protein diets on efficiency of nitrogen utilization has been observed in rats by Forbes <u>et al.</u> (1958).

Dietary energy level has been reported to have the greatest influence on voluntary food consumption although suboptimum dietary protein levels may affect food consumption

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(1) ADE and ADN will refer to Apparent Digestible Energy and Apparent Digestible Nitrogen, respectively, in this thesis.

by limiting growth (Peterson *et al.*, 1954). Conversion of gain and food consumption data to a unit body weight basis essentially removes dietary protein influences on these measures. However, on low protein diets, with data converted to an ABW(1) basis, experimental animals may alter food intake in an attempt to acquire more protein (Sibbald, 1957; Bowland *et al.*, 1958; Likuski, 1959).

The per cent ADN of a diet increases with nitrogen intake. This relationship has been demonstrated in sheep (Homb and Breirem, 1952), swine (Lloyd and Crampton, 1955; Likuski, 1959), and rats (Crampton and Rutherford, 1954; Bowland *et al.*, 1958; Likuski, 1959). Lassiter *et al.* (1956), however, reported no per cent ADN differences corresponding to ration protein variation in swine. It has been suggested that per cent ADN response to protein intake does not represent a true digestibility change but reflects a relatively constant metabolic fecal nitrogen (Crampton and Rutherford, 1954). Graphically represented, the protein intake to per cent ADN relationship is curvilinear (Crampton and Rutherford, 1954; Homb and Breirem, 1952) and it was suggested by Crampton and Rutherford that for their studies true protein digestibility was 100 per cent.

II. Influence of Sex on Gain, Food Consumption, Digestibility, and Nitrogen Retention.

During early growth phases sex differences may not be apparent but during more advanced stages of growth males

(1) ABW will refer to Average Body Weight in this thesis.

attain greater body weight gains, consume more food, and require more food per pound of gain than females. This has been demonstrated in swine by Bell et al. (1958) and Bowland and Berg (1959). In a report at this institution, dealing with Sprague-Dawley rats, Hussar and Bowland (1959) found significant differences in favour of males in rate of gain but not food consumption from weaning to 4 weeks of age when data were converted to 100 grams ABW.

In the same report male superiority for apparent digestibility of food and nitrogen was demonstrated. The same authors found efficiency of nitrogen utilization differences between sexes but trends were not reported. Other reports by Sibbald (1957) and Bowland et al. (1958) at this institution, using the same line of rats as Hussar and Bowland, indicated no sex influence on nitrogen retention. On this basis experiments were designed by these workers to include sex balance but no further analysis of the effects of sex on metabolic measures was made.

III. Maternal Influence on Performance of Offspring.

It is possible to estimate maternal ability by comparison of cross fostered young (Butler and Metrakos, 1950; Cox et al., 1959) or by reciprocal crosses (Butler, 1952; Chai, 1955). Schultze (1954) found that as litter size increased so did total litter weight, although gain per individual offspring decreased. He suggested that total litter weight to weaning of large litters is a better estimate

of maternal lactation than is average weight gain of individual animals.

Performance of animals from birth to weaning may depend upon both their own genetic potential and the environment afforded by the dam. The maternal environment may consist of both pre-natal and post-natal influences (Butler, 1952; Cox *et al.*, 1959).

Pre-weaning performance may in turn be reflected in post-weaning performance (Butler and Metrakos, 1950; Butler, 1952; Chai, 1956; Cox *et al.*, 1959). Using cross fostered young from two lines of mice of different size, Butler and Metrakos (1950) found offspring suckled on the large line females had a greater rate of gain to 16 days, and a slower rate of gain from 16 to 32 days, than offspring suckled on the small line females. Analysis of variance for mature body weights in mice by Chai (1959) indicated maternal influences present but of less magnitude than genotypic influences. On the other hand, Cox *et al.* (1959) attributed 68 per cent of the variation in 4-week weights of mice to pre-weaning environment. The report by Butler (1952) indicated maternal influences were recognizable in the F_2 generation. Honeyman (1957), relative to weight gains in mice between 21 and 51 days of age, found no reciprocal crossbred differences although parental strain differences were evident.

IV. Inherited Nature of Metabolism.

(a) Levels of Metabolic Measures as Affected by Genotype.

A detailed account of strain differences in rats with respect to nitrogen and energy metabolism has been reported by Palmer *et al.* (1946). These workers developed two strains from a single mating, having high and low food conversion ratios and analyzed the biochemical and physiological factors which characterized the two strains. Differences were found between strains for gain, food consumption, proportion of nitrogen in gains, percentage body protein and ether extract, energy requirement for maintenance, basal metabolism, total and endogenous nitrogen excretion, and body temperature. No differences occurred for apparent food or nitrogen digestibility. More recently a series of reports has appeared in the literature in which metabolic differences in rat lines of varying degrees of obesity have been studied (Bloom and Fenton, 1956; Fenton, 1956; Fenton *et al.*, 1951a and b, 1953, and 1956; Lyon, 1957; Lyon *et al.*, 1953, 1956, and 1958). Strain differences in utilization of protein, fat, and glucose as measured by gain, food consumption, efficiency of protein utilization, food conversion, nitrogen retention, and pyridoxine metabolism were found. Bowland and Berg (1959) working with swine found genotypic variation in ability to utilize dietary protein.

(b) Variation in Genotypic Response to Environmental Fluctuation.

It has been demonstrated that genotypic response to environmental fluctuation may vary. Housing by genotype interactions have been reported by breeds, with sheep (King and Young, 1955), by strains, with poultry (Gowe, 1956), and by sire, with swine (Connelly, 1959). Cipolloni et al. (1951) have reported a species by feed interaction for sheep and cattle, and Lucas and Calder (1956) found differing breed response to different planes of feeding in swine. Although it is not reported as such, differential genetic gain response in mice to dietary protein is evident in a report by Fenton and Carr (1951). Bowland and Berg (1959) worked with swine and reported ration by strain interactions for average daily gain and feed consumption during the growing period to 110 pounds and for feed conversion during the finishing period to 200 pounds. Carcass measurements and scores in the above report did not show ration x strain interactions. In another report by Bowland and Berg (1958) with swine no strain differences in relative response to frequency of feeding were found. Although studies of differential genetic response to environment have not been frequent, a survey of literature presently available would suggest that genotype by environment interactions are not as prevalent as gross differences in requirement and productive traits between genotypes.

V. Thyroid Activity.

(a) Genetic Control of Thyroid Activity.

It has been demonstrated that thyroid activity is a heritable character (Shaklee and Shaffner, 1952; Shaklee and Knox, 1956; Chai, 1958a). Chai studied thyroid I^{131} decay in mice and arrived at a heritability estimate of 14 per cent while Shaklee and Knox measured thyroid weight in poultry and arrived at a heritability of 92 per cent.

(b) Genetic Differences in Thyroid Activity.

Species differences in thyroid activity exist on a unit body weight basis according to Pipes and Turner (1956) and Stevens and D'Angelo (1954). Of considerably greater academic and practical interest, however, are comparisons of animals having more similar genetic constitutions. Strain and breed differences for various measures of thyroid activity have been found in mice (Lyon, 1956; Amin *et al.*, 1957; Chai, 1958b), poultry (Mixner *et al.*, 1944; Mixner and Upp, 1947; Glazener *et al.*, 1949; Schultze and Turner, 1945), cattle (Johnson and Ragsdale, 1959), and sheep (Henneman *et al.*, 1955). Amin *et al.* (1944) reported thyroid activity of F_1 intermediate to the parental strains. In a report by Mixner and Upp (1947) thyroid activity was associated with F_1 heterosis for weight gain.

Premechandra *et al.* (1959b) were able to differentiate between mono- and dizygous cattle twins on the basis of thyroid function.

(c) Relationship of Thyroid Activity to Gain and Body Weight.

High relationships between thyroid activity and gains have been reported (Shaklee and Knox, 1956; Singh et al., 1956; Draper and Firth, 1957). It is not clear in Draper's report whether the comparison is based on a unit body weight basis.

It is possible to alter rate of gain by experimentally inducing hypo- or hyperthyroidism. Feeding thiouracil to lambs and ewes (Vander Noot et al., 1950b) reduced gains when initial body weight was less than 146 pounds and increased gains when initial body weight was greater than 146 pounds. Terrill et al. (1950) reported a similar effect from thiouracil in fattening swine. Thiouracil has also been effective in reducing gains in growing chickens (Mellen and Hill, 1953). Perry et al. (1950) found thyroprotein effective in fattening but not growing swine. It would seem reasonable that mild hyperthyroidism in early stages of growth would increase gains.

Positive feed conversion responses to thiouracil were reported by Vander Noot et al. (1950a and b). Contrastingly, the work by Perry et al. (1950) and Glazener et al. (1949) indicated increased efficiency of feed conversion from thyroprotein administration.

It has been reported that thyroxine secretion rate on a unit body weight basis is positively related to differential growth between poultry strains (Mixner and Upp, 1947;

Glazener et al., 1949; Premechandra et al., 1959a). Premechandra et al. (1959a) indicated that the difference in thyroid activity between 2 New Hampshire strains was more pronounced than the difference in body weight. Leghorn birds were reported by Schultze and Turner (1945) to have a higher thyroxine secretion rate than heavier strains.

(d) Influence of Sex on Thyroid Activity.

Biellier and Turner (1957) tabulated a review in which practically all workers noted greater thyroid activity in female compared to male chickens. Three papers dealing with sex influence in mice report conflicting results. Hurst and Turner (1947) found no sex differences to 5 weeks of age but at muturity male thyroid activity exceeded female activity. On the other hand, Monroe and Turner (1946) found that young female mice secreted more thyroxine than males but that this situation reversed as the animals grew older. No attempt was made in either of the preceding reports to relate hormone production to unit body weight. Amin et al. (1957) related hormone production to unit body weight and found male secretion exceeded female secretion in mice approximately 3 months of age.

During the period of estrus in females, thyroid activity increases (Soliman and Reineke, 1954; Soliman and Badawi, 1956). This observation is in agreement with Mellen and Hill (1953) who demonstrated added estrogens increased basal metabolic rate while reducing goitrogenic effects of

thiouracil. Therefore, thyroid activity would be more variable in sexually mature females than males.

(e) Influence of Age on Thyroid Activity.

The effect of increasing age is to decrease thyroid activity relative to unit body weight. This has been demonstrated in mice (Hurst and Turner, 1947), rats (Monroe and Turner, 1946; Cottle and Carlson, 1956), poultry (Schultze and Turner, 1945), and cattle (Johnson and Ragsdale, 1959).

(f) Influence of Thyroid on Nitrogen Metabolism.

Sternheimer (1939) found that added thyroxine resulted in increased liver protein and assumed incorrectly that there was a similar effect of thyroxine on total body protein. Earlier, however, Addis _et al._ (1938) had shown no corresponding trends in liver and total body protein from exogenous thyroxine. More conclusive studies found increased endogenous nitrogen excretion from thyroxine administration (Devel, 1928; Makherjee and Mitchell, 1951) and, reciprocally, increased nitrogen retention from thiouracil administration (Terrill _et al._, 1950). Such effects, however, are the results of experimentally induced hypo- or hyperthyroidism and differences in thyroid activity between genotypes will not necessarily be related to nitrogen metabolism differences in the manner reported above.

EXPERIMENTAL

I. Introduction.

The original intent of this study was to ascertain whether performance differed between the Wistar(1) and Sprague-Dawley(2) lines of rats and their crossbreds and to determine to what extent differences, if evident, were reflected in protein and energy metabolism. In addition, it was hoped to gain information on the effects of severe inbreeding in lines presumably reasonably homozygous at the outset. Alterations were made in the original plan, such that the scope of the experiment has been broadened to include thyroid studies, but narrowed relative to energy metabolism and inbreeding effects.

II. Description of Breeding Stock Animals.

A. The Original Breeding Stock.

One male and five females, and four males and six females, from the Sprague and Wistar lines respectively, constituted the breeding material for these experiments. Each line had 4 successive generations of brother-sister matings in their immediate past. At the time the Wistar rats were transferred to this colony their temperament was excitable by comparison with the Sprague. However, throughout the entire period of experimentation the reverse situation

(1) Acquired from the Department of Biochemistry, University of Alberta.

(2) Stock colony rats in the Department of Animal Science and hereafter referred to as Sprague.

existed, although in general both lines were docile and easy to handle. Phenotypically, other than temperament, the only obvious distinguishing feature between the two lines was head shape, the Sprague head being longer and narrower.

B. Maintenance of Breeding Stock.

Throughout the experimental period, random breeding within the 2 inbred lines was the practice followed. Fertility problems, which are discussed later, demanded an indefinite rather than the standard 21 day mating period.

During mating periods females were checked every 2 days for signs of pregnancy and were transferred to littering tins 3 to 4 days before parturition. These tins were then checked periodically throughout the day until late evening for litters. Newborn were often placed in an incubation room at 100°F. for 1 or 2 hours. At birth litters were killed down to 7 or 8 and weaning occurred at 21 days of age at which time each rat was individually identified and weighed. Selection of test rats occurred at weaning.

All animals when not being fed an experimental diet were supplied with large type fox chow checkers(1) and were housed in grouped maintenance cages 24" by 16" by 13" high. The routine department practice of adding alfalfa hay and chopped liver to the breeding cages was followed.

(1) Ralston Purina Company of Canada Limited, Woodstock, Ontario, Canada.

C. Health and Reproductive Problems.

Three distinct phenomena which impaired the fitness of the stock rats arose during the period of experimentation. They were: (a) reproduction problems; (b) diarrhea; and (c) a bronchial disease.

(a) Reproductive Problems.

The reproductive problem consisted of infertility, abortion or absorption of fetuses, and still births. No estimate of the interrelationship of these phenomena could be made other than attributing them to inbreeding. The problem became manifest in the 4th generation of brother x sister matings when only 3 out of 8 potential litters were obtained. At the same time matings in the main Sprague colony were successful. After one month of mating only 4 of the 11 available females had littered giving 2 Sprague litters of 2 and 5 live young, and 2 Wistar litters of 6 and 7 live young.

One aborting Sprague female and one Wistar female with a single aborted fetus were examined(1) in an attempt to ascertain the internal conditions leading to abortion and still births. However, gross, bacteriological, and histological examinations did not reveal the cause of the problem.

In addition to the routine practice of adding alfalfa hay and chopped liver to the breeding cages, whole grain was added to the breeding diet and a vitamin supplement, listed in Table 1, given orally to breeding and pregnant females.

(1) Examinations were conducted by the Alberta Department of Agriculture, Veterinary Services Branch, Edmonton, Alberta.

Table 1. Vitamin Supplement(1) for Breeding Rats.

	PER GM.	PER DROP (APPROX.)
Vitamin A (I.U.)	25,000	750
Vitamin D (I.U.)	10,000	300
Vitamin E (Mgm.)	166	5

(1) Two drops per rat given bi-weekly.

The practice of vitamin supplementation to pregnant females was later discontinued due to struggling of animals at time of treatment.

Although abortions and still births decreased throughout the period of experimentation, the incidence of sterility remained fairly high. However, during the course of one year a sufficient population had been built up by frequent matings and repetitive breeding of females. When final matings for the thyroid study were made the composite reproduction problem had eased somewhat.

(b) Diarrhea.

Most females resulting from the initial matings developed an anal discharge which was, apparently, severe diarrhea. Coccidiosis was suspected and sulfaquinoxiline(1) was added to the drinking water but this treatment proved ineffective. Rats were generally weak but no deaths could be

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(1) Merck and Co. Limited, P.O. Box 899, Montreal 3, Montreal, Canada. One litre of drinking water contained 5.9 grams sulfaquinoxaline and 1.5 grams sodium hydroxide.

attributed to this condition nor was it obvious that reproduction was affected. The condition cleared up naturally in the next generation.

(c) Bronchial Disease.

Throughout the 2 year period of these experiments a bronchial disease was prevalent in the inbred rats. Symptoms consisted of snuffling in early stages and heavy breathing, often with a mucous nasal discharge, clawing at the nose, extreme lethargy, and finally death. Examination(1) of 26 specimens in various stages of infection indicated severe lung congestion. Pasteurella multocida was isolated which is a common organism responsible for rat pneumonia. The condition is contagious and whole litters were often noted to acquire the disease. Precautions of disinfection(2) of cages and isolation of animals were adhered to. During the 2 year period very few cases of severe pneumonia were noted in the main department colony rats housed in the same room.

III. Statistical Analysis.

An analysis of variance program for use with LGP-30 electronic computers was prepared by Dale Bent(3) and used

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(1) Examinations were conducted by the Alberta Department of Agriculture, Veterinary Services Branch, Edmonton, Alberta.

(2) R2L Bactericide: G. H. Wood and Co., Queen Elizabeth Way, Box 34, Toronto 14, Canada.

(3) Interprovincial Pipe Line Company, Edmonton, Alberta, Canada.

extensively in the analysis of both metabolism and thyroid data collected in this study. The method of analysis is that described by Goulden (1956) for multiple classification of data with proportionate sub-class numbers. The program will handle a 10 factor classification of data and up to 10 first-order interactions. Results are significant to 7 figures. A complete analysis of variance table with corresponding population, main factor, and sub-plot means and variances is printed out.

IV. Metabolism Studies.

A. Materials and Methods.

The handling of animals during acclimatization and trial periods and the collection and analysis of urine and feces for nitrogen content was similar to that described by Sibbald (1957). No energy determinations were carried out. Minor alterations in method were made in that no glass wool plugs were used in urine funnels and feces were ground through a 40 rather than a 20 mesh screen. The reason for removing the glass wool was based on the observation that urine appearing in the Erlenmeyer flasks was delayed, in some cases, up to one day, which could result in evaporation losses.

Purified diets containing 20 per cent alphacel(1) based on formulae by Sibbald (1957) were used. One minor alteration to Sibbald's vitamin mix was made in that d-l-vitamin E succinate replaced α-tocopherol acetate.

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(1) Nutritional Biochemicals Corporation, Cleveland, Ohio, U.S.A.

The nitrogen source and salt mix were prepared once, the vitamin mix at 6 month intervals, and the completed diet every 2 weeks. Storage of all components was at a temperature of 0°C. Care was taken to bring food bottles to room temperature before weighing.

B. Preliminary Metabolism Experiment.

(a) Object

The purpose of the first series of trials was to determine whether gains, food utilization, and nitrogen metabolism differed between the 4 genotypic groups(1) studied when fed the same diet. If differences did occur an estimate of the numbers of experimental animals required for sound statistical design of further experiments could be made.

(b) Procedure

Throughout the preliminary trials litters were killed back to 8 at birth. Due to breeding problems, only small animal numbers could be obtained in any reasonable time range. Grouped as closely together in time as possible, 3 preliminary metabolism trials were run and analyzed separately:

Trial 1: "Pure line comparison." Four rats, sex balanced, from each of 2 Wistar and 1 Sprague litters, replicated once.

Trial 2: "Reciprocal crossbred comparison." Four rats, sex balanced, from 1 litter representing each reciprocal crossbred group, replicated once.

Trial 3: "Comparison of all breeding groups." Four rats from 1 litter, sex balanced, from each of the 4 breeding groups, not replicated.

(1) For purposes of this thesis each of the inbred lines and their reciprocal crosses will be said to constitute a genotypic group.

Acclimatization periods for trials 1 and 2 and all metabolism periods were 1 week in duration. During trials 1 and 2 all rats were placed on the experimental diet listed in Table 2 for acclimatization when the average weight of rats in each litter reached 50 grams. For trial 3, animals were fed the experimental diet from weaning and were put on metabolism test individually as they reached a weight of 65 grams. Nearly one-half of the rats placed on the experimental diet at weaning died within 2 or 3 days. Examination of the intestinal tract revealed that blockage was not occurring and that failure to eat was probably the cause of death. Breaking up the fox chow checkers and adding them to the experimental diet circumvented the problem.

Table 2. Diet Fed in the Preliminary Metabolism Experiment.

	PER CENT
Nitrogen Source "A"(1)	15.2
Sucrose	54.8
Alphacel	20.0
Mazola oil	5.0
Salts(1)	4.0
Vitamin mix(1)	1.0

(1) Sibbald (1957).

(c) Results

Summarized results for the 3 preliminary trials are presented in Table 3. Corresponding mean squares and tests of significance are recorded in Table 4. Because of numbers involved few significant differences resulted although some trends may be noted.

Table 3. Summary of Gain, Food, and Metabolism Data from the Preliminary Metabolism Experiment.

			PER 100 GM. ABW				APPARENT DIGESTIBLE		RETENTION		
	NO. OF LITTERS	NO. OF RATS	7-DAY TRIAL GAIN	FOOD CONS. (O.D.BASIS)	GROSS N CONS.	FOOD CONS.PER GM.GAIN	FOOD	N	TOTAL N(1)	GROSS N	ADN
			gm.	gm.	gm.	gm.	%	%	gm.	%	%
Trial 1											
Genotype											
W	4	16	26.7	84.0	1.69	3.3	76	89	1.00	59	65
S	2	8	30.6	74.9	1.49	2.7	76	91	0.93	62	68
Sex											
M	-	12	28.6	78.5	1.58	3.1	76	90	0.99	62	69
F	-	12	27.4	83.4	1.67	3.2	77	90	0.96	57	63
Replicate											
1	3	12	27.9	75.0	1.47	3.0	75	89	0.85	58	65
2	3	12	28.0	87.0	1.78	3.2	77	91	1.10	62	68
Trial 2											
Genotype(2)											
W x S	2	7	26.8	87.5	1.77	3.4	77	89	1.04	59	67
S x W	2	8	33.6	92.4	1.87	2.9	76	89	1.07	57	64
Sex											
M	-	8	28.4	89.6	1.81	3.3	77	89	1.07	59	66
F	-	7	32.7	90.7	1.84	2.9	76	88	1.05	57	64
Replicate											
1	2	7	31.2	86.5	1.75	2.9	78	90	1.07	61	68
2	2	8	29.7	93.2	1.89	3.3	75	87	1.05	55	63

(Continued)

Table 3 (Continued). Summary of Gain, Food, and Metabolism Data from the Preliminary Metabolism Experiment.

			PER 100 GM. ABW				APPARENT DIGESTIBLE		RETENTION		
	NO. OF LITTERS	NO. OF RATS	7-DAY TRIAL GAIN	FOOD CONS. (O.D.BASIS)	GROSS N CONS.	FOOD CONS.PER GM.GAIN	FOOD	N	TOTAL N(1)	GROSS N	ADN
			gm.	gm.	gm.	gm.	%	%	gm.	%	%
Trial 3											
Genotype											
W	1	4	42.2	105.9	2.16	2.5	78	90	1.33	62	68
S	1	4	29.0	90.7	1.86	4.0	74	89	0.99	53	59
W x S	1	4	40.2	98.7	2.03	2.7	77	91	1.23	60	67
S x W	1	4	42.3	98.3	2.01	2.4	77	89	1.22	60	68
Sex											
M	-	8	38.7	95.9	1.96	2.7	76	90	1.20	60	67
F	-	8	38.1	100.9	2.06	3.1	77	89	1.18	57	64

(1) Per 100 gm. ABW.
(2) The last appearing letter represents the dam line.

Table 4. Mean Squares and Tests of Significance for Data Summarized in Table 3.

		PER 100 GM. ABW				APPARENT DIGESTIBLE		RETENTION		
SOURCE OF VARIATION	Df	7-DAY TRIAL GAIN	FOOD CONS. (O.D.BASIS)	GROSS N CONS.	FOOD CONS. PER GM.GAIN	FOOD	N	TOTAL N(1)	GROSS N	ADN
Trial 1										
Total	23	64.9	139.8	0.070	0.900	4.11	2.00	0.048	33.3	36.2
Main Effects										
Genotype	1	83.5	438.0*	0.205*	1.908	0.27	15.45**	0.023	53.0	25.8
Sex	1	7.7	141.7	0.055	0.045	7.00	2.89	0.005	127.4*	189.3*
Replicate	1	0.1	860.5**	0.611**	0.160	21.91*	9.97**	0.382**	90.9	68.7
Interactions										
G x S	1	12.4	151.3	0.055	0.197	2.33	--(2)	0.029	69.0	76.5
G x R	1	301.5*	3.3	0.004	4.314*	5.58	0.41	0.020	34.3	46.0
S x R	1	10.1	9.2	0.005	0.912	0.09	0.23	0.000	2.3	1.7
Error	17	63.4	94.8	0.040	0.764	3.37	1.11	0.038	22.9	24.8
Trial 2										
Total	14	47.3	63.3	0.026	0.566	6.13	5.25	0.024	47.3	43.4
Main Effects										
Genotype	1	173.7*	90.2	0.037	1.018	6.24	0.03	0.003	13.8	20.1
Sex	1	69.1	4.9	0.002	0.572	3.65	0.54	0.001	14.8	16.5
Replicate	1	8.5	168.1	0.068	0.869	26.38*	25.37	0.002	135.1	90.3
Interactions										
G x S	1	124.0	215.4*	0.088*	1.096	3.07	--(2)	0.090	50.7	69.7
G x R	1	59.7	154.7	0.063	0.004	6.42	0.70	0.052	32.2	47.4
S x R	1	4.5	--(2)	--(2)	0.109	3.07	6.27	0.019	65.5	55.7
Error	8	27.8	31.9	0.013	0.532	4.62	5.07	0.022	43.7	38.4

(Continued)

Table 4 (Continued). Mean Squares and Tests of Significance for Data Summarized in Table 3.

		PER 100 GM. ABW				APPARENT DIGESTIBLE		RETENTION		
SOURCE OF VARIATION	Df	7-DAY TRIAL GAIN	FOOD CONS. (O.D.BASIS)	GROSS N CONS.	FOOD CONS. PER GM.GAIN	FOOD	N	TOTAL N(1)	GROSS N	ADN
Trial 3										
Total	15	130.2	127.5	0.053	2.106	12.24	2.22	0.066	81.0	91.5
Main Effects										
Genotype	3	161.4	154.3	0.061	2.358*	11.56	4.33	0.086	70.4	79.2
Sex	1	1.6	103.0	0.041	0.744	7.58	1.00	0.001	30.8	28.4
Interaction										
G x S	3	191.3	117.7	0.050	6.775*	--(2)	0.89	0.037	76.2	91.0
Error	8	111.7	124.2	0.052	0.429	17.72	2.08	0.077	93.1	104.3

(1) Per 100 gm. ABW.
(2) Negative sums of squares.
* Significant at the 5 per cent level.
** Significant at the 1 per cent level.

The data from trial 1 suggested superiority of the Sprague line over the Wistar line for gains, efficiency of food conversion, per cent ADN, and efficiency of nitrogen utilization as measured by per cent of gross nitrogen and ADN retained. The Wistar consumed more food than the Sprague. In trial 2 surprisingly large differences between the reciprocal crossbreds were evident where the S x W(1) group exceeded the W x S for gain, consumption of food and nitrogen, and had a lower food conversion ratio. Trial 3 is the least sound trial statistically since only 1 litter from each breeding group was represented. An exceptionally poor Sprague litter was represented on this trial which it is expected was in part the result of a health problem. However, in accordance with trial 2, the S x W group had greater gain on trial and a smaller food conversion ratio. With the exception of a 2 per cent difference in per cent ADN in favour of the W x S no other reciprocal crossbred differences were apparent for any of the other measures made in trial 3.

Trials 1 and 3 indicated that gains were slightly in favour of males although a reverse situation occurred in trial 2. In all 3 trials female food consumption was greatest which resulted in a smaller food conversion ratio for males. Males were slightly superior to females for total nitrogen retention per unit body weight as well as efficiency of protein utilization in all 3 trials.

(1) The last appearing letter represents the dam line.

C. Main Metabolism Experiment.

(a) Object

Observations in the Preliminary Experiment indicated that genotypic differences existed for gains, food conversion, and nitrogen metabolism. This experiment was designed to study these phenomena further and to uncover any possible interactions of genotype with dietary protein levels. An attempt was also made to determine the causes of reciprocal crossbred differences.

(b) Methods and Procedure

Methods and procedures as modified for trial 3 in the Preliminary Experiment were followed. A further alteration was the implementation of a 5-day metabolism period. Seven-day as opposed to 5-day metabolism periods offer no advantage with swine (Lassiter et al., 1956) and probably the same situation exists with rats. St. Lawrence Oil(1) was substituted for Mazola Oil in the preparation of diets. Litters were weighed at birth and subsequently killed back to 7 at 1 week of age. Milk supply should not have been limiting at this early stage.

Test diets consisted of 10 per cent, 20 per cent, and 30 per cent protein varied at the expense of sucrose, (Table 5). Sibbald (1957) used this means of varying dietary protein and found the influence on ADE to be small. Four replicates composed of one male and 1 female from 1 litter of each breeding group were allotted to each of the 3 experimental diets.

(1) St. Lawrence Starch Company Limited, Port Credit, Ontario, Canada.

Table 5. Diets Fed in the Main Metabolism Experiment.

	LP(1)	MP(1)	HP(1)
	%	%	%
Nitrogen source "A"(2)	10.2	20.2	30.2
Sucrose	59.8	49.8	39.8
Alphacel	20.0	20.0	20.0
St. Lawrence Oil	5.0	5.0	5.0
Salts(2)	4.0	4.0	4.0
Vitamin mix(2)	1.0	1.0	1.0
Nitrogen analysis (O.D. Basis)	1.36	2.69	4.01
Moisture analysis	2.02	2.53	3.11
Approx. gross energy (Cal./gm.)	4.33	4.33	4.33

(1) Refers to Low (LP), Medium (MP), and High (HP) Protein.

(2) Sibbald (1957).

The experimental design called for 96 rats but 2 Sprague on LP and 1 S x W on MP lost weight during the trial and were not used in the analysis. Two Wistar litters weaned only 5 young, the deficiency being taken up by the MP group. In total, 91 rats were used. Sex balance was only partial due to the difficulty of acquiring sexually balanced litters at weaning.

During the collection of data, food consumption for the Sprague male, Replicate 2, MP, and feces weight for

the W x S female, Replicate 3, MP, were lost through error. Missing values were calculated by the method of Goulden (1956) for missing data.

Six cases of mild diarrhea were noted during the test period, and were not confined to any dietary group. Four cases occurred in the Sprague and 2 in the Wistar line. In only 1 case, where the animal acquired pneumonia and died, was diarrhea related to poor gain.

Due to unavoidable sex imbalance, mean squares for genotype, diet, and replicate interaction with sex were often found to be negative. Therefore, variance due to sex interactions was not removed in the process of partitioning sources of variation.

(c) Results

Data from the Main Metabolism Experiment are summarized in Tables 6 and 8 and in Appendix Tables 1 (A,B,C,D) and 2 (A,B,C,D). Mean squares and tests of significance are given in Tables 7 and 9. To facilitate interpretation, graphs representing genotype by diet interactions are presented in Figures 1 to 9, inclusive.

(1) Replicate Variation

For all cases measured replicate effects were significant at either the 5 per cent or 1 per cent level. It was not possible to determine the exact causes of replication variation.

Table 6. Summary of Gain and Food Data from the Main Metabolism Experiment.

			PER 100 GM. ABW			
	NO. OF RATS	ABW ON TRIAL	5-DAY TRIAL GAIN	FOOD CONS. (O.D. BASIS)	GROSS N CONS.	FOOD CONS. PER GM. GAIN
		gm.	gm.	gm.	gm.	gm.
Replicate			**	**	**	**
1	23	77.1	32.4	72.1	1.95	2.3
2	23	77.7	29.2	70.0	1.94	2.6
3	22	77.1	28.2	69.1	1.89	2.9
4	23	80.0	33.6	77.2	2.06	2.4
Genotype			**	**	NS	**
W	22	75.0	25.4	70.3	1.93	3.1
S	22	77.6	30.0	68.6	1.93	2.6
W x S	24	79.9	33.0	73.3	1.97	2.3
S x W	23	79.3	34.8	76.2	2.01	2.3
Diet			**	NS	**	**
LP	30	75.2	25.2	71.6	0.98	3.2
MP	29	79.0	32.7	72.9	1.96	2.4
HP	32	79.7	34.6	72.0	2.88	2.1
Sex			**	**	**	NS
M	41	79.4	32.6	74.2	2.03	2.5
F	50	76.9	29.4	70.5	1.90	2.6
R x G			*	**	**	*
R x D			NS	NS	**	NS
G x D			NS	NS	*	NS

NS Not significant.
* Significant at the 5 per cent level.
** Significant at the 1 per cent level.

Table 7. Mean Squares and Tests of Significance for Data Summarized in Table 6.

	"F" VALUE FOR SIGNIFICANCE			PER 100 GM. ABW			
SOURCE OF VARIATION	5%	1%	Df	5-DAY TRIAL GAIN	FOOD CONS. (O.D. BASIS)	GROSS N CONS.	FOOD CONS. PER GM. GAIN
Total			90	62.1	60.0	0.660	0.894
Main Effects							
Replicate	2.76	4.13	3	150.9**	295.8**	0.121**	2.008**
Genotype	2.76	4.13	3	378.4**	255.9**	0.034	3.476**
Diet	3.15	4.98	2	743.3**	14.0	27.836**	9.094**
Sex	4.00	7.08	1	233.3**	305.1**	0.393**	0.663
Interactions							
R x G	2.04	2.72	9	57.6*	95.9**	0.096**	1.085*
R x D	2.25	3.12	6	48.4	67.5	0.078**	0.748
G x D	2.25	3.12	6	14.9	41.8	0.061*	0.730
Error			60	23.0	31.6	0.020	0.442

* Significant at the 5 per cent level.
** Significant at the 1 per cent level.

Table 8. Summary of Apparent Digestibility and Nitrogen Retention from the Main Metabolism Experiment.

	NO. OF RATS	ABW ON TRIAL	APPARENT DIGESTIBLE		RETENTION		
			FOOD	N	TOTAL N(1)	GROSS N	ADN
		gm.	%	%	gm.	%	%
Replicate			*	**	**	**	**
1	23	77.1	77.4	91.1	0.98	57.0	63.1
2	23	77.7	76.7	88.1	1.12	61.0	69.7
3	22	77.1	77.7	89.6	0.93	54.4	61.0
4	23	80.0	78.6	92.1	1.08	58.5	63.8
Genotype			NS	**	**	**	**
W	22	75.0	77.2	89.2	0.91	53.2	60.1
S	22	77.6	77.8	89.4	1.05	57.5	64.6
W x S	24	79.9	77.3	90.5	1.05	59.4	66.1
S x W	23	79.3	78.0	91.6	1.10	60.7	66.7
Diet			NS	**	**	**	**
LP	30	75.2	78.4	87.4	0.75	76.0	86.9
MP	29	79.0	77.2	90.5	1.08	55.0	60.8
HP	32	79.7	77.2	92.5	1.24	43.1	46.7
Sex			NS	NS	**	NS	NS
M	41	79.4	77.5	90.6	1.09	58.6	64.9
F	50	76.9	77.6	89.9	0.98	57.1	64.0
R x G			NS	*	**	**	**
R x D			NS	NS	**	**	**
G x D			NS	NS	NS	*	**

(1) Per 100 gm. ABW.
NS Not significant.
* Significant at the 5 per cent level.
** Significant at the 1 per cent level.

Table 9. Mean Squares and Tests of Significance for Data Summarized in Table 8.

SOURCE OF VARIATION	"F" VALUE FOR SIGNIFICANCE 5%	1%	Df	APPARENT DIGESTIBLE FOOD	N	RETENTION TOTAL N(1)	GROSS N	ADN
Total			90	5.50	13.03	0.082	241.3	342.9
Main Effects								
Replicate	2.76	4.13	3	16.06*	71.27**	0.170**	170.9**	318.3**
Genotype	2.76	4.13	3	4.10	28.56**	0.152**	245.3**	201.9**
Diet	3.15	4.98	2	14.44	201.50**	1.895**	8,553.7**	12,812.8**
Sex	4.00	7.08	1	1.13	14.81	0.279**	49.0	17.4
Interactions								
R x G	2.04	2.72	9	1.64	9.83*	0.076**	98.1**	124.5**
R x D	2.25	3.12	6	6.90	7.40	0.092**	144.3**	148.1**
G x D	2.25	3.12	6	5.95	7.21	0.015	54.3*	227.6**
Error			60	5.21	4.66	0.016	20.6	20.3

(1) Per 100 gm. ABW.
* Significant at the 5 per cent level.
** Significant at the 1 per cent level.

GENOTYPE X DIET INTERACTIONS FROM THE MAIN METABOLISM EXPERIMENT

Figure 1 - Gain (per 100 gm. ABW)

Legend

W x S ·- - - - -·
S x W ×- - - - -×
S ×——×
W ●——●

Figure 2 - Food Cons. (per 100 gm. ABW)

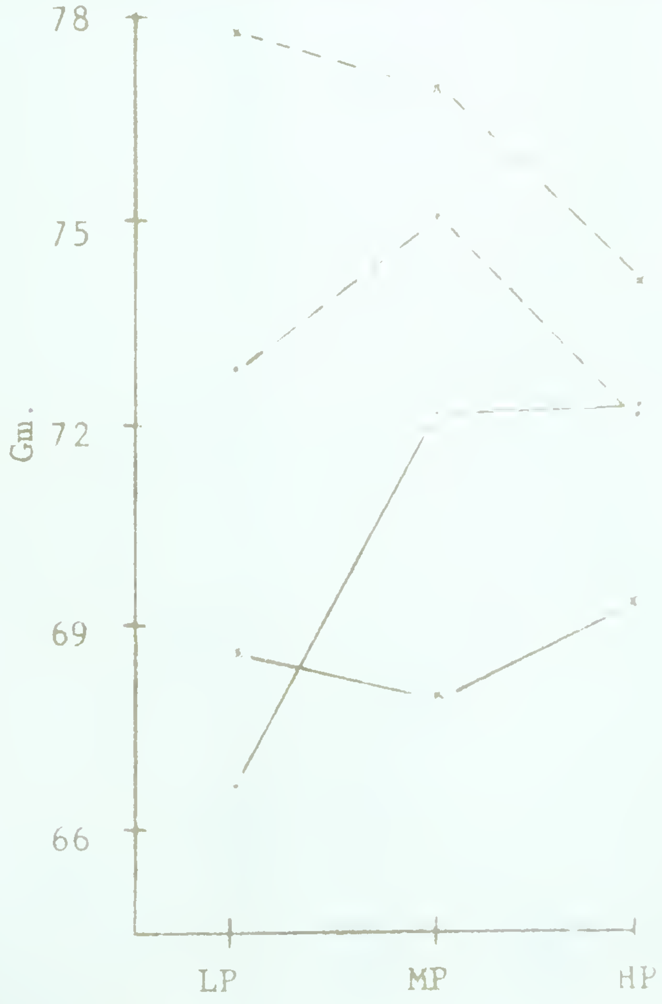
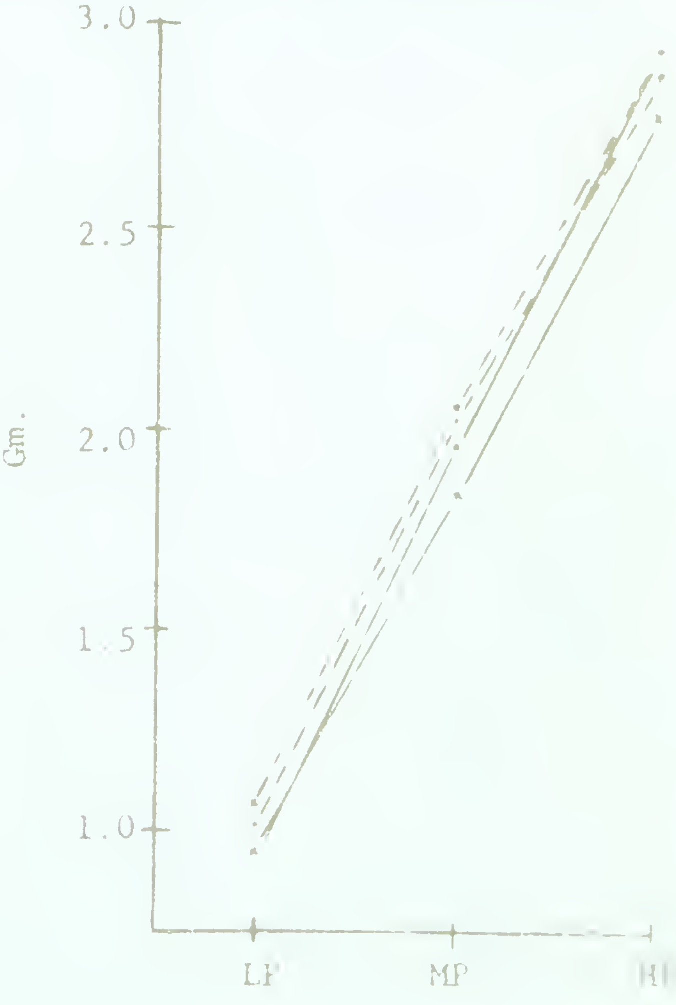


Figure 3 - N Cons. (per 100 gm. ABW)



GENOTYPE X DIET INTERACTIONS FROM THE MAIN METABOLISM EXPERIMENT

Figure 4 - Gm. Food per Gm. Gain

Legend

W x S •- - - - -•
S x W ×- - - - -×
S ×———×
W ●———●

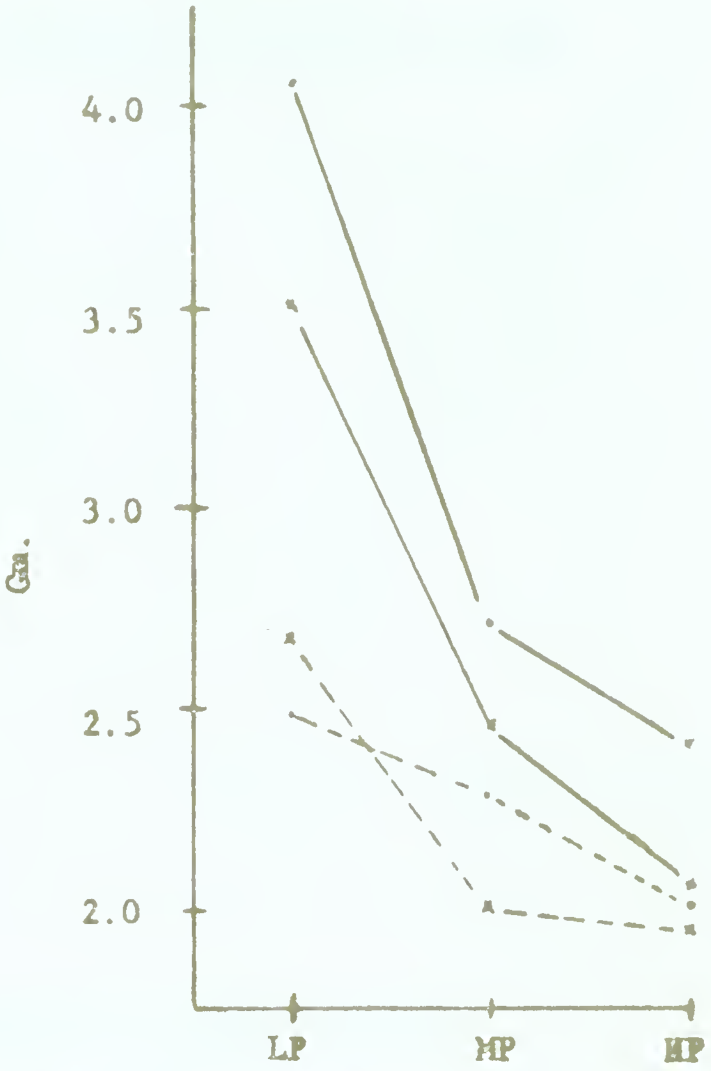


Figure 5 - % ADN

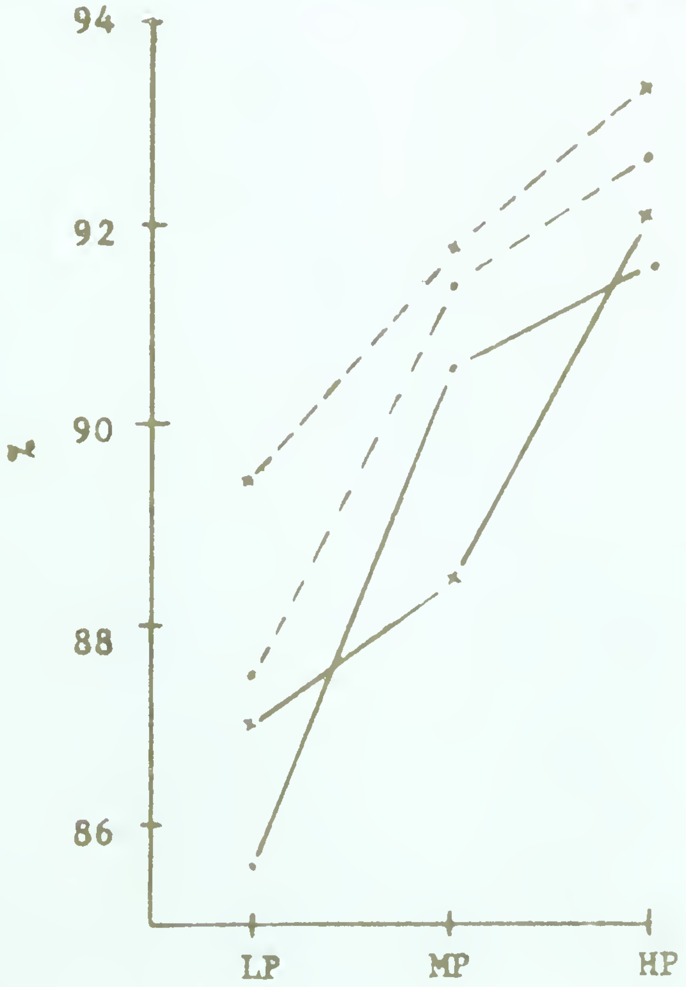
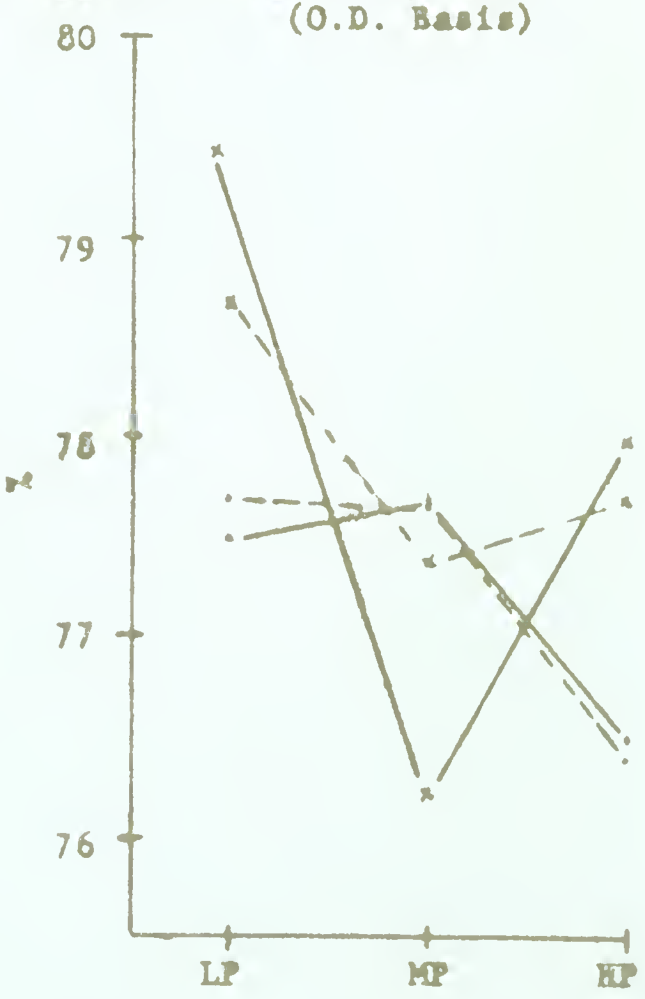


Figure 6 - % Apparent Digestible Food (O.D. Basis)



GENOTYPE X DIET INTERACTIONS FROM THE MAIN METABOLISM EXPERIMENT

Figure 7 - Mg. N Retained (Per 100 gm. ABW)

Legend

W x S • - - - - •
S x W x - - - - x
S x —— x
W ● —— ●

Figure 9 - % ADN Retained

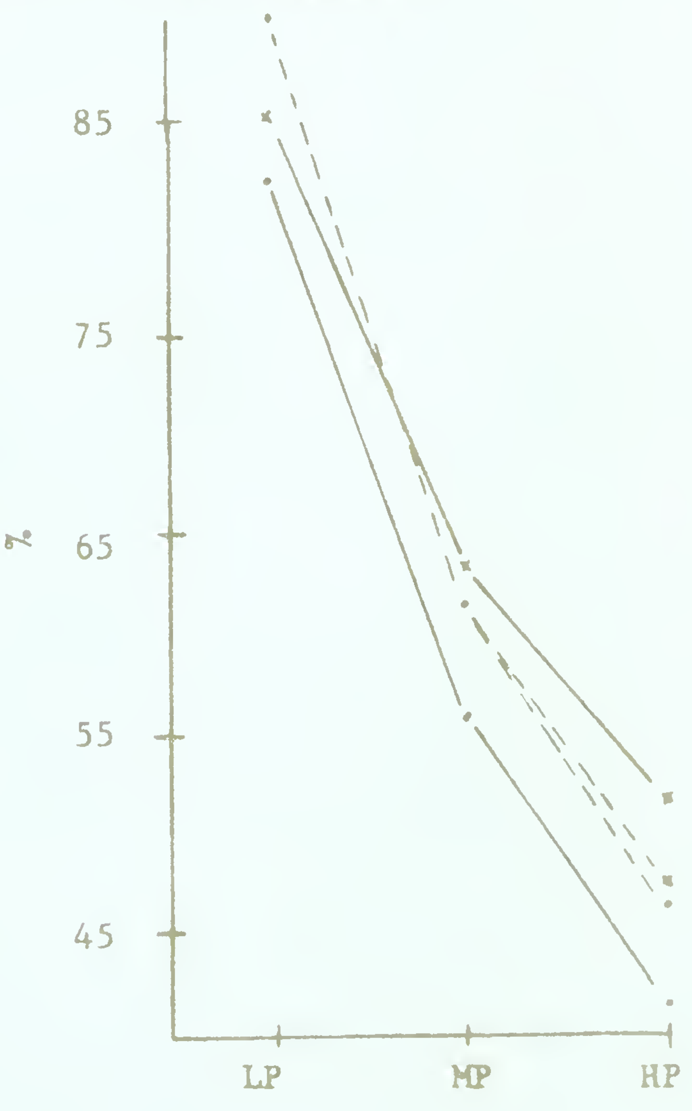
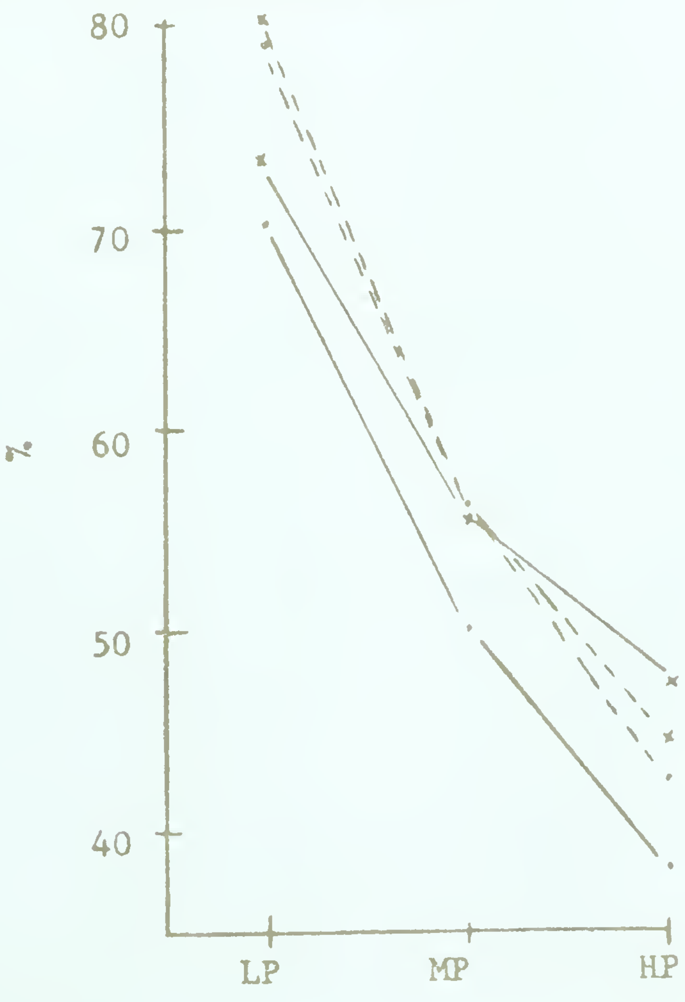


Figure 8 - % Gross N Retained



(2) Influence of Dietary Protein Level on Gains, Food Consumption, Apparent Digestibility, and Nitrogen Retention.

Consistent with increasing levels of dietary protein were increases in rates of gain, particularly between 10 and 20 per cent protein. These gains were not related to food consumption, which did not show dietary variation, but were in response to greater intakes of nitrogen at higher levels of dietary protein. As expected, food conversion was positively related to dietary protein level.

Apparent food digestibility was not influenced by the protein level of the diet. However, an increase in per cent ADN was observed with increasing dietary protein levels. This finding is in accordance with expectation and probably reflects the effect of a relatively constant metabolic fecal nitrogen (Crampton and Rutherford, 1954).

Efficiency of nitrogen utilization, measured by retention as a percentage of either gross nitrogen or ADN, was inversely related to dietary protein levels. At the same time, total nitrogen retention increased with corresponding increases in dietary protein and was related to nitrogen intake and reflected in gains. Other workers at this institution have noted a similar influence on efficiency of protein utilization in rats (Sibbald, 1957; Likuski, 1959).

(3) Influence of Sex on Gains, Food Consumption, Apparent Digestibility, and Nitrogen Retention.

There was a small but highly significant difference in trial gains between sexes in favour of males. This was true at all protein levels, although the LP diet seemed to have a greater depressing effect on female gains. Food and, therefore, nitrogen consumption were significantly greater for males and were apparently related to superior male gains. Food conversion was slightly in favour of males but the mean square was not significant.

Mean differences between sexes for apparent digestibility of food or nitrogen did not exist. Total nitrogen retention was significantly greater in the males which was expected on the basis of consumption differences. Both sexes utilized gross or apparently absorbed nitrogen equally well.

(4) Influence of Genotype on Gains, Food Consumption, Apparent Digestibility, and Nitrogen Retention.

There were statistically significant differences in gains, food consumption, and food conversion between genotypic groups in this experiment. Rates of gain were greater for the Sprague than the Wistar line. Sprague rats consumed smaller quantities of food which, when combined with greater gain, resulted in less food required per unit of gain by Sprague in comparison with Wistar. Small reciprocal crossbred differences were evident for gains and when crossbreds were

compared to inbreds crossbred superiority was evident. Crossbreds were also more efficient in food conversion. No statistically significant genotype x diet interaction existed for gain, food consumption, or food conversion. Figure 1 demonstrates this finding for gain but Figures 2 and 4 indicate a change in the relative position of inbreds on the LP diet for food consumption and food conversion. No statistically significant differences between genotypes for nitrogen consumption existed but the mean square for genotype x diet was significant at the 5 per cent level. Figure 3 illustrates this interaction in that Wistar rats consumed less nitrogen than Sprague rats on the LP diet but on MP and HP diets the relative position reversed. In fact, Wistar intake on the HP diet increased to the extent that means for crossbred and Wistar were approximately equal.

Significant differences in favour of crossbreds over inbreds were evident for per cent ADN. No mean differences between inbreds existed but between reciprocal crossbreds S x W exceeded W x S for per cent ADN. The mean square for genotype x diet interaction was not significant although comparison of sub-group means indicated a change in the relative position of each inbred line on the MP diet (See Figure 5).

No trends between genotypic groups for apparent ability to digest food existed. However, Figure 6 illustrates a varying genetic response at different dietary protein levels, although the mean square was not significant.

Total nitrogen retention per unit weight was least for Wistar and greatest for S x W with Sprague and W x S means equal and intermediate. Between genotypes total nitrogen retention at each protein level showed generally similar differences, (Figure 7) although on HP Sprague retention equalled S x W and the W x S group had dropped comparatively.

Mean values for efficiency of protein utilization indicated pure line differences in favour of the Sprague group and small reciprocal crossbred differences in favour of the S x W group. Crossbreds had a greater efficiency of protein utilization than inbreds. The values for the Wistar were relatively much lower than for the other 3 groups. Efficiency of protein utilization mean squares for genotype by diet interaction were significant for both per cent gross nitrogen and ADN retained at the 5 per cent and 1 per cent levels, respectively. Comparison of sub-group means, Figures 8 and 9, revealed that this interaction was not due to pure line or reciprocal crossbred differences, which remained consistent at each protein level. However, on the LP diet crossbreds were superior to inbreds but on the HP diet crossbreds were intermediate to the inbred lines.

(5) Pre-weaning Performance.

Results obtained from birth to weaning are summarized in Table 10. Total numbers of live offspring born and average rat weight at birth were depressed in the Wistar group. In addition crossbreds were superior for both of these measures

Table 10. Pre-weaning Data for the Main Metabolism Experiment.

	GENOTYPE			
	W	S	W x S	S x W
I. Birth				
No. of litters	9	13	10	7
Total no. of offspring	58	101	89	57
Av. no. of offspring per litter	6.4	7.8	8.9	8.1
Av. rat weight (gm.)	6.1	6.5	6.7	6.2
Av. litter weight (gm.)	39.2	50.5	59.9	50.3
II. Weaning				
No. of litters(1)	5	8	5	5
Total no. of offspring weaned	29	54	34	34
Av. no. of offspring weaned per litter	5.8	6.8	6.8	6.8
Av. rat weight (gm.)	36.5	36.8	37.7	36.7
Av. litter weight (gm.)	210	249	257	249
III. Birth to Weaning				
Av. gain per rat	30.8	30.5	31.1	30.2
Av. gain per litter	173	198	197	199

(1) The disparity between number of litters born and number of litters weaned is due to litters being killed when not needed for test.

which would suggest that hybrid vigor was active in the pre-natal environment.

Comparison of individual rat gain and total litter gain to weaning did not reveal any differences in maternal ability of the two lines.

D. Discussion and Conclusions.

(a) Influence of Dietary Protein Level on Gains, Food Consumption, Apparent Digestibility, and Nitrogen Retention.

On the basis of material presented by Sibbald (1957) for the weanling rat the finding in this thesis that diet had no effect on food consumption was expected. Sibbald pointed out that weanling rats have specific energy requirements and within physiological limits will adapt food consumption to meet those energy requirements. There was no evidence in the data collected for this thesis to support the suggestion by Bowland *et al.* (1958) at this institution than on low protein diets rats may increase intake in order to obtain more protein. Bowland *et al.* (1958), however, used diets as low as 6.1 per cent protein which may account for the different results. Greater gains on high protein diets in this experiment reflect, therefore, greater nitrogen intake, which was incidental to food consumption. Similarly, relationships between dietary protein levels and

per cent ADN, total nitrogen retention, and efficiency of protein utilization were dependent upon greater nitrogen intake.

A statistical attempt was made to relate per cent ADN retained to trial gains per 100 grams ABW, Table 11.

Table 11. Correlations Between Per Cent ADN Retained and Trial Gains(1)

	Df	r_{xy}
Gross	89	-0.316
Between diet	1	-0.987
Within diet	88	+0.418
Between genotype	2	+0.977
Within genotype	87	-0.428
Within genotype and diet	79	+0.302

(1) Per 100 grams ABW.

The gross correlation was -0.316 which was negative due to the effect of dietary protein which is indicated by the large negative between diet correlation of -0.987. This effect was expected since an increase in dietary protein in these trials resulted in increased nitrogen consumed and increased trial gains with a concomitant decrease in per cent ADN retained. When the effect of diet was

statistically removed the correlation became positive indicating that animals on the same diet which are most efficient in protein utilization tend to have the greatest trial gains. (Further discussion of Table 11 is presented on page 45.)

(b) Influence of Sex on Gains, Food Consumption, Apparent Digestibility, and Nitrogen Retention.

Trends noted in the Preliminary Experiment for gains and total nitrogen retained between sexes were substantiated in the Main Experiment. Sex differences were not large for either of these measures, male superiority being less than 10 per cent in both cases. Total food and nitrogen consumption were greater in females than males in the preliminary work, but significant differences for the reverse situation were found in the Main Experiment.

Trends toward sex differences in efficiency of protein utilization in the Preliminary Experiment may have been random occurrences in view of results from the Main Experiment in which there were no sex differences for this measure. It may be concluded that in the Main Experiment the gain difference between sexes was related to greater consumption of food by males but not to differential ability to utilize dietary protein.

(c) Influence of Genotype on Gains, Food Consumption, Apparent Digestibility, and Nitrogen Retention.

Trends reported between inbred lines and between reciprocal crossbreds in the Preliminary Experiment for gains, food consumption, and food conversion were substantiated in the Main Experiment. In addition, both experiments indicated the same trends in efficiency of protein utilization between inbreds but between reciprocal crossbreds mean values in the Preliminary Experiment were in favour of the W x S group, whereas in the Main Experiment they were in favour of the S x W group.

Comparison of crossbreds versus inbreds and comparison of reciprocal crossbreds indicated that the group which gained the most consumed the most food. However, between inbreds this was not the case where it was found that gain differences were 4.6 grams in favour of the Sprague group but food consumption was 1.7 grams greater in the Wistar group. Each consumed equal amounts of nitrogen. Greater gains and more efficient utilization of protein by Sprague rats indicate superior ability to utilize ingested energy and protein. Differences between genotypes other than Wistar for efficiency of protein utilization were small but, nevertheless, varied in the same direction as gains.

The statistical correlation, Table 11, of percentage ADN retained and gains between genetic groups resulted in an $r_{xy} = +0.98$. Within genetic groups the correlation was $r_{xy} = -0.43$ which is a reflection of the influence of dietary protein level. Removing further the effects of diet and genotype by diet interaction gave an $r_{xy} = +0.302$. The implication is that between genotypes gains and efficiency of protein utilization vary in the same direction and, further, that individuals within a genotypic population fed the same diet exhibit the same relationship, but to a lesser degree. Because those genotypes with greatest efficiency of nitrogen utilization tended to consume the most nitrogen it may be concluded that efficiency of nitrogen utilization between genotypes is not related to nitrogen intake in the manner previously described for diets.

A small total genetic difference of approximately 2 per cent for per cent ADN was evident in the Main Experiment but no obvious differences were apparent in the Preliminary Experiment. It was noted that differences in per cent ADN were positively and proportionately related to nitrogen intake differences between the genetic groups and on this basis the suggestion is made that true digestibility differences between the genetic groups were non-existent.

Genotypic interactions with dietary protein level did not exist for measures of gain and food utilization.

However, differential genetic ability to consume and utilize gross nitrogen or ADN was evident. Because crossbreds were intermediate to the parental lines on MP and HP diets and superior on the LP diet it may be said that crossbreds adapted better to diets low in protein than did inbreds. Genetic variations in response to dietary fluctuations of this nature indicate the necessity of specifying and controlling the genotypes involved in nutritional experiments.

(d) Maternal Influence on Reciprocal Crossbred Differences.

Reciprocal crossbred differences were apparent for nearly all measures in which genotypic differences were evident and were consistently in favour of the S x W group. These differences did not appear to reflect maternal environment and no other satisfactory explanation presents itself.

V. Thyroid I^{131} Decay Studies.

A. Materials and Methods.

Before the introduction of radioactive iodine into the field of research, gross observation of thyroid weight was the most commonly used method to estimate thyroid activity (Dempsey and Astwood, 1943). During the last decade I^{131} techniques have been used for numerous studies involving thyroid activity. The simplest and quickest of these is an in vivo technique developed by Stevens and D'Angelo (1954) based on preliminary work by others (Wolff, 1951; Perry, 1951; Albert, 1951; Fish et al., 1952) in which the rate of release of I^{131} from the thyroid is determined. The assumption is made that decay rates are proportional to the production of thyroxine. This method has been used with rats (Cottle and Carlson, 1956), guinea pigs (Stevens et al., 1955), and cattle (Johnson and Ragsdale, 1959; Premechandra et al., 1959b).

It is possible that artifacts may arise, particularly from organic iodine introduced into the blood stream from the diet, or that small glands may show a greater decay rate in order to maintain quantitative thyroxine production similar to larger glands. The original workers who developed this technique, however, made comparisons with other thyroid activity criteria and concluded that thyroid I^{131} decay estimates were valid. Recently, Flamboe and Reineke (1959) studied thyroid activity in dairy goats and found a poor relationship between thyroid decay and actual thyroxine production as

estimated by the effect of thyroxine substitution on thyroid I^{131} decay. Premechandra et al. (1959b) are of the opinion that thyroid I^{131} decay is not an accurate measure of thyroid activity, although in their report this method compared favourably with the thyroxine substitution technique as a method of identifying mono- and dizygous twins. Notwithstanding these criticisms, the thyroid I^{131} decay technique was chosen to estimate thyroid activity in the four genotypic groups under study in these experiments.

The procedure most commonly followed is to make an initial base count and relate counts at successive intervals thereafter to the base count. The base count should be taken at a time when maximum uptake of injected I^{131} has occurred. There is some conflict in the literature regarding the time interval between injection and maximum thyroid uptake. Using rats as the experimental animal, Wolff (1951) reported that 90 per cent uptake occurred at 40 hours post injection, while Chaikoff et al. (1947) indicated that in 24 hours 90 per cent of the injected dose appears in the thyroid as protein bound iodine. Perry (1951) made the base reading at 24 hours, whereas Cottle and Carlson (1956) used either 12 or 24 hours. Amin and Chai (1957) made a base reading at 72 hours with mice. It seemed that a valid base count could be obtained anytime after equilibrium had been attained by the rat to the injected load of I^{131} and on this basis a 48-hour interval between injection and base count reading was chosen. Counts were taken thereafter at 4 consecutive intervals of 2 days each, giving a total test

period of 10 days from time of injection.

An injection dose of approximately 10 µc. per 100 grams body weight has commonly been used to obtain adequate I^{131} activity (Wolff, 1951; Albert *et al.*, 1952; Pipes and Turner, 1956). Preliminary tests in these experiments indicated that 5 µc. in mature and 10 µc. in weanling rats gave desirable base day thyroid counts in the region of 80,000 counts per minute. Each dose was made up to a 0.2 ml. volume, dilutions being made in the radiation laboratory. Injections were made with a 1 ml. syringe fitted with a 1 inch number 24 needle in the animal room of this department.

A standard solution was made at the time of dose preparation. This standard served as a check on the dose injected and, by comparing the standard count each day with the base day reading, fluctuations in machine function due to line voltage variation could be estimated and eliminated.

Background counts are most often taken over the epigastric region in rats (Wolff, 1951; Stevens and D'Angelo, 1954; Reineke and Singh, 1955). However, because feces and urine containing I^{131} were often spread on the phosphor cover and transferred to the gloved hands, it was believed that a better background measure would be one taken with the gloved hand placed over the phosphor cover in a simulation of the position during rat counting. This system was used to estimate thyroid background. Background for the standard was similarly determined but with the gloved hand removed.

Counts were made on a 2½ inch sodium iodide, thallium activated, well type phosphor counter(1) linked with a decade scaler(2), see Figure 10. Two 1 minute counts were made in all cases and averaged. All counts were corrected for background as well as for isotope decay and machine fluctuations, which were corrected to the base reading, and the resulting values reported as either per cent of previous count, per cent of base count, or log (per cent of base count). An example of the procedure followed in these experiments for correction of counts and calculation of thyroid I^{131} decay is illustrated in Appendix Table 3.

In order to reduce movement, and thereby experimental error, a number of workers have anesthetized animals prior to counting (Perry, 1951; Wolff, 1951; Reineke and Singh, 1955). However, the rats used in this experiment were quite docile, with the result that sufficiently accurate counts were obtained without the use of anesthetic procedures.

Rats were transferred to the radiation laboratory in a metal carrying cage(3) lined with paper. This paper was replaced daily and stored with the feces. A plastic(4) phosphor cover, complete with stand and tray for collection

(1) Nuclear of Chicago, 223 West Erie Street, Chicago 10, Illinois, U.S.A.
(2) Tracerlab Inc., 1601 Trapelo Road, Waltham 54, Mass., U.S.A.
(3) Animal Cage, Type LC-57: Geo. H. Wahmann Manufacturing Co., 1123 East Baltimore Street, Baltimore 2, Maryland, U.S.A.
(4) Designed by Dr. W.H. Cottle, Department of Physiology, University of Alberta, Edmonton, Canada.

EQUIPMENT AND METHOD FOR DETERMINING THYROID I^{131} DECAY

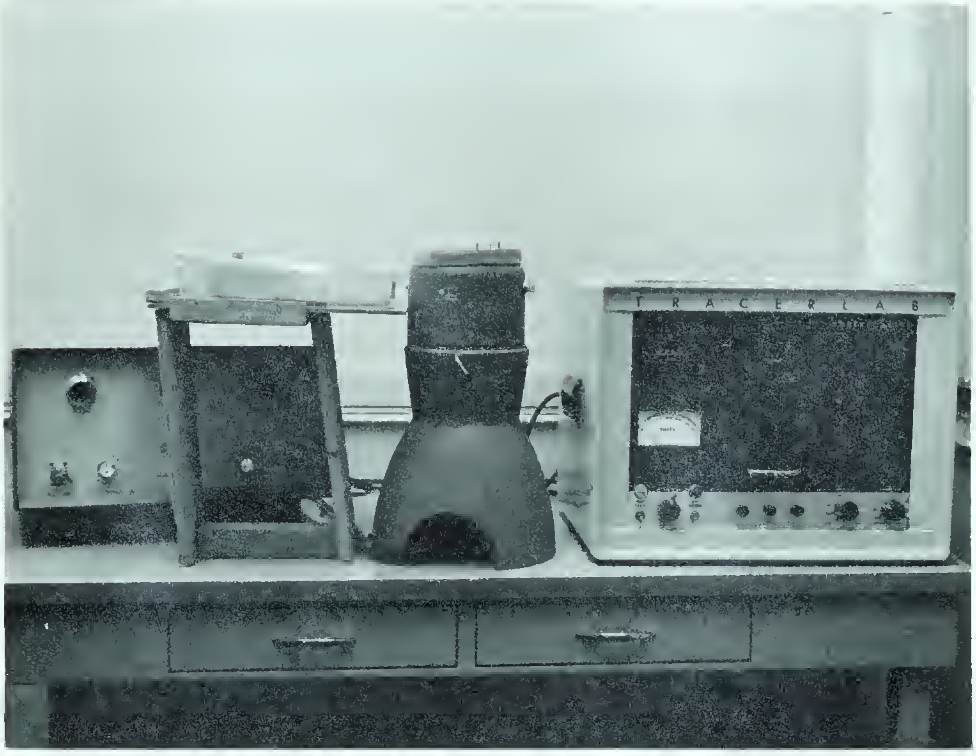


Figure 10 - I^{131} Counting Equipment

Figure 11 - Procedure for Determining <u>In Vivo</u> Thyroid I^{131} Activity

of feces and urine during counting was used. Feces and urine were collected on paper towelling and transferred to radioactive waste tins in the radiation laboratory. The apparatus and method used to determine in vivo thyroid radioactivity is shown in Figure 11. All I^{131} decay determinations were made in the forenoon. The electric current to the counting apparatus was turned on one-half hour before counting was begun in order to allow the apparatus to warm up.

Radioactive materials from the animal rooms were stored in metal garbage containers kept in an isolated location for a minimum of 2 isotopic half lives or approximately 16 days. These wastes were subsequently disposed of through normal garbage disposal channels.

B. Preliminary Thyroid I^{131} Decay Experiment.

(a) Object

The object of this trial was to evaluate the thyroidal I^{131} decay technique for the purpose of genotypic and sex comparisons and to arrive at an estimate of experimental numbers required for valid estimates.

(b) Procedure

Five replicates composed of one male and one female mature rat, ranging in age from 5 to 15 months, from each of the Wistar, Sprague, and crossbred groups were tested for thyroid I^{131} decay rate. No consideration was given to the dam line in the choice of crossbreds. Wistar and Sprague

females with a poor history of reproductive performance were selectively chosen for test in order that the maximum number of fertile females would be retained for further matings. Males from both inbred lines and animals of each sex from the crossbreds were randomly chosen. Males and females were injected and counted on alternate days. A counting order which was followed during each day's counting was established for each rat within a sex group. During the test period rats were housed individually or in pairs in metabolism cages, described in the metabolism section. Urine was washed down a sink daily with large quantities of water and feces were collected every 2 days and stored.

Upon completion of the trial, rats were transferred to grouped maintenance cages for 2 weeks at the end of which time they were killed and disposed of along with other waste material. During the 2 week post-test period, urine and feces were collected on shavings which were added to the radioactive wastes every 2 days.

(c) Results

Mean results are listed in Table 12 and represented graphically in Figures 12, 13, and 14. Mean squares and tests of significance are presented in Table 13.

Data from the Preliminary Experiment were first plotted as per cent of previous count and as per cent of base count (Figure 12). Per cent of base count values resulted in apparently logarithmic curves, although the

Table 12. Summary of Thyroid I^{131} Decay Data from the Preliminary Thyroid Experiment.

GENOTYPE	DAYS AFTER INJECTION	PER CENT OF BASE COUNT	PER CENT OF PREVIOUS COUNT	LOG (PER CENT OF BASE COUNT)
W	4	70.9	70.9	1.851
	6	52.3	73.8	1.719
	8	39.8	76.1	1.600
	10	33.3	83.7	1.522
S	4	66.8	66.8	1.825
	6	48.2	72.2	1.683
	8	39.2	81.3	1.593
	10	33.3	84.9	1.522
C(1)	4	67.5	67.5	1.829
	6	49.3	73.0	1.693
	8	38.5	78.1	1.586
	10	32.1	83.4	1.507
SEX				
M	4	63.4	63.4	1.802
	6	45.9	72.4	1.662
	8	37.9	82.6	1.579
	10	31.0	81.8	1.491
F	4	74.0	74.0	1.869
	6	54.4	73.5	1.736
	8	40.7	74.8	1.610
	10	34.3	84.3	1.535

(1) C refers to Crossbred.

Table 13. Mean Squares(1)(2) and Tests of Significance for Data Summarized in Table 12.

SOURCE OF VARIATION	Df	MS	F
Total	119	1.784	
Main Effects			
Replicate	4	0.640*	3.36
Genotype	2	1.251**	6.57
Sex	1	6.659**	34.96
Day	3	56.332**	295.72
Interactions			
R x G	8	0.964**	5.06
R x S	4	1.523**	7.99
R x D	12	0.184	0.97
G x S	2	0.133	0.70
G x D	6	0.037	0.19
S x D	3	0.319	1.68
Error	74	0.190	

(1) Data converted to log (per cent of base count).
(2) Mean square multiplied by 100.
* Significant at the 5 per cent level.
** Significant at the 1 per cent level.

THYROID I^{131} DECAY DURING THE PRELIMINARY THYROID EXPERIMENT

Figure 12 - Genotype Comparison

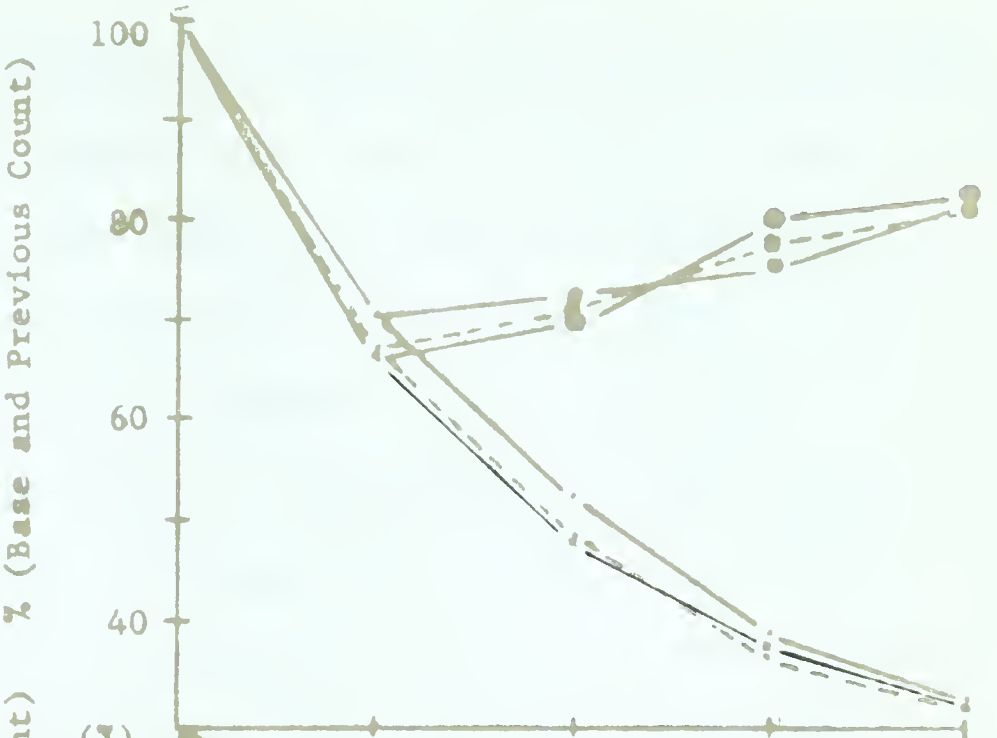


Figure 13 - Sex Comparison

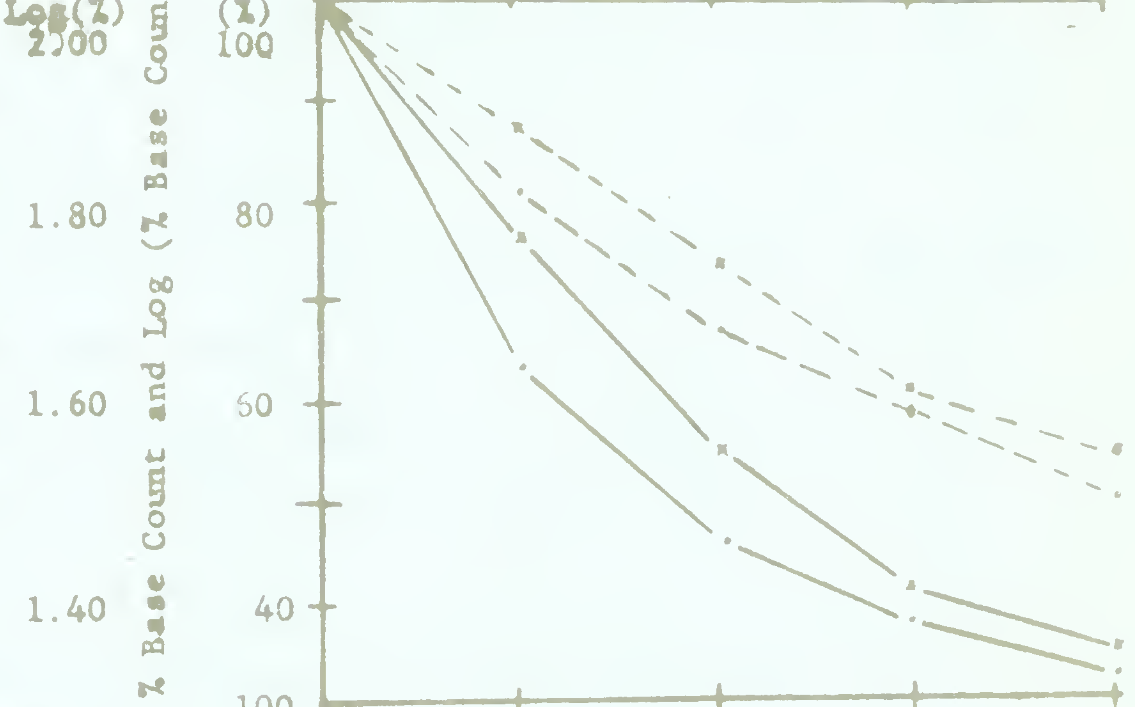
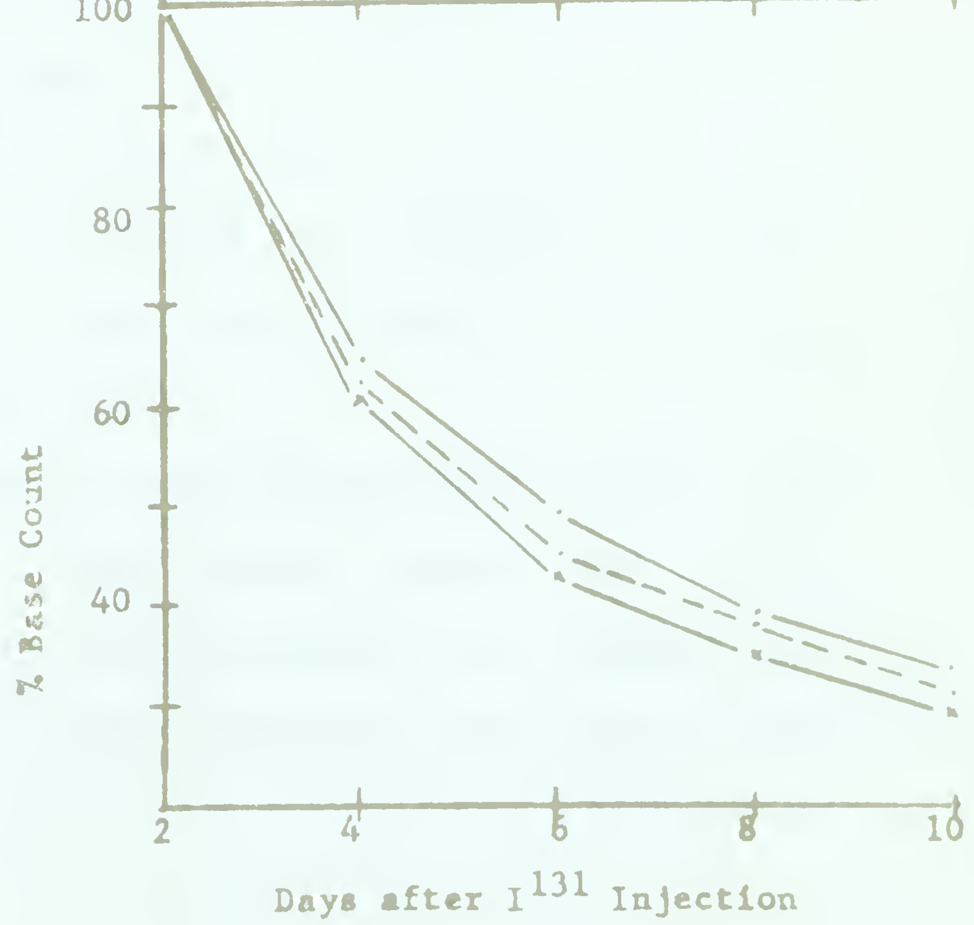


Figure 14 - Genotype Comparison (Females Excluded)



upward trend for the per cent of previous count plot suggested a departure from a true logarithmic thyroid decay picture. Plotting results as log (per cent base count), Figure 13, bore out this expectation; however, decay curves were straightened to a considerable degree. It was further anticipated that data converted to logs would be more valid for variance analysis since the mean and standard deviation are highly positively correlated when counts per minute are used as the criterion. Therefore, the log (per cent base count) was considered to be the best method of representing the data from these experiments.

Strain comparisons with sexes combined, represented in Figure 12, indicated a switch in the relative positions of the crossbred and Sprague decay curves between the 6th and 8th day. Separating males from females and plotting male curves for the 3 genotypes, represented in Figure 14, indicated small but consistent differences between the 3 genetic groups. Sprague males had a greater rate of decay than the Wistar males, and crossbreds were intermediate. The confounding effects of sex may be attributed to estrus cycle influence on thyroid activity (Soliman and Reineke, 1954; Soliman and Badawi, 1956).

Male decay rates were consistently greater than decay rates for females throughout the 10-day test period.

Differences were small between test groups but the mean square for both sexes and genotypes was highly significant.

C. Main Thyroid I^{131} Decay Experiment.

(a) Object

Based on results obtained in the preliminary trial, this experiment was undertaken to study thyroid activity at approximately the same stage of growth that metabolic measures were made in the metabolism section. The effect of age on thyroid activity was also studied by conducting trials at 2 later stages of growth.

(b) Methods and Procedure

Estimates were made on thyroid activity during 3 stages of growth. These stages were at approximately 6 weeks of age, Period 1; 3 months of age, Period 2; and 5 months of age, Period 3. Before decay tests were begun in Period 1, animals were housed in the metabolism cages, described in the metabolism section, for 5 days and fed the same diet used during the Preliminary Metabolism Experiment. During this time, gains and food consumption were recorded. Following collection of this data, animals were injected with I^{131} and thyroid decays estimated. During Period 2 the first decay count was made at 5, rather than 4, days post injection. During the time of thyroid estimation, animals were housed in maintenance cages. The methods of waste disposal and the procedure for determining thyroid I^{131} decay were as described in the Preliminary Experiment. During the latter 2 periods of thyroid activity estimation a radiation analyzer(1),

(1) Nuclear of Chicago, 223 West Erie Street, Chicago 10, Illinois, U.S.A.

illustrated in Figure 10, was connected between the counter and scaler.

Two males and two females from one litter in the inbred lines and one male and one female per litter from each of the reciprocal crossbreds were tested and replicated 3 times giving a total of 36 rats. During Period 1, replicates were placed in metabolism cages at 5 day intervals in an attempt to equalize their average body weights.

A 10 μc. dose of I^{131} was required on weanling rats to obtain adequate counts. During the final two periods all animals were injected on a single day with a 5 μc. dose of I^{131}. Females and males were housed separately and were injected and counted in that order. Because the time required to count each sex group was less than 2 hours, no rat counting order was followed. Where possible, the same animals were tested for thyroid decay at each period. However, 7 deaths occurred following Period 1 and an additional 5 deaths occurred during Period 3. Animals which died following Period 1 were replaced by animals of the same age, sex, and genotype for the remaining 2 periods. These deaths were apparently precipitated by exposure to low outdoor temperatures during transfer to the counting laboratory.

A break in the decay curves on the 6th day following injection was noted during Period 3. The recorded rat background count for this day was considerably lower than for other counting days during this period and would account for the aberrant results. It is probable that

experimental error was involved. Because all test groups were similarly affected, comparative results within Period 3 would not be disturbed although a comparison of results between periods would be affected.

(c) Results

Mean gain and food consumption data from Period 1 as well as mean thyroid I^{131} decay data from all 3 periods are presented in Table 14. Summarized data by counting day are listed in Appendix Table 3. Mean squares and tests of significance for these data are presented in Table 15. Graphical representation during specific periods is given in Figures 15 to 20, inclusive, and between periods in Figures 21 to 23, inclusive.

Sprague test animals gained more, consumed less food, and had a smaller food conversion ratio than the Wistar. There was a difference in gain between reciprocal crossbreds in favour of S x W although food conversion was about the same for both groups. Crossbreds by comparison with inbreds were inferior for both measures. Males exhibited a statistically non-significant tendency to exceed females for gains and food conversion.

During Period 1 thyroid I^{131} decay rates showed consistent genotypic differences, see Figure 15. The pattern of differences was of the following order, $S > W > S \times W > W \times S$. During Period 2 genotypic differences were not as clearly defined. Figure 17 illustrates a change in the relative position of the inbreds on day 6 and thereafter

Table 14. Summary of Gain and Food Data During Period 1 and Mean Thyroid I^{131} Decay Data from all Periods in the Main Thyroid Experiment.

	PERIOD 1 PER 100 GRAMS ABW			THYROID I^{131} DECAY		
	5-DAY TRIAL GAIN	FOOD CONS.	FOOD CONS. PER GM.GAIN	PERIOD 1	PERIOD 2	PERIOD 3
	gm.	gm.	gm.	Log (% Base Count)		
Replicate						
1	21.9	63.4	3.1	1.631	1.734	1.708
2	22.8	64.0	3.0	1.669	1.708	1.650
3	21.3	58.9	2.9	1.660	1.763	1.691
Genotype						
W	21.8	65.1	3.0	1.645	1.727	1.675
S	25.7	63.5	2.5	1.618	1.745	1.709
W x S	16.5	53.2	3.3	1.708	1.734	1.679
S x W	19.6	62.6	3.5	1.694	1.739	1.664
Sex						
M	22.1	59.8	2.9	1.628	1.728	1.678
F	21.9	63.9	3.0	1.671	1.743	1.688
Days after Injection						
2				1.831	1.845	1.823
4				1.693	1.781	1.770
6				1.580	1.683	1.606
8				1.508	1.636	1.535
Mean Thyroid I^{131} Decay by Periods				1.653	1.736	1.684

Table 15. Mean Squares and Tests of Significance for Data Summarized in Table 14.

SOURCE OF VARIATION	GAIN AND FOOD CONSUMPTION FOR PERIOD 1				THYROID I^{131} DECAY -- ALL DAYS(1)					
					PERIOD 1		PERIOD 2		PERIOD 3	
	Df	5-DAY TRIAL GAIN	FOOD CONS.	FOOD CONS. PER GM.GAIN	Df	MS	Df	MS	Df	MS
Total	33	31.4	59.9	0.513	139	1.857	139	0.856	123	1.634
Main Effects										
Replicate	2	6.3	87.1**	0.080	2	1.941**	2	3.563**	2	3.757**
Genotype	3	123.9**	203.3**	1.390*	3	5.506**	3	0.311**	3	1.245**
Sex	1	0.2	141.3**	0.229	1	6.464**	1	0.861**	1	0.397
Day					3	69.859**	3	31.318**	3	57.031**
Interactions										
R x G	6	39.4	73.9**	0.714	6	1.226**	6	0.681**		---(2)
R x S	2	9.5	37.0**	0.620	2	---(3)	2	0.070	2	0.162
R x D					6	0.203	6	---(3)	6	0.175
G x S	3	26.3	155.0**	0.366	3	0.919**	3	0.459**	3	0.270
G x D					9	---(3)	9	0.277**	9	0.288
S x D					3	0.295*	3	0.769**	3	0.395
Error	16	19.9	4.4	0.359	101	0.100	101	0.057	91	0.134

(1) Mean square multiplied by 100.
(2) Incomplete subplot (W x S from Replicate 3).
(3) Negative sums of squares.
* Significant at the 5 per cent level.
** Significant at the 1 per cent level.

THYROID I^{131} DECAY DURING "PERIOD 1" OF THE MAIN THYROID EXPERIMENT

Figure 15 - Genotype Comparison

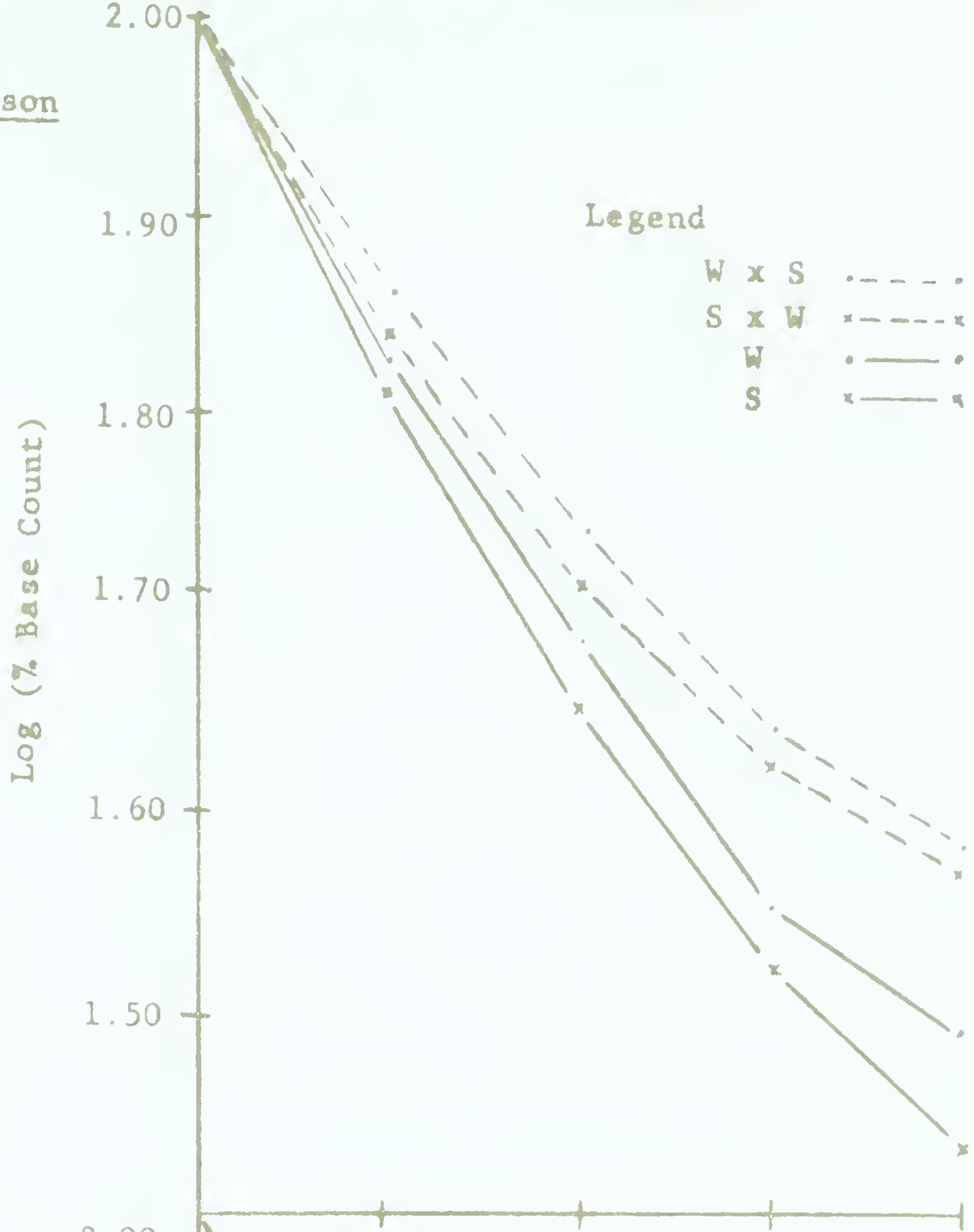
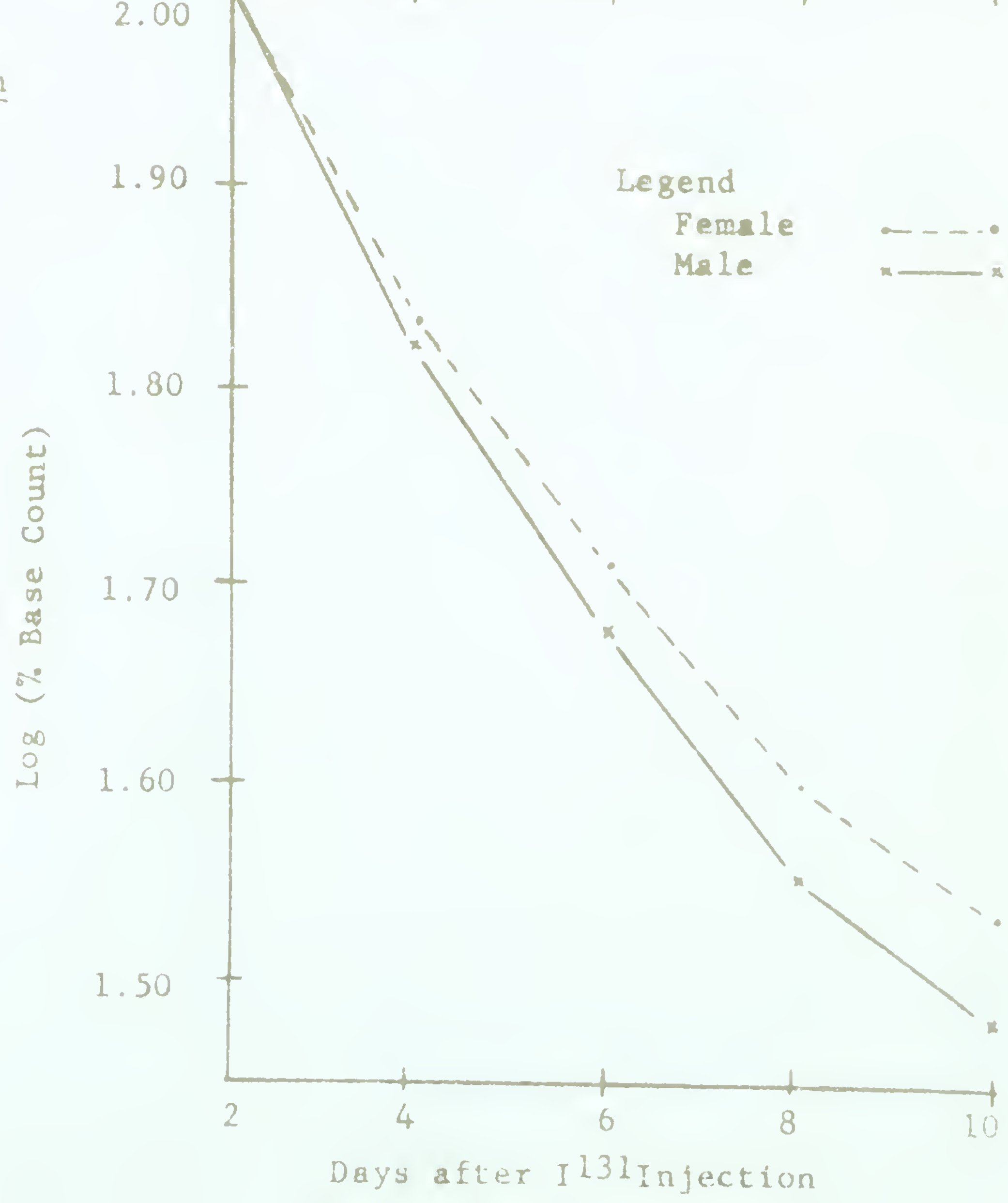


Figure 16 - Sex Comparison



THYROID I^{131} DECAY DURING "PERIOD 2" OF THE MAIN THYROID EXPERIMENT

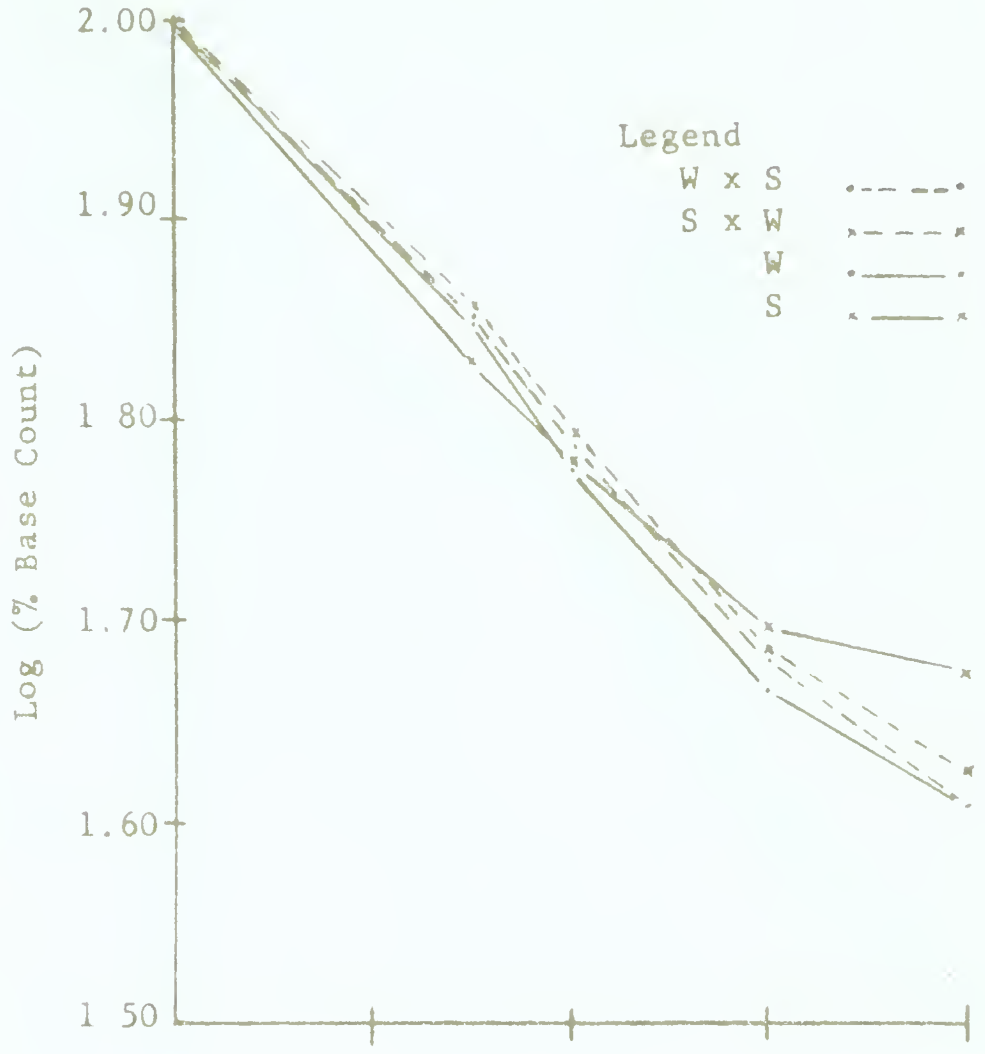


Figure 17 - Genotype Comparison

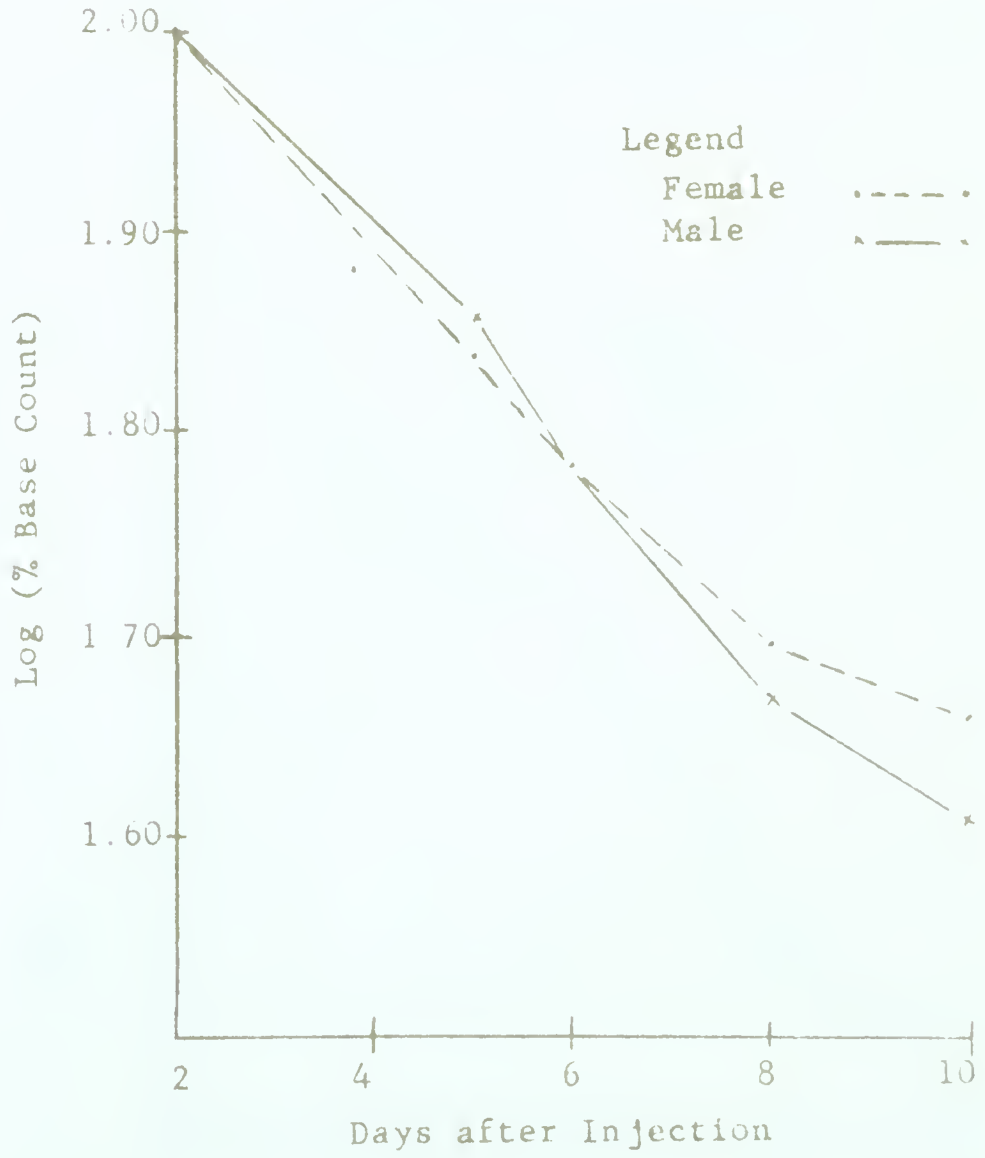


Figure 18 - Sex Comparison

THYROID I^{131} DECAY DURING "PERIOD 3" OF THE MAIN THYROID EXPERIMENT

Figure 19 - Genotype Comparison

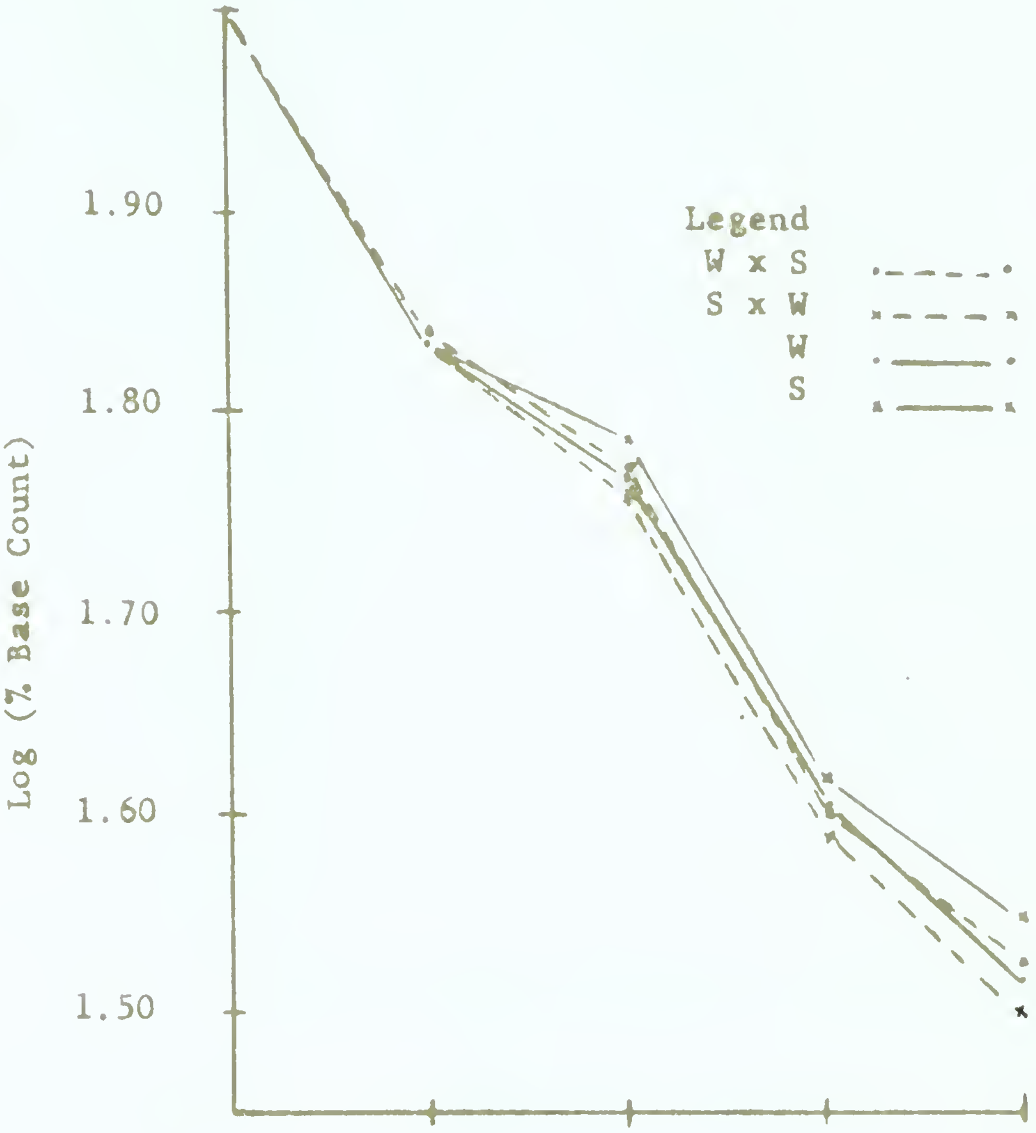
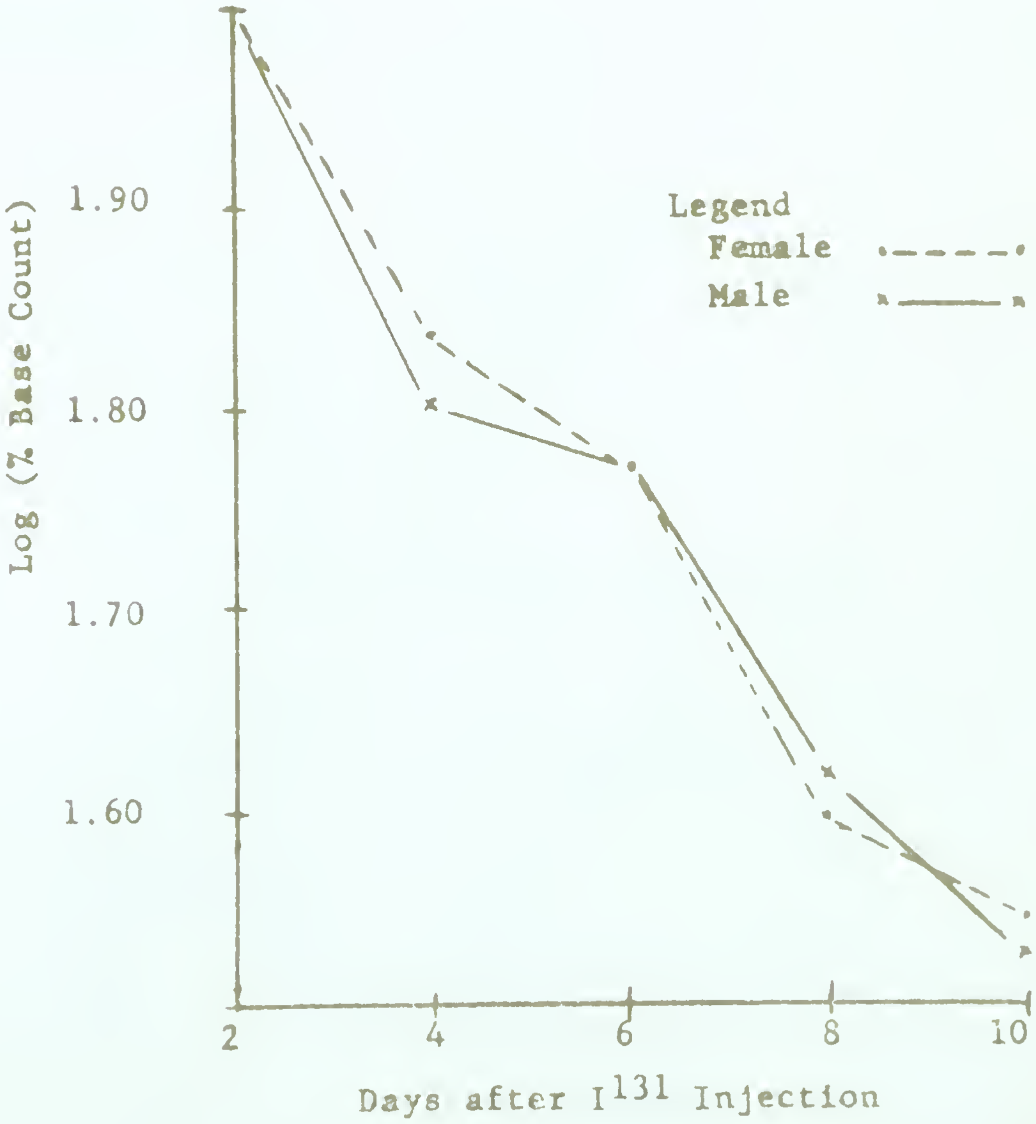
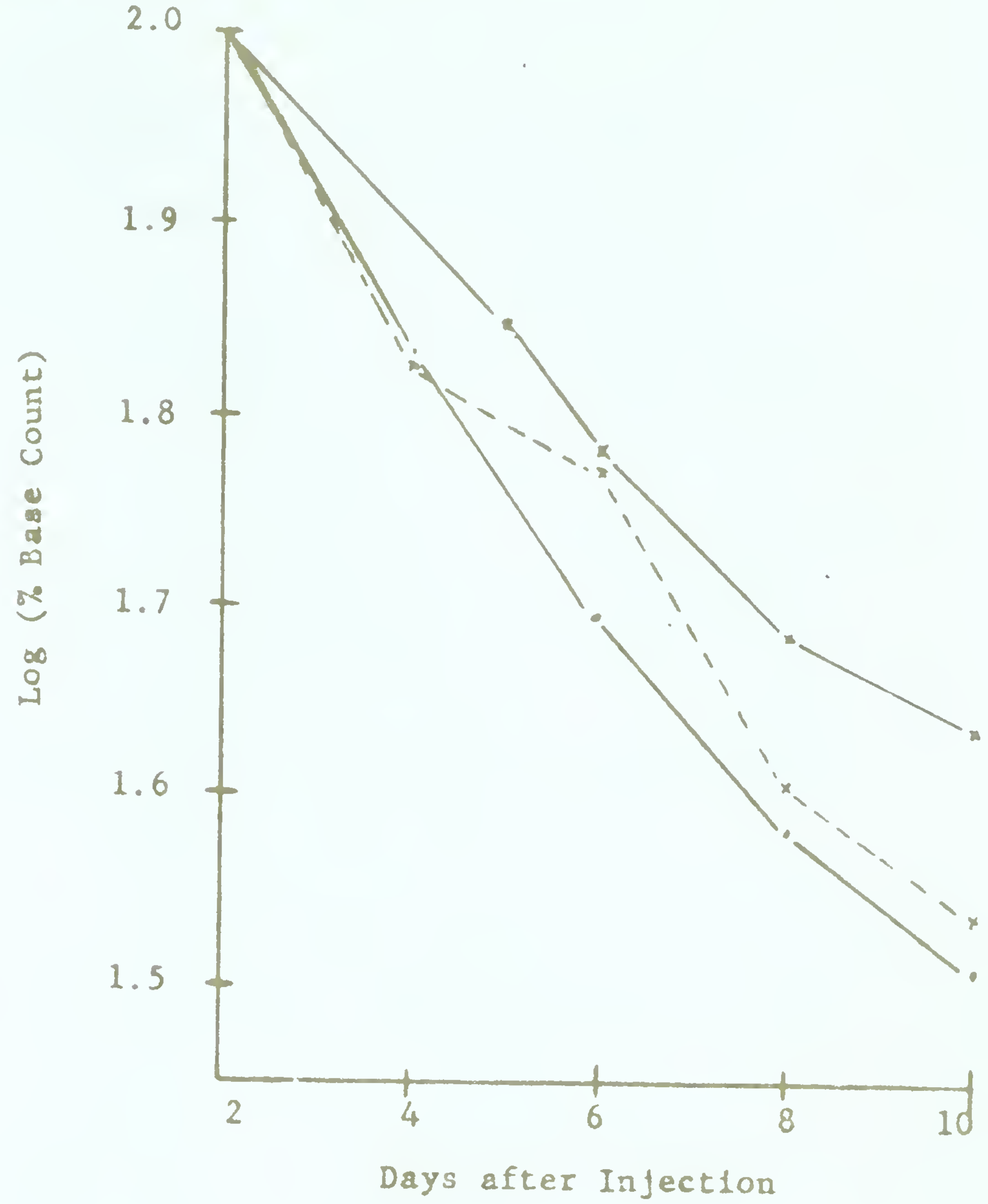


Figure 20 - Sex Comparison



INFLUENCE OF AGE ON THYROID I^{131} DECAY

Figure 21 - Period Comparison



INTERACTIONS WITH AGE

Figure 22 - Genotype x Age

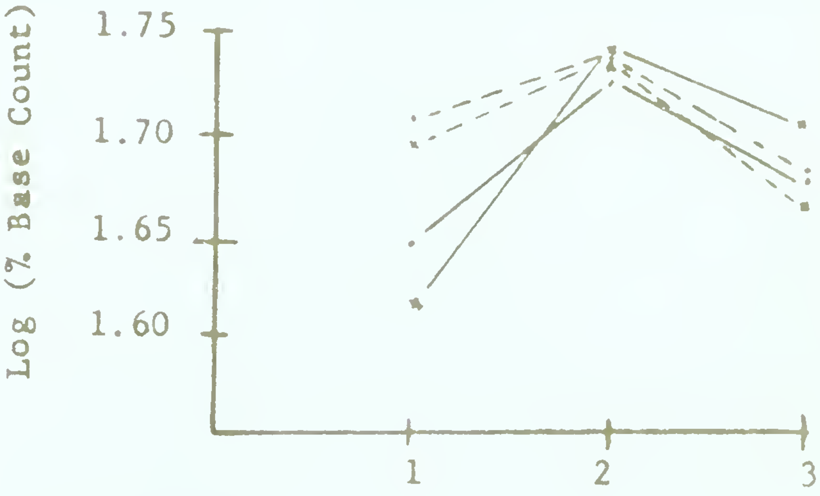
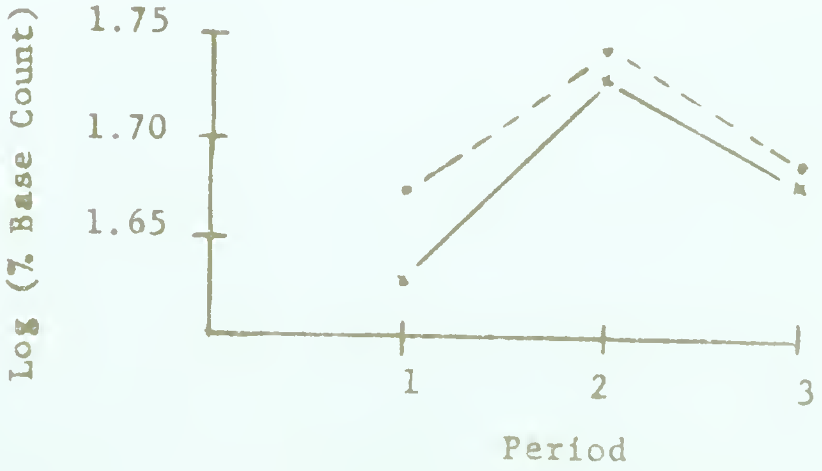


Figure 23 - Sex x Age



throughout the remainder of the 10-day test period. On days 8 and 10 it would appear that the Wistar had the greatest rate of decay and the Sprague the least with crossbreds intermediate. Reciprocal crossbred differences were notably uniform throughout the test period with W x S having a greater decay rate than the S x W. During Period 3 the Wistar decay rate was clearly greater than the Sprague and crossbreds were about equal to the Wistar. Reciprocal crossbred differences, with S x W having the greatest decay rate, were again uniform throughout the test period.

Male decay rates were markedly greater in Period 1, see Figure 16. Figure 18 illustrates that in Period 2 sex decay curves crossed one another on day 6 although mean values for the entire period were greater for the males. During Period 3, Figure 20, decay curves for the sexes crossed at 2 points and did not appear to exhibit any real sex trends although mean sex values indicated males had the greatest decay rate.

D. Discussion and Conclusions

During Period 1 high and low rates of gain for genotypic groups corresponded to fast and slow rates of thyroid I^{131} decay. The statistical correlation between these 2 measures was calculated and is presented in Table 16.

Table 16. Correlations Between Log (Per Cent Base Count) and Gains(1) During Period 1.

	Df	r_{xy}
Total	33	-0.426
Between Genotype	2	-0.981
Within Genotype	31	-0.086

(1) Per 100 grams ABW.

Between genotypes the correlation was $r_{xy} = -0.981$. The negative values are dependent upon greater decay rates, which have smaller log (per cent base count) values, being positively related to gains. The gross correlation was $r_{xy} = -0.426$. Removing the effect of genotype from this value resulted in a correlation $r_{xy} = -0.086$. This small correlation means that within a genotype gains cannot be predicted from estimates of thyroid activity, made at approximately the same time as gains are measured. Kunkel (1953) who studied feedlot gains in 2 breeds of beef cattle reported small correlations between gains and plasma bound

iodine levels within each breed group, but each breed group had an optimum level which was associated with gains. The relationship reported by Kunkel is similar to that reported herein.

A high positive relationship between gains and per cent ADN retained for genotypes was reported in the metabolism section of this thesis and it may be that greater decay rates between genotypes are related to greater efficiency of protein utilization.

Between genotypes in the Main Thyroid Experiment food conversion ratio, as well, was apparently related to thyroid decay. The order of decreasing decay rate as well as increasing food conversion ratio was Sprague, Wistar, and crossbred which indicates that thyroid activity of each genotype may be associated with optimum food conversion.

Figure 21 suggests that decay rates decreased with age although Period 2 was considerably reduced by comparison with Period 3. It was noted that base day counts were lower for Period 2 which may have been responsible for the lowered rates of decay in Period 2.

An age x genotype interaction is apparent in Figure 22 for the Main Experiment which indicates greater decay rates for the Sprague during the early post-weaning period and for the Wistar during later periods. In addition crossbred decay rates increased relative to the inbreds with increasing age in this trial. The relationship which these values may have had to rate of gain was not studied.

Figure 23 indicates that the effect of age on sexes was to reduce earlier sex differences in rate of I^{131} decay.

Between experiments certain disparities in results arose. During the Preliminary Thyroid Experiment rates of thyroid I^{131} decay were greater for Sprague than Wistar whereas during Period 3 of the Main Thyroid Experiment Wistar exceeded Sprague. In addition crossbreds gained less than inbreds during Period 1 which was unexpected. It is possible that low performance repeatability by the experimental genotypes may have had some bearing on the deviate results. Such a situation has been reported by Warwick and Lewis (1954) for 60-day body weights in inbred lines of mice and their crosses.

A more likely explanation presents itself, however. Between experiments it was not possible to control the physiological age of the test animals. This may be an important factor if different genotypes have different developmental patterns. During the Preliminary Thyroid Experiment rats ranged from 5 to 15 months of age whereas in Period 3 all animals were approximately 5 months of age. Also, during the Main Metabolism Experiment rats were specifically allotted on test at 65 grams whereas during Period 1 of the Main Thyroid Experiment considerable variation in rat weight on test existed and in addition rat weights were considerably greater than 65 grams. However, it was noted that crossbreds

had greater body weights throughout both experiments. This would suggest that the disparity in gain between the Metabolism and Thyroid Experiments may have been due to a temporary alteration in rate of gain between the genotypes.

Possible differences in growth patterns between genotypes suggest that experiments of this nature should be designed to assess the entire growth period. Single measurements based on standardized weight or age may only apply to that specific period of growth.

GENERAL SUMMARY AND CONCLUSIONS

The results from a series of studies on dietary protein utilization and thyroid activity on two phenotypically alike and relatively homozygous lines of rats and their reciprocal crossbreds have been presented. A summary of the results and their implications is presented in this section.

I. The Influence of Dietary Protein Level on Gain, Food Consumption, Apparent Digestibility, and Nitrogen Retention.

Dietary energy levels were held approximately constant with the result that diet had no noticeable effect on food consumption. Nitrogen intake, therefore, varied directly with the protein level of the diet and on this basis it was found that increasing protein levels resulted in greater nitrogen retention, greater gains, and lower food conversion ratios.

Although apparent food digestibility was unaffected by dietary protein level apparent nitrogen digestibility was increased with increasing dietary protein levels. It was suggested that this increase reflected a relatively constant metabolic fecal nitrogen excretion and that true protein digestibility was probably unaffected by dietary protein level.

Efficiency of nitrogen utilization decreased sharply at higher levels of protein reflecting greater wastage of nitrogen. Statistical correlation of per cent ADN retained and gain between diets was high and negative.

II. The Influence of Sex on Gain, Food Consumption, Apparent Digestibility, Nitrogen Retention, and Thyroid Activity.

Males gained more than females during the metabolism experiment. With the exception of food consumption, and, thereby, consumption and total retention of nitrogen per unit body weight, no statistically significant differences between sexes were found for any of the other measures made. On this basis it was deduced that gain difference between sexes was primarily the result of greater food consumption by males.

Evidence was obtained in the Main Thyroid Experiment which suggested that greater male gain during the early post-weaning period was related to greater thyroid activity. However, the effect of sex on thyroid activity was more clearly demarcated than the effect on gain. It was found that in later stages of growth thyroid activity was not significantly different between sexes, a finding which may be interpreted as a sex x age interaction.

III. The Influence of Genotype on Gain, Food Consumption, Apparent Digestibility, Nitrogen Retention, and Thyroid Activity.

Genotypic variation in gain and food conversion was evident. Crossbred heterosis existed for these measures but was of less magnitude than were differences between the parental lines, where Sprague rats were superior to the Wistar. Crossbreds consumed more food than inbreds and on this basis hybrid vigour for gain may be said to reflect, in part, greater

crossbred food consumption. Between inbreds, however, food consumption was inversely related to gain which means that Sprague superiority cannot be explained in terms of food consumption. However, per cent gross nitrogen or per cent ADN retained was greater for the Sprague. This resulted in greater total nitrogen retention and will have been partially responsible for superior Sprague gain.

Heterosis for efficiency of nitrogen utilization was evident and, therefore, complementary to food consumption in producing crossbred gain and food conversion superiority. The statistical correlation of gain and per cent ADN retained between genotypes was high and positive, which served to confirm the deductions made relative to the factors underlying genetic gain differences. A similar smaller correlation was found for individuals within a genotype fed the same diet which means that individual rat differences in gain were positively related to efficiency of nitrogen utilization.

Each genotype during the early post-weaning period had a characteristic thyroid activity which appeared to be related to optimal food conversion ratio and was found to be highly correlated to gain. It was conjectured that between genotypes thyroid activity and efficiency of protein utilization may have been similarly related. Within genotypes gain and thyroid activity showed no correlation.

No genetic difference for per cent food digestibility was found but crossbreds had a higher per cent ADN than did inbreds. This difference appeared to be the product of

nitrogen intake which would mean that true genetic nitrogen digestibility differences did not exist.

Differential genetic response to dietary protein levels was found for nitrogen consumption and efficiency of protein utilization such that crossbreds had a higher per cent retention on low protein diets but were intermediate to inbreds on high protein diets. This would suggest that crossbreds were more adaptable to diets low in protein. A further environmental interaction with genotype was found where relative thyroid activity of the inbred lines reversed during later growth stages and crossbreds increased relative to inbreds.

Reciprocal crossbreds differed from one another for most measures made and were consistently in favour of the S x W group. The cause of these differences was not determined.

IV. The Influence of Inbreeding on Fitness.

Deleterious effects from inbreeding occurred and were manifest by impaired reproductive potential and lowered resistance to bronchial disorders. Apparently a certain about of heterozygosity for deleterious recessives existed in the original stock.

BIBLIOGRAPHY

Addis, T.D.Karnofshy, W. Lew and L.J. Poo. 1938. The protein content of the organs and tissues of the body after administration of thyroxine and dinitrophenol and after thyroidectomy. J. Biol. Chem. 124:33-41.

Albert, A. 1951. The in vivo determination of the biological decay of thyroidal radioiodine. Endocrin. 48:334-338.

Allison, James B. and J.A. Anderson. 1945. The relation between absorbed nitrogen, nitrogen balance, and biological value of proteins in adult dogs. J. Nutr. 29:413-420.

Amin, A., C.K. Chai and E.P. Reineke. 1957. Differences in thyroid activity of several strains of mice and F_1 hybrids. Amer. J. Physiol. 191:34-36.

Armstrong, David G. and H.H. Mitchell. 1955. Protein nutrition and the utilization of dietary protein at different levels of intake by growing swine. J. Animal Sci. 14:49-68.

Bell, J.M., J.P. Bowland, C.O.T. Cameron, E.W. Crampton, L.E. Lloyd, M.E. Seale and F. Whiting. 1958. Nutrient requirements for Canadian Yorkshire swine. II. The effects of barley and oat finishing rations on rate of gain, feed utilization and carcass quality of pigs marketed at 185 and 200 pounds liveweight. Can. J. Animal Sci. 38:73-83.

Biellier, H.V. and C.W. Turner. 1957. The thyroid secretion rate of domestic fowls as determined by radioiodine techniques. Res. Bull. 622, Univ. of Missouri, Coll. of Agr. Exp. Sta., Columbia, Missouri.

Bloom, Roy A. and Paul F. Fenton. 1956. Glucose tolerance in relation to obesity and food intake. Amer. J. Physiol. 184:438-440.

Bowland, J.P. and R.T. Berg. 1958. Restricted feed intake in market pigs. Proc. West. Sect., Amer. Soc. Animal Prod. 9:XIX. 1-5.

Bowland, J.P. and R.T. Berg. 1959. Influence of strain and sex on the relationship of protein to energy in the rations of growing and finishing bacon hogs. Can. J. Animal Sci. 39:102-114.

Bowland, J.P., I.R. Sibbald, R.T. Berg and N. Hussar. 1958. Influence of dietary fat on energy consumption and digestion and on nitrogen utilization of weanling rats. Can. J. Animal Sci. 38:187-193.

Butler, L. 1952. A study of size inheritance in the house mouse. II. Analysis of 5 preliminary crosses. Can. J. Zoology 30:154-171.

Butler, L. and J.D. Metrakos. 1950. A study of size inheritance in the house mouse. I. The effect of milk source. Can. J. Res. 28:D.16-34.

Chai, C.K. 1955. Analysis of quantitative inheritance of body size in mice. I. Hybridization and maternal influence. Genetics 41:157-164.

Chai, C.K. 1958a. Heritability of iodine metabolism in the thyroids of mice. 4th Int. Biometric Conference and Symposium on Biometrical Genetics (Abstr.).

Chai, C.K. 1958b. Endocrine Variation. Thyroid function in inbred and F_1 hybrid mice. J. Heredity 49:143-148.

Chaikoff, J.L., A. Taurog and W.O. Reinhardt. 1947. The metabolic significance of plasma bound iodine of plasma: A study of its concentration under various conditions and of its rate of formation as measured with radioactive iodine. Endocrin. 40:47-54.

Cipolloni, Mary Ann, Burch H. Schneider, Henry L. Lucas and Helen M. Pavlech. 1951. Significance of the differences in digestibility of feeds by cattle and sheep. J. Animal Sci. 10:337-343.

Connelly, F.M. 1959. Indoor versus outdoor farrowing and raising of swine. Project Report, Univ. of Alta., Edmonton, Canada.

Cottle, Merva and L.D. Carlson. 1956. Turnover of thyroid hormone in cold-exposed rats determined by radioactive iodine studies. Endocrin. 59:1-11.

Cox, D.F., J.E. Legales and C. Clark Cockerham. 1959. Maternal influence of body weight. J. Animal Sci. 18: 519-527.

Crampton, E.W. and B. Elaine Rutherford. 1954. Apparent digestibility of dietary protein as a function of protein level. J. Nutr. 54:445-451.

Dempsey, E.W. and E.B. Astwood. 1943. Determination of the rate of thyroid hormone secretion at various environmental temperatures. Endocrin. 32:509-518.

Devel, Harry J. Jr., Irene Sandiford, Kathleen Sandiford and Walter M. Boothby. 1928. The effect of thyroxine on the respiratory and nitrogen metabolism of a normal subject following prolonged nitrogen free diet. J. Biol. Chem. 76:407-414.

Draper, H.H. and J.A. Firth. 1957. The regression of thyroid weight on body weight in the growing chick and the influence of penicillin. Poultry Sci. 36:42-45.

Fenton, Paul F. 1956. Growth and fat deposition in the mouse. A definition of obesity. Amer. J. Physiol. 184:52-54.

Fenton, Paul F. and Claire J. Carr. 1951a. The nutrition of the mouse. X. Studies on the utilization of high and moderately low protein diets for growth in four strains of mice. J. Nutr. 43:441-450.

Fenton, Paul F. and Claire J. Carr. 1951b. The nutrition of the mouse. XI. Response of four strains to diets differing in fat content. J. Nutr. 45:223-233.

Fenton, Paul F. and Marian T. Dowling. 1953. Studies on Obesity. I. Nutritional obesity in mice. J. Nutr. 49:319-331.

Fenton, Paul F. and John M. Marsh. 1956. Inherited metabolic patterns in mice. Caloric requirements for protein utilization and determination of protein minima. J. Nutr. 60:465-472.

Fish, W.A., H. Carlin, and F.C. Hickey, O.P. 1952. The thyroidal uptake, retention and iodine pool in rats under controlled iodide diet. Endocrin. 51:282-292.

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

Forbes, R.M., Lucille Vaughan and Martha Yohe. 1958. Dependence of biological value on protein concentration in the diet of the growing rat. J. Nutr. 64:291-302.

Glazener, E.W., C.G. Shaffner and M.A. Jull. 1949. Thyroid activity as related to strain differences in growing chicks. Poultry Sci. 28:834-849.

Goulden, C.H. 1956. Methods of statistical analyses, 2nd Ed. John Wiley and Sons, Inc., London.

Gowe, R.G. 1956. Environment and poultry breeding problems. 2. A comparison of the egg production of 7 s.c. White Leghorn strains housed in laying batteries and floor pens. Poultry Sci. 35:430-435.

Henneman, H.A., E.P. Reineke and S.A. Griffin. 1955. The thyroid secretion rate of sheep as affected by season, age, breed, pregnancy and lactation. J. Animal Sci. 14:419-434.

Homb, T. and K. Breirem. 1952. The use of fecal nitrogen as a measure of dry matter intake and of digestibility of organic matter in forage. J. Animal Sci. 11:496-500.

Honeyman, Merton S. 1957. A quantitative genetic study of a nutritional strain difference in mice. J. Heredity 48:84-87.

Hurst, Victor and C.W. Turner. 1947. The thyroid secretion rate of growing and mature mice. Am. J. Physiol. 150: 686-692.

Hussar, N. and J.P. Bowland. 1959. Rapeseed oil meal as a protein supplement for swine and rats. II. Energy and nitrogen digestibility and nitrogen retention. Can. J. Animal Sci. 39:94-101.

Johnson, H.D. and A.C. Ragsdale. 1959. Changes in thyroid I^{131} release rate during growth in Holstein, Brown Swiss, and Jersey calves at constant environmental temperatures of 50° and 80°F. J. Dairy Sci. 42:1821-1830.

King, J.W.B. and G.B. Young. 1955. A study of three breeds of sheep wintered in four environments. J. Agr. Sci. 45:331-338.

Kunkel, H.O., R.W. Colby and C.M. Lyman. 1953. The relationship of serum protein bound iodine levels to rates of gain in beef cattle. J. Animal Sci. 12:3-9.

Lassiter, J.W., S.W. Terrill, D.E. Becker and H.W. Norton. 1956. Protein levels for pigs as studied by nitrogen balance. J. Animal Sci. 15:392-399.

Likuski, H.J. 1959. Energy utilization and nitrogen retention by swine and rats fed rations varying in energy and protein level. M.Sc. thesis, Univ. of Alta., Edmonton, Canada.

Lloyd, L.E. and E.W. Crampton. 1955. The apparent digestibility of the crude protein of the pig ration as a function of its crude protein and crude fibre content. J. Animal Sci. 14:693-699.

Lucas, I.A.M. and H.F.C. Calder. 1956. The response of different types of pigs to varying levels of feeding from weaning to bacon weight, with particular reference to carcass quality. J. Agr. Sci. 47:287-323.

Lyon, J.B., Jr. 1956. Thyroid activity of inbred strains of mice. Fed. Proc. 15:122.

Lyon, John B., Jr. 1957. Muscle and liver glycogen levels in lean and obese strains of mice. Amer. J. Physiol. 190:434-438.

Lyon, John B., Jr., Marion T. Dowling and Paul F. Fenton. 1953. Studies on obesity. II. Food intake and oxygen consumption. J. Nutr. 51:65-70.

Lyon, John B., Jr., and Paul F. Fenton. 1956. Muscle and liver glycogen of mouse strains susceptible or resistant to nutritionally induced obesity. Am. J. Physiol. 187:415-416.

Lyon, J. B., Jr., Harry L. Williams and Eugene A. Arnold. 1958. The pyradoxine-deficient state in two strains of inbred mice. J. Nutr. 66:261-275.

Mellen, W.J. and F.W. Hill. 1953. Effects of thiouracil, thyroprotein and estrogen upon the basal metabolism and thyroid size of growing chickens. Poultry Sci. 32:994-1001.

Mixner, J.P., E.P. Reineke and C.W. Turner. 1944. Effect of thiouracil and thiourea on the thyroid gland of the chick. Endocrin. 34:168-174.

Mixner, John P. and Charles W. Upp. 1947. Increased rate of thyroxin secretion by hybrid chicks as a factor in heterosis. Poultry Sci. 26:389-395.

Monroe, R.A. and C.W. Turner. 1946. Research Bull. 403. Univ. of Missouri, Coll. of Agr. Exp. Station, Columbia, Missouri. (As reported by Hurst and Turner, 1947)

Mukherjee, R. and H.H. Mitchell. 1951. The basal energy and nitrogen metabolism of dairy calves in hyperthyroidism induced by thyroprotein administration. J. Animal Sci. 10:149-162.

Palmer, Leroy S., Cornelia Kennedy, Charles E. Calverley, Cecil Lohn and Paul H. Weswig. 1946. Genetic differences in the biochemistry and physiology influencing food utilization for growth in rats. Tech. Bull. 176. Univ. of Minnesota Ag. Exp. Sta., St. Paul, Minnesota.

Perry, William F. 1951. A method for measuring thyroid hormone secretion in the rat with its application to the bio assay of thyroid extracts. Endocrin. 48: 643-650.

Perry, T.M., W.M. Beeson and F.N. Andrews. 1950. The effect of thyroprotein on the growth and carcass composition of swine. J. Animal Sci. 9:48-53.

Peterson, D.W., C.R. Grau and N.F. Peek. 1954. Growth and food consumption in relation to dietary levels of protein and fibrous bulk. J. Nutr. 52:241-257.

Pipes, G.W. and C.W. Turner. 1956. The effect of thyroxin on thyroid function. Res. Bull. 617. Univ. of Missouri, Coll. of Agr. Exp. Sta., Columbia, Missouri.

Premechandra, B.N., G.W. Pipes and C.W. Turner. 1959a. Study of growth in New Hampshire chickens with varying thyroid status. Poultry Sci. 38:795-798.

Premechandra, B.N., G.W. Pipes and C.W. Turner. 1959b. Thyroid function of mono- and dizygous cattle twins. J. Dairy Sci. 42:1346-1350.

Reineke, E.P. and O.N. Singh. 1955. Estimation of thyroid hormone secretion rate of intact rat. Proc. of the Soc. for Exp. Biol. and Med. 88:203-207.

Schultze, M.O. 1954. Weight increments of suckling rats as affected by litter size and maternal diet. J. Nutr. 54:453-460.

Schultze, A.B. and C.W. Turner. 1945. The determination of the rate of thyroxin secretion by certain domestic animals. Res. Bull. 392. Univ. of Missouri, Coll. of Agr. Exp. Sta., Columbia, Missouri.

Shaklee, William C. and C.W. Knox. 1956. Selection for thyroid weight in New Hampshire chickens. J. Heredity 47:211-212.

Shaklee, William E. and C.G. Shaffner. 1952. High and low thyroidal response to the feeding of thiouracil to New Hampshire chickens. J. Heredity 43:238-242.

Sibbald, I.R. 1957. Energy and nitrogen in the food of the weanling rat. Ph.D. thesis, Univ. of Alta., Edmonton, Canada.

Singh, O.N., H.A. Henneman and E.P. Reineke. 1956. The relationship of thyroid activity to lactation, growth, and sex in sheep. J. Animal Sci. 15:625-630.

Soliman, F.A. and H.M. Badawi. 1956. Levels of thyroid and thyrotrophic hormones in blood of rats at various stages of estrus cycle. Nature 177:235.

Soliman, F.A. and E.P. Reineke. 1954. Changes in uptake of radicactive iodine by the thyroid of the rat during the estrus cycle. Amer. J. Physiol. 178:89-90.

Sternheimer, Richard. 1939. The effect of a single injection of thyroxin on carbohydrates, protein and growth in the rat liver. Endocrin. 25:899-908.

Stevens, Catherine E. and S.A. D'Angelo. 1954. Relative rates of biological decay of thyroidal I^{131} in rat and guinea pig. Proc. Soc. Exp. Biol. Med. 85:633-635.

Terrill, S.W., T.S. Hamilton, J.L. Krider and W.A. Carroll. 1950. Carcass composition and nitrogen balances of swine fed thiouracil. J. Animal Sci. 9:58-65.

Vander Noot, George W., Ralph P. Reece and William C. Skelley. 1950a. Effects of methyl thiouracil in the rations of lambs and aged ewes. J. Animal Sci. 9:3-6.

Vander Noot, George W., Ralph P. Reece and William C. Skelley. 1950b. Effect of methyl thiouracil and thiouracil in swine. J. Animal Sci. 9:54-57.

Warwick, E.J. and W.L. Lewis. 1954. Growth and reproductive rates of mice produced by intercrossing inbreds and outbreds of 3 size groups. J. Heredity 45:35-38.

Wolff, J. 1951. Some factors that influence the release of iodine from the thyroid gland. Endocrin. 48:284-297.

Appendix Table 1A. Gain and Food Data from the Main Metabolism Experiment --- Genotype x Replicate Means.

GENOTYPE	REPLICATE	NO. OF RATS	ABW ON TRIAL	PER 100 GM. ABW			FOOD CONS. PER GM.GAIN
				5-DAY TRIAL GAIN	FOOD CONS. (O.D.BASIS)	GROSS N CONS.	
			gm.	gm.	gm.	gm.	gm.
W	1	5	77.2	29.6	71.5	1.91	2.4
	2	6	73.3	23.1	69.4	1.92	3.5
	3	5	73.7	19.1	62.8	1.78	3.8
	4	6	76.0	29.4	76.3	2.10	2.7
	Mean		75.0	25.4	70.3	1.93	3.1
S	1	6	75.6	31.0	68.7	1.85	2.3
	2	5	79.3	31.4	70.9	2.12	2.3
	3	5	72.4	23.3	63.4	1.87	3.7
	4	6	82.5	33.3	70.9	1.89	2.2
	Mean		77.6	30.0	68.6	1.93	2.6
W x S	1	6	77.5	34.2	72.1	1.97	2.1
	2	6	79.7	30.7	66.4	1.76	2.2
	3	6	79.7	30.4	70.9	1.91	2.4
	4	6	82.6	36.6	83.7	2.23	2.3
	Mean		79.9	33.0	73.3	1.97	2.3
S x W	1	6	78.0	34.5	76.1	2.04	2.3
	2	6	78.8	31.9	73.6	1.99	2.4
	3	6	81.4	37.5	77.5	1.99	2.1
	4	5	78.8	35.4	78.0	2.03	2.2
	Mean		79.3	34.8	76.2	2.01	2.3

Appendix Table 1B. Gain and Food Data from the Main Metabolism Experiment --- Diet x Replicate Means.

				PER 100 GM. ABW			
DIET	REPLICATE	NO. OF RATS	ABW ON TRIAL	5-DAY TRIAL GAIN	FOOD CONS. (O.D.BASIS)	GROSS N CONS.	FOOD CONS. PER GM.GAIN
			gm.	gm.	gm.	gm.	gm.
LP	1	8	75.3	27.7	70.1	0.97	2.6
	2	7	75.2	20.9	66.8	0.93	3.5
	3	7	73.5	24.1	71.3	0.97	3.8
	4	8	76.7	27.5	77.4	1.05	2.9
	Mean		75.2	25.2	71.6	0.98	3.2
MP	1	7	78.3	35.4	75.0	2.01	2.1
	2	8	78.7	32.9	72.2	1.94	2.2
	3	7	77.8	26.4	65.2	1.74	3.0
	4	7	81.0	36.0	79.4	2.14	2.2
	Mean		79.0	32.7	72.9	1.96	2.4
HP	1	8	77.9	34.6	71.6	2.86	2.1
	2	8	78.9	32.8	70.7	2.82	2.2
	3	8	79.7	33.2	70.7	2.83	2.2
	4	8	82.4	37.6	75.0	3.01	2.0
	Mean		79.7	34.6	72.0	2.88	2.1

Appendix Table 1C. Gain and Food Data from the Main Metabolism Experiment --- Sex x Replicate Means.

				PER 100 GM. ABW			
SEX	REPLICATE	NO. OF RATS	ABW ON TRIAL	5-DAY TRIAL GAIN	FOOD CONS. (O.D.BASIS)	GROSS N CONS.	FOOD CONS. PER GM.GAIN
			gm.	gm.	gm.	gm.	gm.
M	1	11	78.7	34.6	73.9	1.97	2.1
	2	8	80.1	30.7	73.1	1.99	2.7
	3	11	77.4	29.6	69.6	1.93	2.7
	4	11	81.6	35.1	79.8	2.23	2.3
	Mean		79.4	32.6	74.2	2.03	2.5
F	1	12	75.6	30.4	70.5	1.92	2.4
	2	15	76.5	28.4	68.4	1.91	2.5
	3	11	76.8	26.7	68.7	1.85	3.2
	4	12	78.6	32.2	74.8	1.91	2.4
	Mean		76.9	29.4	70.5	1.90	2.6

Appendix Table 1D. Gain and Food Data from the Main Metabolism Experiment --- Genotype x Diet and Sex x Diet Means.

				PER 100 GM. ABW			
GENOTYPE	DIET	NO. OF RATS	ABW ON TRIAL	5-DAY TRIAL GAIN	FOOD CONS. (O.D.BASIS)	GROSS N CONS.	FOOD CONS. PER GM.GAIN
			gm.	gm.	gm.	gm.	gm.
W	LP	8	71.8	18.9	66.5	0.91	4.0
	MP	6	75.8	27.8	72.2	1.95	2.7
	HP	8	77.7	30.1	72.5	2.94	2.4
S	LP	6	72.7	22.3	68.5	0.94	3.5
	MP	8	77.9	31.5	67.9	1.83	2.5
	HP	8	80.9	34.2	69.4	2.77	2.1
W x S	LP	8	79.6	29.4	72.7	1.00	2.5
	MP	8	81.1	34.0	75.0	2.02	2.3
	HP	8	78.9	35.6	72.1	2.88	2.0
S x W	LP	8	76.2	29.6	77.7	1.06	2.7
	MP	7	80.4	36.7	76.9	2.04	2.1
	HP	8	81.4	38.3	74.1	2.93	1.9
SEX							
M	LP	13	78.3	27.9	74.4	1.02	2.9
	MP	13	78.8	33.5	75.1	2.02	2.5
	HP	15	80.8	35.9	73.2	2.92	2.1
F	LP	17	72.9	23.1	69.4	0.95	3.4
	MP	16	79.1	32.0	71.1	1.91	2.3
	HP	17	78.7	33.3	71.0	2.84	2.2

Appendix Table 2A. Apparent Digestibility and Nitrogen Retention from the Main Metabolism Experiment --- Genotype x Replicate Means.

GENOTYPE	REPLICATE	NO. OF RATS	ABW ON TRIAL	APPARENT DIGESTIBLE FOOD	APPARENT DIGESTIBLE N	RETENTION TOTAL N(1)	RETENTION GROSS N	RETENTION ADN
			gm.	%	%	gm.	%	%
W	1	5	77.2	77.3	91.3	0.92	55.2	60.9
	2	6	73.3	75.9	87.5	0.85	49.7	57.5
	3	5	73.7	77.1	86.3	0.81	51.6	60.6
	4	6	76.0	78.6	91.5	1.05	56.1	61.5
	Mean		75.0	77.2	89.2	0.91	53.2	60.1
S	1	6	75.6	77.2	89.9	0.95	56.8	63.8
	2	5	79.3	76.5	87.0	1.36	64.6	74.7
	3	5	72.4	78.0	87.7	0.88	49.0	55.6
	4	6	82.5	79.3	92.5	1.03	59.2	64.3
	Mean		77.6	77.8	89.4	1.05	57.5	64.6
W x S	1	6	77.5	77.5	90.9	1.00	58.0	64.2
	2	6	79.7	76.2	88.3	1.13	67.0	76.5
	3	6	79.7	77.5	91.2	0.90	54.6	60.3
	4	6	82.6	77.9	91.8	1.16	58.0	63.5
	Mean		79.9	77.3	90.5	1.05	59.4	66.1
S x W	1	6	78.0	77.5	92.2	1.05	57.7	62.9
	2	6	78.8	78.1	89.3	1.17	63.1	71.0
	3	6	81.4	78.0	92.4	1.11	61.0	66.3
	4	5	78.8	78.7	92.7	1.09	61.1	66.3
	Mean		79.3	78.0	91.6	1.10	60.7	66.7

(1) Per 100 gm. ABW.

Appendix Table 2B. Apparent Digestibility and Nitrogen Retention from the Main Metabolism Experiment --- Diet x Replicate Means.

DIET	REPLICATE	NO. OF RATS	ABW ON TRIAL	APPARENT DIGESTIBLE		RETENTION		
				FOOD	N	TOTAL N(1)	GROSS N	ADN
			gm.	%	%	gm.	%	%
LP	1	8	75.3	77.8	88.4	0.76	77.9	88.1
	2	7	75.2	77.2	84.2	0.68	72.5	86.1
	3	7	73.5	79.0	86.9	0.74	75.3	86.4
	4	8	76.7	79.4	89.8	0.82	78.0	86.8
	Mean		75.2	78.4	87.4	0.75	76.0	86.9
MP	1	7	78.3	77.4	91.7	1.08	53.9	58.7
	2	8	78.7	75.2	88.0	1.17	60.2	68.6
	3	7	77.8	77.9	90.1	0.88	49.6	54.8
	4	7	81.0	78.7	92.8	1.19	55.5	59.8
	Mean		79.0	77.2	90.5	1.08	55.0	60.8
HP	1	8	77.9	76.9	93.2	1.11	38.9	41.8
	2	8	78.9	77.7	91.5	1.45	51.6	56.4
	3	8	79.7	76.2	91.6	1.14	40.3	44.1
	4	8	82.4	77.9	93.7	1.25	41.6	44.4
	Mean		79.7	77.2	92.5	1.24	43.1	46.7

(1) Per 100 gm. ABW.

Appendix Table 2C. Apparent Digestibility and Nitrogen Retention from the Main Metabolism Experiment --- Sex x Replicate Means.

				APPARENT DIGESTIBLE		RETENTION		
SEX	REPLICATE	NO. OF RATS	ABW ON TRIAL	FOOD	N	TOTAL N[1]	GROSS N	ADN
			gm.	%	%	gm.	%	%
M	1	11	78.7	77.5	91.4	1.00	57.9	63.6
	2	8	80.1	76.9	88.0	1.29	67.3	76.8
	3	11	77.4	77.0	90.0	0.95	53.8	59.8
	4	11	81.6	78.4	92.4	1.17	57.7	62.6
	Mean		79.4	77.5	90.6	1.09	58.6	64.9
F	1	12	75.6	77.2	90.7	0.96	56.3	62.5
	2	15	76.5	76.5	88.1	1.03	57.6	65.9
	3	11	76.8	78.3	89.2	0.91	55.0	62.1
	4	12	78.6	78.8	91.8	1.00	59.2	65.0
	Mean		76.9	77.6	89.9	0.98	57.1	64.0

(1) Per 100 gm. ABW.

Appendix Table 2D. Apparent Digestibility and Nitrogen Retention from the Main Metabolism Experiment --- Genotype x Diet and Sex x Diet Means.

GENOTYPE	DIET	NO. OF RATS	ABW ON TRIAL	APPARENT DIGESTIBLE		RETENTION		
				FOOD	N	TOTAL N(1)	GROSS N	ADN
			gm.	%	%	gm.	%	%
W	LP	8	71.8	77.5	85.6	0.65	70.5	82.1
	MP	6	75.8	77.7	90.6	0.98	50.2	55.4
	HP	8	77.7	76.5	91.6	1.12	38.0	41.5
S	LP	6	72.7	79.5	87.0	0.70	73.4	84.3
	MP	8	77.9	76.2	88.5	1.04	55.7	63.0
	HP	8	80.9	78.0	92.1	1.31	47.3	51.4
W x S	LP	8	79.6	77.7	87.5	0.79	79.0	90.4
	MP	8	81.1	77.7	91.4	1.14	56.3	61.7
	HP	8	78.9	76.4	92.7	1.22	42.8	46.3
S x W	LP	8	76.2	79.0	89.5	0.86	80.6	90.1
	MP	7	80.4	77.4	91.8	1.16	56.7	61.7
	HP	8	81.4	77.7	93.5	1.31	44.4	47.5
SEX								
M	LP	13	78.3	78.5	88.5	0.80	78.3	88.5
	MP	13	78.8	77.4	90.8	1.11	54.5	59.8
	HP	15	80.8	76.8	92.3	1.32	45.0	48.9
F	LP	17	72.9	78.3	86.6	0.71	74.3	85.7
	MP	16	79.1	77.1	90.3	1.06	55.4	61.6
	HP	17	78.7	77.5	92.7	1.17	41.5	44.7

(1) Per 100 gm. ABW.

Appendix Table 3. Series of Example Calculations Illustrating the Procedure for Correcting Counts(1) and Calculating Thyroid I^{131} Decay

	1 INITIAL	2 BACKGROUND	3 CC_1(2)	4 ISOTOPE DECAY FACTOR*	5 CC_2	6 MACHINE FACTOR**	7 CC_3
			(1-2)		(3÷4)		(5x6)
Standard (Hypothetical)							
Base day	61,000	1,000	60,000	1.0000	60,000	1.0000	
Base plus 2 days	52,300	1,100	51,200	0.8434	60,707	0.9884	
Rat "x" (Hypothetical)							
Base day	82,000	2,000	80,000	1.0000	80,000	1.0000	80,000
Base plus 2 days	55,000	1,900	53,100	0.8434	62,959	0.9884	62,229

Thyroid I^{131} Decay for Rat "x" on Base Plus 2 Days

Per cent of Base Count = 62,229 ÷ 80,000 x 100 = 77.8%

Log (per cent of Base Count) = log (77.8%) = 1.891

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(1) All counts as counts per minute.
(2) CC refers to corrected count.
* Derived from decay tables.
** Machine factor = CC_2 base day ÷ CC_2 base plus 2 days (Standard). This factor is then inserted in the same column for Rat "x".

Appendix Table 4. Summary of Thyroid I^{131} Decay Data from the Main Thyroid Experiment.

REPLICATE	DAYS AFTER I^{131} INJECTION	PERIOD 1	PERIOD 2	PERIOD 3
		Log (% Base Count)		
1	4	1.813	1.841	1.830
	6	1.675	1.780	1.796
	8	1.557	1.682	1.634
	10	1.477	1.635	1.572
2	4	1.849	1.824	1.808
	6	1.701	1.753	1.737
	8	1.582	1.651	1.567
	10	1.545	1.606	1.487
3	4	1.833	1.868	1.832
	6	1.703	1.810	1.775
	8	1.601	1.712	1.613
	10	1.504	1.664	1.543
GENOTYPE				
W	4	1.829	1.849	1.822
	6	1.686	1.776	1.766
	8	1.570	1.667	1.599
	10	1.495	1.618	1.515
S	4	1.817	1.830	1.821
	6	1.662	1.779	1.785
	8	1.538	1.699	1.637
	10	1.452	1.674	1.594
W x S	4	1.857	1.853	1.836
	6	1.742	1.787	1.768
	8	1.642	1.680	1.591
	10	1.584	1.618	1.521
S x W	4	1.841	1.856	1.821
	6	1.725	1.793	1.756
	8	1.631	1.686	1.582
	10	1.578	1.622	1.497

(Continued)

Appendix Table 4 (Continued). Summary of Thyroid I^{131} Decay Data from the Main Thyroid Experiment.

SEX	DAYS AFTER I^{131} INJECTION	PERIOD 1	PERIOD 2	PERIOD 3
		Log (% Base Count)		
M	4	1.821	1.854	1.802
	6	1.671	1.782	1.769
	8	1.549	1.669	1.616
	10	1.472	1.606	1.524
F	4	1.839	1.837	1.839
	6	1.709	1.782	1.770
	8	1.603	1.693	1.599
	10	1.534	1.659	1.543
DAYS AFTER INJECTION				
	4	1.831	1.845	1.823
	6	1.693	1.782	1.770
	8	1.580	1.685	1.606
	10	1.508	1.634	1.535