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Title: COMPARATIVE VALUES OF LIVER NICOTINAMIDE NUCLEOTIDE COENZYMES, ENDOCRINE GLANDS, AND THEIR RELATIONSHIPS WITH PRODUCTION TRAITS OF DIALLEL CROSSED BEEF BULLS

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This investigation for the first time reports information on the correlation of various forms and ratios of nicotinamide nucleotide coenzymes in the liver tissue and endocrine glands weight with various production traits. Fifty-five inbred and line cross beef bulls raised under standard management conditions and slaughtered at 1000 lbs. body weight were used in the study.

Fluorometeric determinations of the nicotinamide nucleotide coenzymes showed that NADH was present in lower concentration per gram of frozen liver tissue than NAD^+ while the concentration of $NADP^+$ was lower than NADPH per gram of frozen liver tissue. All forms of coenzymes showed a positive significant correlation with age (at 1000 lbs. body weight) and percent fat in the body. The coenzymes in the meantime also showed a significant negative correlation with percent lean in the carcass. Thyroid gland weight also showed a

negative correlation with age, percent fat in the carcass and all the coenzymes which were positively associated with age and percent fat in the carcass. Thyroid gland weight was positively correlated with the $NADP^+$/NADPH ratio since this ratio had a negative association with percent fat in the carcass.

There was a positive correlation of thyroid weight with the $NADP^+$/NADPH ratio. The latter ratio showed a significant positive association with feed per unit gain. Feed per unit gain, in the meantime, was positively associated with $NADP^+$ and negatively associated with NAD^+, percent lean in the carcass and gain per day.

As age at 1000 lbs. body weight increases, the amount of thyroid tissue at 1000 lbs. body weight decreases and simultaneously feed per unit gain and percent fat in the carcass increases while percent lean and gain per day decreases.

No significant correlation of any kind was observed between pituitary gland weight and production traits. This was attributed to the involvement of this gland with various hormones that may have antagonistic inducing action on these coenzymes. Evidence obtained, however, indicates both hormones (STH and TSH) increases percent lean, water content and gain per day and decreases percent fat in the carcass. Based on this evidence a model for the action of these hormones was presented.

Inbreeding showed a negative significant correlation with percent lean in the carcass. In the meantime inbreeding was

positively associated with total coenzymes ($NAD^+ + NADP^+$, NADH + NADPH). These coenzymes all have a significant negative correlation with percent lean in the carcass. Heterozygosity, therefore, seems to have a heterotic effect on growth as it increases percent lean in the carcass whereas it shows a negative heterotic effect on coenzyme concentration in liver tissue. This seems proper since the above coenzymes have a significant negative correlation with percent lean in the carcass. High percent lean and low coenzyme levels were characteristics associated with the line crosses while the opposite was true of inbred animals.

Lines and line crosses showed statistically significant differences in coenzymes and production traits. This trend was also true for the average of parents compared with their offspring. Although statistically significant differences were not obtained when all inbreds of 17 and 21 percent inbreeding were compared with line crosses, a trend indicating higher gain per day, lower feed per unit gain and lower coenzyme levels, was noted in the line crosses. Diallel analysis showed no general combining ability for either the coenzymes or production traits. Specific combining ability and reciprocal effects were shown to exist for various coenzymes and production traits.

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INTRODUCTION

Higher organisms are made up of well organized groups of cells specialized to carry out specific functional activities which are needed for the survival of the organism. The health of the organism depends upon the health of the cells of which it is composed. Life of higher organisms depends on organized, coordinated and integrated functional activities of their cellular constitutions. If certain cells in certain critical parts (brain, respiration, heart) die, the organism dies, but death of the cell at other parts of the body might not bring immediate death to the organism. The loss of cells will have an impact on the general well-being of the individual and such an individual will usually deviate in its behavior from the one which is normal. Studies at the cellular level reveal that the death of a cell is the result of loss of function of the cell to maintain itself. As the death of the organism can be explained on the basis of its cellular constitution, the death of a cell can also be studied in terms of its components which enable the cell to function. The animal system is a mechanical structure operated through chemical reactions carried out on the intracellular level. When we consider biochemical reactions as the bases of functional activities of the cell, two components of the cell

namely, enzymes and cellular organelle, (as mechanical means within which biochemical reactions are carried out) attract our attention. Loss of function of a cell can be attributed to the loss of any of the components of the cell which would cause the death of the cell in the same manner as the death of cells lead to the death of the organism. From the foregoing discussion it is logical to conclude that the answer to life and death is to be investigated at the intracellular level.

Disruption in the function and also in the coordination of the integrated, organized system which is supposed to be under the hormonal and nervous control in higher organisms also leads to abnormalities and lethalities. It is now known that control mechanisms also exist in simple forms of life (unicellular) which do not have nerves and presumed not to have hormones. According to Krebs (1958) energy can be obtained by fermentation if air is not available or by oxidation if air is available. He indicates that air in this case, serves as a control which stops fermentation. Other control mechanisms in lower organisms, according to the author, are those processes which coordinate the chemical synthesis of elements in relation to the requirement. Krebs indicates that although feedback inhibition is the fundamental controlling mechanism of biochemical reactions, it is different than the regulation by hormones which usually act by their direct effect on enzymes.

If enzyme reactions can be granted as basis of cellular activities, one would expect that any change in the quality and quantity of these catalysts would bring about a change in the biochemical reactions and thus would regulate the living process. It is now fairly clear that the synthesis of enzymes, as well as their relationship to biochemical reactions and metabolism is under genetic control (Garrod, 1963; Beadle and Tatum, 1941; Beadle, 1945; Beadle, 1959; Yonofsky and Crawford, 1959; Jacob and Monad, 1961).

The function of an animal breeder is to manipulate the genetic constitution of his livestock with the objective of developing the kind of genetic combination that has the type of metabolism which is quantitatively and qualitatively superior for efficiency of production. Developing purebred sires possessing desirable genetic material for use in commercial herds or for the improvement of the progenies of scrub females at this stage of animal genetics is a must. For the development and maintenance of such pure sires, however, the development of lines within a breed is the result of a wise program of inbreeding coupled with selection. Although inbreeding is a part of animal improvement, it often carries with it the characteristic side effect of loss of vigor in various aspects of animal production. Although the definite basis and the consequences resulting from various degrees of homozygosity and heterozygosity are not well established, the loss of vigor, especially in early life traits, are

attributed to the fixation of loci, and heterozygosity is considered to overcome this problem. Alexander and Bogart (1961), for example, showed that suckling gain in inbred calves was significantly depressed compared to linecross calves. They also showed that age at 500 lb. and 800 lb. body weight was affected. The development of inbred lines serves a useful purpose in making all possible line crosses when the economic traits under studies have plateaued. All possible line crosses (diallel crosses) make it possible to exploit further heterotic effect on one hand, and on the other hand, these crosses serve as a basis for the evaluation of genetic composition of the lines. In addition, diallel crosses make it possible to evaluate differences in various genetic combinations as general and specific combining abilities and maternal effects. The diallel method of study was originally proposed by Schmidt (1922).

Variation in economic traits seems to exist and they can be demonstrated to have a genetic basis. The basis for these variations however lies in those biochemical reaction regulated through various other agents making up the metabolism of the organism, the complexity of which has made the life of a scientist more interesting and his time well spent. Although the ease of handling associated with simple organisms and laboratory animals to be used for metabolic studies have provided us with ample information, more knowledge is needed regarding the type of metabolism of large animals. Lindsay

(1959) indicates that carbohydrate metabolism of ruminants is different from that of non-ruminants. Laboratory animals use carbohydrates for the production of glucose, part of which is oxidized, the rest stored as fat, whereas utilization of carbohydrate by ruminant predominantly results in volatile fatty acids. Of these, mainly propronic acid may be converted to glucose while most of the acetate is used for fat deposit.

Changes in the metabolic pattern of an animal takes place as a part of the aging process. Changes in the level of endocrine secretion and in enzyme level takes place from the embryonic stages of life to adulthood and maturity. Changes in the metabolic pattern too is associated with the normal aging process from a young animal to maturity. It is now fairly well known that as an animal reaches maturity,rate of gain and feed efficiency in beef cattle decrease. Blood and urine constituents as metabolic end products differ in a good doing animal from that in an animal that gains slowly. Several studies have shown that (Price, et al., 1956; Bogart, et al., 1963; and Clark, et al., 1963), calves gaining at a rapid rate have low urea excretion and lower amino acid and urea nitrogen in the blood and that the metabolism of rapidly and economically gaining animals resemble those of young calves. On the other hand, the metabolism of poor doing animals resembles the metabolism of mature animals. The basis of the foregoing observation and other aspects of

metabolism may be understood from the development of the enzymatic pattern at various stages of development in various animals. The hexokinase activity in the intestinal mucosa and brain of rats, lambs, and sheep was studied (Jarrett and Filsell, 1958) to determine the level of activity in both tissues of these species. It was shown that the activity was greater in the rat than in lambs, and that the activity for suckling lambs (one-half to five days old) was higher than the activity for either adult sheep or 7 to 35 day-old lambs. The increase in activity of hexokinase and phosphoglucose isomerase in the lactating rat mammary gland tissue at various stages was shown (McLean 1958a) to be much smaller than the increase in activity of both glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase in this tissue. Gallagher and Buttery (1959) showed that sheep liver tissue has, in general, 1/2 to 1/3 the enzymatic activities reported for rats and that young sheep had lower cytochrome-c-oxidase than adult sheep. Studying a number of enzymes at various age groups of rats Burch et al. 1963) concluded that the fetal stages had a moderate capacity for glycolysis with a transition starting just before birth to a capability for utilization of lactic acid and conversion of both lactate and glycogen to glucose. Dennis (1966) using liver biopsies on calves 2 days through eight-weeks old analyzed 4 Kreb-cycle enzymes plus 3 glycolytic enzymes and G-6 phosphate dehydrogenase (as a measure of pentose-phosphate

pathway) and showed that all except the phospho-glycerate kinase and isocitric dehydrogenase, both of which increased the activity of all the rest of the enzymes under investigation, decreased with age. It is suggested that the decrease in activity of glycolytic enzymes is due to the development of the rumen, the reduction in absorption of glucose, and the dependency of the animal on volatile fatty acids. According to this study, the Kreb cycle enzymes were also decreased over the eight weeks study period which was attributed to the normal aging process. The difference was in the high level of G-6 phosphate dehydrogenase in the newborn calf indicating the pentose phosphate pathway. Dennis (1966) suggests that since the level of G-6-phosphate dehydrogenase reported in the liver of newborn rats and guinea pigs is very low, the level of this enzyme in the newborn seems to be the only difference between ruminants and monogastric animals. Young lambs removed acetate from the blood stream within 30 minutes after the injection of acetic acid in their jugular veins while in the same experiment (Jarrett and Filsell, 1960) showed that it required 60 minutes for older sheep to do so. They concluded that the young behave in many respects like non-ruminants and gradually develop some of the metabolic characteristics of the adult sheep. The metabolic adaptation of calves and cows was studied (Bartley, et al., 1966) with the infusion of glucose in the jugular vein and determination of glucose-6-phosphatase and

phosphorylase in the liver biopsies. Their study showed that the activity of glucose-6-phosphatase increased for 8-12 weeks in calves indicating that a metabolic adaptation for the decrease in availability of glucose from the intestines is associated with rumen development. They also demonstrated that the infusion of glucose intra-duodenally into calves over 15 weeks of age and in cows decreased the activity of both enzymes to the level observed in the calves with non-functional rumen, providing evidence that the response was due to the availability of glucose.

Biochemical pathways clearly demonstrate that nicotinamide nucleotide coenzymes play an important role as cofactors of many dehydrogenase enzymes in biochemical reactions. Nicotinamide nucleotide coenzyme, the presence of which is being studied in beef liver in the present investigation is also known by various names. Objections to the various early terms designating these coenzymes warranted a new terminology. The nomenclature used in the present study are those proposed in a report by the Commission on Enzymes in (1961). The commission suggested that nicotinamide adenine dinucleotide (NAD) be used instead of the early terminologies for this coenzyme which were diphosphopyridine nucleotide (DPN), cozymase, codehydrogenase I or codehydrase I and coenzyme I (Col) and that nicotinamide adenine dinucleotide phosphate (NADP) be used in place of triphosphopyridine nucleotide phosphate (TPN),

phosphocozymase, codehydrogenase II or codehydrase II and coenzyme II. The reduced forms of the above coenzymes are designated as NADH and NADPH for NAD and NADP respectively. The term nicotinamide nucleotide coenzymes is used to designate the sum of both of these coenzymes.

In this study NAD^+ and $NADP^+$ are used to designate the oxidized forms of nicotinamide adenine dinucleotide and nicotinamide adenine dinucleotide phosphate respectively. The total oxidized coenzymes are indicated by the abbreviation $[(NAD^+) + (NADP^+)]$, while the abbreviations [(NADH) + (NADPH)] indicate total reduced coenzymes. The term total coenzymes or nicotinamide nucleotide coenzymes include all the reduced and oxidized forms of NAD and NADP. NAD stands for the sum total of oxidized and reduced forms of NAD, while NADP stands for the sum of oxidized and reduced forms of NADP. NADH or NADPH as such stands for the reduced forms of nicotinamide adenine dinucleotide and nicotinamide dinucleotide phosphate respectively.

Racker (1955) reviews the enzymes with which these nucleotides serve as cofactors. The role of these coenzymes in limiting the rate due to the competitive action of many dehydrogenases is indicated by Dixon (1949). Sir Hans Krebs (1958) presented evidence that nicotinamide nucleotides are rate limiting in the interaction of substrates with dehydrogenases. Dickens, (1959, 1961) explained

the significance of these coenzymes in their relation to the regulation of metabolic pathways of cells and in the metabolism of carbohydrates and their important role as an electron transfer system in the respiratory chain for the production of the major source of energy (ATP).

Morton (1958) indicates that NAD is synthesized in the nucleus of the cell and that its synthetic activity increases during growth. He proposed a mechanism by which cell division seems to be regulated by the level of NAD. The fact that investigations have shown lower levels of NAD in tumor and embryonic than in normal or older tissues suggests that tumor tissue represents a change from adult tissue to embryonic tissue (see Morton, 1958). The nucleus is a unique cellular organelle and it is devoted to the synthesis of DNA, RNA, and NAD, and each of these has a specific and direct effect on the reactions which are carried out in the cytoplasm. DNA and RNA serve as template and messenger for the synthesis of protein including enzymes. The coenzymes influence a dynamic relation between enzymes and substrate involved in these reactions (Morton 1961). The author also indicates that these coenzymes are closely related to the rate of cell division.

It now seems clear that there is a close relationship between nicotinamide nucleotide coenzymes and growth. Enzymes and other regulatory mechanisms operating through the endocrine system,

affect the level of the coenzymes. Thus, they have an effect on rate of growth and efficiency of production. Although information on the level of the nicotinamide nucleotides in the laboratory animal is available, there is limited information on the level of reduced and oxidized forms of these coenzymes in ruminants raised under standard conditions belonging to certain definite genetic groups on which other physiological information under standard conditions is also available. To the knowledge of the author, to date there has been no report on these coenzymes in beef cattle. One of the reasons may be the lack of experimental work with these animals. It was therefore decided that the levels of oxidized and reduced forms of the nicotinamide nucleotide coenzymes be investigated in the liver tissues of a number of calves produced in a diallel cross of beef cattle. These calves were raised under standard experimental conditions and further data on certain economic traits and endocrine glands were also available from them. This thesis presents the above investigation and the interpretation of the results obtained in terms of genetic relationships and physiological importance. The level of these coenzymes in various genetic groups and the relation of the coenzymes to various traits such as rate of gain, feed efficiency, wither height, age, carcass composition, and various endocrine gland weights have received special attention. The methodology in case of practical importance might be adopted as testing

procedures through liver biopsies to serve as guide lines for picking up those differences which cannot be identified with routine testing procedures and thus would enable one to identify those individuals that one wants to serve as the parents of the next generation.

REVIEW OF LITERATURE

When Harden and Young in 1905 (1905a,b) added fresh yeast juice to a solution containing glucose, fermentation immediately began. When they dialyzed the juice they discovered that the juice could no longer continue fermentation of the glucose solution. They showed that although the dialyzed material inside the bag cannot ferment, its fermenting ability can be restored by the addition of the material which had escaped outside into the water as a result of dialysis. They further demonstrated that the dialyzed material can be easily destroyed by heating, but that the material which had diffused out was thermostable. On the basis of these experiments the discoverer concluded that in addition to the undialyzable large thermolabile molecule inside the bag, the yeast juice also contained dialyzable thermostabile material which is required for the fermentation process. The large molecules which were thermolabile and undialyzable in the yeast juice is now known to be a mixture of various enzymes. The dialyzable thermostable small molecule portion of the yeast juice according to McElroy,(1965)are now known to be NAD which was later identified and shown to be a compound containing adenylic, acid nicotinic acid (B Vitamin) and phosphate. Kaplan (1960) in a review on the chemical structures and properties of these coenzymes indicated that NADP can be synthesized from NAD

enzymatically and the difference between NAD and NADP is the additional phosphate which is attached to the adenylic acid. The author indicates that the reactive group for the hydrogen transfer for both of these coenzymes is the pyridine ring which undergoes oxidation and reduction in biological reactions by accepting or donating hydrogen.

The importance of these nucleotides as coenzymes in metabolism as rate limiting factors, their availability in the oxidized and reduced forms as an important regulatory mechanism of cell behavior, their role as an electron transfer system in connection with the respiratory chain, and finally their relationship with cell division and rate of growth is clearly explained (see Krebs, 1958; Dickens, 1959, and 1961; Morton, 1958, and 1961).

Krebs and Hem (1964), in their in vitro study, further demonstrated that NADH was limiting in gluconeogenesis. They showed that the addition of oxaloacetate and pyruvate to a preparation of liver and kidney brought a drastic reduction in the level of NADH. Since NADH is needed for gluconeogenesis to reduce diphosphoglycerate to glyceraldehyde phosphate, the reduction in gluconeogenesis was attributed to the fact that the added acids competed with diphosphoglycerate for NADH and thus limited the availability of NADH for diphosphoglycerate.

Information on the level of nicotinamide coenzymes in

ruminants comes from the work of Caiger, et al., (1962) who analyzed liver tissue of both young and adult sheep and rats for oxidized and reduced forms of nicotinamide nucleotides. There were four merino sheep three years of age and four wether lambs ranging in age from four days to eight weeks. They concluded that in both sheep and rats the concentration of total nicotinamide nucleotides increases during normal growth of the liver and that maximum level was reached when growth ceased. The concentration was similar in the adult sheep and rat and was twice the level present in the liver of young animals on the basis of concentration per (10^8) nuclei. The sheep liver had a higher level of NAD^+ while the rat liver contained a higher concentration of NADPH. They also indicated that the low concentration of nicotinamide nucleotides was a characteristic of rapidly growing animals since young rats and lambs had half the concentration of these coenzymes compared to that of their adulthood. Further study with a large number of animals on this subject came from the work of Filsell, et al., (1963) who assayed the liver of foetal, newborn and adult sheep for the concentration of reduced and oxidized forms of nicotinamide coenzymes. Forty-eight animals ranging in age from two weeks before birth to 50 days after birth and 5 wethers (6 months to 4 years) and 2 adult ewes (5 and 6 year-olds) were used. This work with a large number of animals confirms the earlier work (see Caiger and Morton, 1962) indicating

that rapid rate of growth was associated with low concentration of nicotinamide nucleotides.

Kronfeld and Raggi (1963) in their further investigations to confirm the postulation that a shortage of all forms of nicotinamide nucleotides was the explanation for acetonemia, analyzed the mammary tissues of six ketotic cows before and after treatment. The postulation was based on the fact that any defect in the oxidation and reduction interfering with the Kreb cycle involving nicotinamide nucleotide coenzymes might interfere with the utilization and reaction of aceto-acetic acid with acetyl Co A to enter the Kreb cycle. This interference would lead to the formation of aceto-acetate which results in the formation of large amounts of ketone bodies. They also suggested that any defect in these reactions might cause a defect in lipogenesis which might lead to the accumulation of reduced forms and depletion of the oxidized form of NAD and NADP which would again impair pentose phosphate shunt and Kreb's cycle. As a result of their analysis they showed that the typical case of acetonemia had a lower level of all forms of nicotinamide coenzymes than that of normal animals. They also found that the ratio of oxidized and reduced forms was not changed indicating that it is the shortage of all four forms of nicotinamide nucleotides and not the ability of the animal to convert the oxidized to reduced form or vice versa which impaired oxidation and reduction in acetonemia.

Burch and Dippe (1964) studied 9 groups of rats ranging in age from 5 days before birth to 65 days after birth. They showed that in relation to protein, NAD increased 55% and NADP increased 188% during this growth period. The ratio of $NADP^+$/NADPH approached one with NADPH slightly higher, and the NAD^+/NADH ratio was between 10:1 and 20:1. They do point out, however, that low NADH and high $NADP^+$ is the result of rapid freezing of liver tissue and that ischemia causes an increase in the NADH and a decrease in the $NADP^+$ content. The activities of both NADH and NADPH cytochrome-c-reductase were shown by Lang (1965) to increase in the tissues of liver and heart of neonatal rats 0-25 days of age. Lang (1965) found a sex difference for NADH-cytochrome-c-reductase activities from 25 days post-partum onward. He also found that NADPH-cytochrome-c-reductase activities increased only in the liver. The author concluded that different electron transport pathways predominate according to sex, developmental stages, and tissues of the animals. The conclusion arrived at by McLean (1958b) was that in the lactating mammary tissue of rats at different stages NAD was mainly present in the oxidized form while NADP was present in the reduced form. Her work showed that the concentration of nicotinamide nucleotides in the rat mammary tissue increases from late gestation to the end of lactation when higher concentration of NAD and NADP were found. She showed that the soluble portion

of homogenates of mammary gland which had a high concentration of total NADP which favors reductive synthesizing activities, also has a higher NADPH/$NADP^+$ ratio and that the G-6-phosphate dehydrogenase and 6-phosphogluconic dehydrogenase are also located in this portion of the mammary preparation. This work indicates that the above two enzymes reduce $NADP^+$ at a more rapid rate than it can be oxidized by NADPH-cytochrome-c-reductase or nicotinamide nucleotides transhydrogenase making it possible to have a large supply of reduced NADP to be used for the fatty acid synthesis in the mammary tissue. Branster and Morton (1956) in a comparative study of the rate of NAD synthesis in normal and tumor tissue of mouse mammary glands showed that the rate of synthesis on the basis of (per 10^9) nuclei in the tumor was 1/3 that in mammary glands from the non-lactating mice and about 1/5 that of the lactating mammary gland tissue. These differences according to the authors were constant and statistically significant.

The level of four forms of nicotinamide nucleotides was also determined (Raux, et al., 1962) in the liver of rabbits ranging from 19-28 days before birth to 5 to 40 days after birth. The results indicate that there was a significant increase in all four forms of the coenzyme in the liver of 28 day old fetus over that in the liver of the 19 day old fetus. Although afterwards there was no change in the concentration of NAD^+, NADH, or $NADP^+$, the level of NADPH

increased only until 5 days after birth and then remained constant. They showed that the NAD concentration in the liver of adult rabbits was higher than the concentration in liver of 40 day old rabbits indicating a change that is taking place with age in the hepatic carbohydrate metabolism. The NAD^+ concentration showed a slight increase while the NADH and NADPH increased in the liver tissue of guinea pigs from fetal stages to that of the adult stages (Raiha, 1961). Nemeth and Dickerman (1960), however, had indicated that the NAD concentration had not changed while the NADPH level increased, reaching the level of adulthood in the liver of guinea pigs 24 hours after birth.

The concentration of nicotinamide nucleotide coenzymes was also studied in the life cycle of the blowfly (Lucilia cuprina) in an attempt to understand the changing pattern of these coenzymes in relation to the stages of development. It was shown (Birt, 1966) that during transformation from prepupa to midpupa the concentration of NAD and NADP declined but both were regenerated rapidly during adulthood. The increase in NAD was 3-fold while NADP increased 2-fold.

Various investigators have indicated that there is a relationship of nicotinamide coenzymes with fatty acid synthesis. Some of these reports clearly demonstrate this relation. Longdon (1957) working with rat liver homogenates concluded that the fatty acids

are synthesized in the cytoplasm and that NADPH serves as the electron donor for the reduction of the α and β unsaturated acyl Co-A to their saturated counterparts. He also suggests that abnormal conditions leading to a low rate of $NADP^+$ reduction and high rate of NADPH oxidation may also be accompanied by a low rate of fatty acid synthesis. The requirement for the fatty acid synthesis by pigeon liver homogenates was shown (Porter et al., 1957) to be the following cofactors: ATP, CoA, GSH, NADH, Mn++ and, isocitric and $NADP^+$.

Studies with endocrines indicate that hormones affect growth and metabolism. The mechanism of how hormones bring about their regulatory effects is still under investigation. The association of certain endocrines with nicotinamide coenzymes is pointed out by various investigators which will be presented later. Lucas, et al. (1950) showed that there was a correlation between thyroid weight, adrenal weight, and rate of gain and that smaller type steers had smaller endocrine weights. Mason, et al. (1956), with all possible crosses of four strains of mice, indicated that mice with intermediate thyroid glands had the greatest growth rate and that mice with larger thyroids had slow rate of oxygen consumption. It has been demonstrated that rapid growing beef calves also have higher TSH in their pituitary glands (Burris and Bogart, 1953; and Krueger, et al., 1954). The oxidation of glucose-1-^{14}C was largely stimulated with the treatment of TSH while the oxidation of glucose-6-^{14}C was

affected to a lesser extent. This action was considered to be primarily due to TSH since the above response was observed within 5 minutes after treatment (Field, et al., 1960).

Lee and Shaffer (1934) with a pair feeding program, showed that rats receiving growth hormone had an increased nitrogen and water content and decreased fat content in their body composition compared to the control group. Experiments with intact dogs, rabbits, and rats showed (Young, 1945) that the growth hormone treated animals had increased nitrogen retention. He also found that in the pituitary extracts of the treated animals the catabolism of carbohydrate was replaced by the fat oxidation. Continuous increase in protein content in the body tissues of adult female rats was observed when animals were treated with purified growth hormones (Greenbaum, 1953). A high rate of fat oxidation in rat liver homogenate was reported (Greenbaum and McLean, 1953) to occur due to treatment with pituitary growth hormones. Lipogenesis of fat in the liver of rats treated with growth hormones also was depressed (Greenbaum and Glascock, 1957). Bogart, (1959) indicates that the types of functions of various endocrine glands, their role in metabolism and their effects on genetic expression is inherited.

Nicotinamide coenzymes are believed to be the means by which hormones influence their regulatory actions. Geengard, et al., (1961) showed that NAD^+ concentration in the liver was greatly

increased with hyphophysectomy and to some extent with adrenolectomy. This experiment suggests that the metabolic control of the pituitary and adrenal is mediated through their effect on these coenzymes. Greengard, et al., (1964) showed that NAD^+ level increased in response to nicotinic acid treatment and that this elevated level remained longer in the hypophysectomized rats compared to normal rats. Of the several organs that were studied in this experiment with hypophysectomized and normal rats, only the liver and kidney showed large increases in NAD^+ concentration in response to nicotinic acid administration. The ratio of endogenous NADPH to endogenous NADH was increased by hypophysectomy. The nicotinic acid administration only increased the NAD concentration in the soluble fraction of the liver in both hypophysectomized and normal rats. The onset of lactation was noted to cause an increase in all four forms of nicotinamide nucleotides in rats (Jarrett and Field, 1965). The maximum level of coenzymes was noted at the 13th day of lactation. The largest increase was in the $NADP^+$ content. The authors showed that when labeled glucose was incubated with mammary gland slices from these rats the oxidation of labeled glucose was increased with the onset of lactation, reaching maximum level at the 13th day of lactation. The addition of prolactin, growth hormone and ACTH to the tissue slices did not significantly increase in oxidation of labeled glucose, which provides evidence that hormones do not

directly affect this oxidation process but rather act indirectly through the nicotinamide nucleotide coenzymes. An interesting study by Greenbaum, et al., (1965) indicates that the nicotinamide nucleotide content in the liver of rats varied with different hormonal conditions. Injection with growth hormones from the pituitary increased the liver NAD^+ and NADH 35% over the control group and it also depressed lipogenesis. The level of total nicotinamide nucleotides was not affected because the increase in $[(NAD^+) + (NADH)]$ was balanced by the decrease in $[(NADP^+) + (NADPH)]$.

Pastan et al., (1961) working with thyroid glands from dogs treated with TSH found that the level of $NADP^+$ was increased in the thyroid slices of the treated animals. There was also an increase in glucose oxidation but they showed that NADPH was not affected. The oxidation of glucose by the thyroid slices from TSH-treated dogs was shown (Field et al., 1963) to increase. This effect was observed even when the glands were removed 15 minutes after the treatment of animals with TSH. This effect of TSH was increased when the glands were pre-incubated with nicotinic acid which by itself also causes an increase in oxidation. The authors showed a correlation between the level of $NADP^+$ in the thyroid and its rate of oxidation of glucose. The authors on these bases indicate that this cofactor is responsible for the oxidation of glucose in these thyroid tissues. TSH treated thyroid slices when incubated in glucose media were

shown (Pastan et al. , 1963) to increase the NADP (mainly the $NADP^+$) concentration. The source of this NADP increase was shown to be NAD because the increase in NADP was coupled with that much reduction in the level of NAD.

Some of the metabolic effects of thyroid hormone (thyroxine) is known, however, some understanding of it to level of enzymes and coenzymes might help clearify the mode of action of this hormone. Lardy and Fildott (1951) showed that incubation of thyroid slices from hyper-thyroid rats inhibited the oxidative phosphorylation. Martius and Hess (1951) working with a similar problem showed that mitochondria from rats isolated 24-27 hours after injection with thyroxine showed much lower oxidative phosphorylation compared to the oxidative phosphorylation of non-treated rats. This experiment suggests that thyroxine inhibits oxidative phosphorylation. Further work (Lehninger et al. , 1955; Dickens, et al. , 1956) on the action of thyroxine showed that oxidative phosphorylation in mitochondria was inhibited by uncoupling the electron transfer system from the phosphorylation system when the mitochondria were isolated from rats treated with source of thyroxine. These authors showed also that thyroxine caused the swelling of the mitochondria resulting in the uncoupling. Field et al. , (1961) showed that additional thyroid hormones added to thyroid slices increased $NADP^+$ concentration. Furthermore, since thyroid hormone increases acetate and pyruvate

oxidation, this effect is not primarily due to the thyroxine but rather to its ability to increase the concentration of $NADP^+$. They showed that the rate of glucose oxidation in the thyroid homogenate is the function of the $NADP^+$ concentration.

MATERIALS AND METHODS

Chemicals Used

NAD^+ with a purity of 98 percent was purchased from Sigma Biochemical Company. Crystalline yeast alcohol dehydrogenase was purchased from Worthington Biochemical Company. Neurospora NAD'ase was purchased from Sigma Biochemical Company. NADH was purchased from Nutritional Biochemical Corporation.

Clostridium kluyveri extract was prepared according to the procedures outlined by Stadtman and Burton (1955) from the dried cells obtained from Sigma Biochemical Company. Methyl-ethyl-ketone (technical grades) was obtained from Van Walters and Rogers, Inc., Industrial Chemicals, Portland, Oregon. All other chemicals were standard materials obtained from the University Chemistry Stores.

Experimental Animals

Fifty-five beef bulls, produced over a two-year period (1964-1965), were used to provide source of liver tissue at a standard live body weight of 1000 lbs. Thirteen of these bulls came from an inbred line of Angus which was closed to outside breeding since 1950 except for one outside bull which was introduced and used to a limited extent in 1953, 1954, and 1955 in this line. The remainder of the

calves were the result of diallel crosses among three inbred lines of Herefords, namely Lionheart, Prince and David. All lines were closed to outside breeding; however, there was an interchange of females between the Prince and David lines prior to 1950.

The coefficient of inbreeding of the bulls varied from 0 to 0.4478 and their ages at 1000 lbs. slaughter weight varied from 350 days to 484 days. The management practices remained constant from year to year. The calves were weaned at 425 lbs. live weight but those calves that could not make this weight due to pasture conditions were weaned at the end of October regardless of weight. An adjustment period was allowed in the barn for the calves to reach 450 lbs. at which time they were put on a standard test ration consisting of 2/3 high quality chopped alfalfa and grass hay with 1/3 concentrated feed all combined into one-inch pellets. The test period covered from 450 lbs. to 800 lbs. body weight during which the bulls were fed individually twice a day. The animals were allowed 3 hours in the morning and 3 hours in the afternoon to eat and water was available at all times. At 800 lbs. the test period was completed. In addition to the pelleted ration given,an additional 3 lbs. of rolled barley was fed to each bull until a standard weight of 1000 lbs. was attained at which time the animals were slaughtered.

There was a total of 10 genetic groups. Nine of these groups were the result of diallel crossing. The types of matings and genetic

combinations are presented in Table I. The 10th group comes from the Angus inbred line of cattle.

Table I. The diallel mating scheme.

		Lines of Sire		
		1	2	3
Lines	1	1x1	1x2	1x3
of	2	2x1	2x2	2x3
Dam	3	3x1	3x2	3x3

The number of animals in each genetic group and the total by line of sires and line of dams are presented in Table II.

Table II. The number of animals in each group of the diallel mating.

		Lines of Sire			
		1	2	3	Total by Dam
Lines	1	5	6	3	14
of	2	3	7	5	15
Dam	3	7	1	5	13
Total by Sire		15	14	13	42

Lines 1, 2, and 3 represent the three inbred Hereford lines, namely Lionheart, Prince and David respectively, while line 4 is used to designate the inbred Angus line.

Records

Records of gain and feed consumption were obtained on each animal during the feed test. Also scores for conformation and body measurements were obtained at 500, 800, and 1000 lb. body weights. Upon slaughter at 1000 lbs. weight the weights of thyroid, adrenal and pituitary glands were obtained. The carcass composition was determined from a standard rib cut. Age at slaughter covers the period in days from birth until the animal reached 1000 lbs. body weight.

From the information obtained on these bulls, the following were used in the present study: Daily gain, feed per unit of gain, percent lean in the carcass, percent fat in the carcass, thyroid gland weight, pituitary gland weight, inbreeding coefficients, age at slaughter and wither height. All these records were obtained at 1000 lbs. body weight.

Collection of Liver Tissue

The bulls were knocked down with a .22 caliber rifle. The animal was then hoisted by the rear legs and bled to death. The small lobe of the liver was then removed from which duplicate samples 1.5-2 inches in size were cut and put in plastic freezer bags which were then immediately placed on blocks of dry ice in an

insulated box. The small lobe was chosen as a means of reducing the variation that might otherwise exist in an arbitrary sample taken from the body of the whole liver. The samples, still inside the ice container, were carried to the laboratory where they were removed from the dry ice and stored in a cold room ($-10^{\circ}F$) until they were analyzed.

Analytical Methods

Of the various methods available for the estimation of nicotinamide nucleotide coenzymes, the fluorometric procedure is recommended as the best procedure (Lowery et al., 1957). The methods chosen for this study were those developed by Jacobson and Astrachan (1957). These are specific methods for the determination of various forms (oxidized and reduced) of nicotinamide nucleotide coenzymes in a quantity as small as $10^{-8}M$. The principle involved is the development of fluorescent compound by reacting the oxidized forms of the nicotinamide nucleotide coenzymes with various agents after which readings are made with a fluorometer equipped with proper filters. For the estimation of individual coenzymes (NAD^{+}, $NADP^{+}$, NADH or NADPH) specific enzymes can be used to interconvert oxidized and reduced forms or to rupture the nicotinamide ribose bond thus yielding information on any specific nicotinamide coenzymes.

Beckman ratio fluorometer equipped with proper primary and secondary filter for the determination of nicotinamide nucleotides was used.

Extraction

The extraction procedures used here were those recommended by Ciotti and Kaplan (1957). Two extractions were needed, one for the oxidized form which was extracted in TCA and one for the reduced form which was extracted with sodium carbonate. These are briefly described below.

1. Extraction of the oxidized form: A sample of up to 700 mg of tissue was added to 5 volumes of 5% TCA and homogenized in a Potter Elvehjem homogenizer with teflon pestle. The denatured protein was removed by centrifugation.
2. Extraction of the reduced forms: Up to 700 mg sample of frozen tissue was immediately placed in a homogenizer with teflon pestle containing five times the volume of the tissue of 0.1 M sodium carbonate (pH 10). The sodium carbonate solution was initially heated in boiling water bath for 3-5 minutes before the tissues were placed in it. The tissue was allowed to stand in this boiling carbonate solution for 30 seconds followed by gentle homogenization for 30 seconds and then the tissue was returned to the

boiling water for 60 seconds. After chilling the sample in crushed ice water it was centrifuged to remove the denatured protein. The extract was ready for assay.

Development of Fluorescence

Fluorescence was measured by the reaction of the oxidized nucleotide with methyl-ethyl ketone. The procedures are those of Ciotti and Kaplan (1957) which was described before. Briefly, it is described as follows:

The sample to be assayed was mixed with 0.2 ml of 1/500 dilution of 0.1 M $MnCl_2$ in methyl-ethyl ketone and 0.6 ml of 3.5 N NaOH. After 5 minutes of room incubation 0.4 N HCl was added to a final volume of 8 ml. The mixture was then heated in a water bath. After cooling, the sample fluorescence was measured in a fluorometer. Although the reduced forms fluoresce but the intensity of its fluorescence is negligible, moreover in the above procedures using methyl-ethyl ketone, the acid treatment destroys the reduced forms allowing only the oxidized form to develop fluorescence. Using various enzymes with subsequent development of fluorescence with methyl-ethyl ketone eliminates any fluorescence with might contribute to the reading by the coenzyme's analogs, thus providing information on any specific coenzymes

(see Ciotti and Kaplan, 1957).

Standard Curve

A standard curve was prepared with NAD^+, 98% purity. The response was linear and reading was taken directly from the curve.

Assay Technique

In this study all the assays described below were carried in triplicates. Two of the tubes underwent enzymatic reactions while the third tube was included to serve as a control. The level of the coenzymes in these tubes was determined in comparison with the standard curve. The procedures followed were strictly those described by Jacobson and Astrachan (1957); however the technique is briefly described.

Assay for Total Oxidized Forms [(NAD^+) + $(NADP^+)$]

A mixture containing 0.4 ml of 5% TCA with tissue extracts, 0.3 ml of 1 M $K_2 Po_4$, with an excess of Neurospora NAD'ase in a total of 1.8 ml at a final pH of 6 was incubated at 37°C for 15 minutes after which 0.2 ml of 50% TCA was added. A third tube similar in content to above, except that 0.2 ml of 50% TCA was added prior to enzymatic reaction, served as a control.

Assay for NAD^+

A mixture containing 0.4 ml of 5% TCA with tissue extracts, 0.07 ml of 2M K_2Co_3, 0.05 ml of 95% ethanol, 0.05 ml of yeast alcohol dehydrogenase (1:40 dilution of the crystalline enzyme) in a total of 1.8 ml at a pH of 10 was incubated at 37°C for 15 minutes, after which 0.2 ml of 50% TCA was added. A third tube with similar contents except that TCA was added before the enzymatic reaction was carried out, was included to serve as a control.

Assay for Total Reduced Forms [(NADH) + (NADPH)]

A mixture containing 0.5 ml of 0.05 M sodium carbonate with tissue extract pH 10, 0.2 ml of 1M potassium phosphate buffer pH 7.5, 0.1 ml of Clostridium kluyveri extract in a total of 0.9 ml at a final pH of 7.5-8 was incubated at 37°C for 15 minutes. After incubation a 0.1 ml of 50% TCA was added. A third tube also containing the above mixture but with the 0.1 ml 50% TCA added before enzyme addition served as a control.

Assay for NADH

A mixture containing 0.5 ml of 0.05 M sodium carbonate with tissue extract buffer pH 10, 0.2 ml of 1 M potassium phosphate buffer (pH 7.5), 0.05 ml of 0.5 M acetaldehyde, and 0.05 ml yeast

alcohol dehydrogenase (1:400 dilution of the crystalline enzymes) was adjusted to a total volume of 0.9 ml and a final pH of 7.5-8 (pH tape was used to adjust the pH in all these assays) and was incubated at 37°C for 1-2 minutes. Upon completion of the incubation, 0.1 ml of 50% TCA was added. A third tube containing above mixture to which 0.1 ml TCA was added before enzyme addition served as a control.

Assay for $NADP^+$ and NADPH

No enzymatic assays were carried to determine these. They were, however, determined by calculations as follows:

(1) Total oxidized coenzymes $[(NAD^+)+(NADP^+)] - NAD^+ = NADP^+$

(2) Total reduced coenzymes $[(NADH)+(NADPH)] - NADH = NADPH$

Information Derived on Various Forms of the Coenzymes

When samples were thus analyzed the following data from the liver tissue of each animal was expressed as combined $[(NAD^+) + (NADP^+)]$, (NAD^+), $(NADP^+)$, combined $[(NADH) + (NADPH)]$, (NADH), (NADPH). These data were used to calculate the various forms and ratios of these coenzymes for each animal. The values are reported as micrograms of nicotinamide nucleotides per gram of frozen tissue. The following is a list of the various forms of coenzymes with the abbreviations used in this study:

1. Total nicotinamide coenzymes = [(NAD^+) + ($NADP^+$) + (NADH) + (NADPH)] .
2. NAD = Oxidized and reduced forms of NAD [(NAD^+) + (NADH)] .
3. NADP = Oxidized and reduced forms of NADP [($NADP^+$)+ (NADPH)] .
4. NAD^+ = Oxidized form of (NAD).
5. $NADP^+$ = Oxidized form of (NADP).
6. NADH = Reduced form of (NAD).
7. NADPH = Reduced form of (NADP).
8. Total oxidized forms of nicotinamide nucleotides = [(NAD^+) + ($NADP^+$)] .
9. Total reduced forms of nicotinamide nucleotides = [(NADH) + (NADPH)] .
10. Ratio of oxidized to the reduced form of NAD = (NAD^+ / NADH).
11. Ratio of oxidized to the reduced form of NADP = ($NADP^+$ / NADPH).
12. Ratio of total oxidized to the total reduced forms of the nicotinamide nucleotide coenzymes = $\frac{[(NAD^+) + (NADPH^+)]}{[(NADH) + (NADPH)]}$.

Statistical Analysis of the Data

All possible simple correlations of the twenty-one traits under

study were made. Analysis of variance was used for studying differences among inbreds, line crosses, and between inbreds and line crosses. Analysis of variance based on the diallel crosses were made to determine if differences exist for general and specific combining abilities and reciprocal effect. Tests were made where the "F" values were significant. Based on the correlations obtained, relationship charts and graphical demonstrations of the coenzymes with endocrine glands and various production traits were prepared.

RESULTS AND DISCUSSION

The mean values for various coenzymes of the different genetic groups are presented in Table III. The mean values for endocrine gland weights, age, wither height, inbreeding coefficients, and various production traits are included in Table IV. The values for coenzymes and production traits when statistically significant were further broken down in various genetic combination as shown in Table V. All three tables (Table III, IV, V) were used as the basis for the "t" test to ascertain the significance of differences between inbred lines, parents vs. offspring and inbred vs. line cross groups. The results of these tests are reported in Table VI. The mean squares for the analysis of variance are presented in Table VII. Those observations that are pointed out as statistically significant in this table were used for "t" test. Analysis of variance based on the diallel crosses are reported in Table VIII. In this table the mean squares for general and specific combining abilities and reciprocal effects are shown. All the simple correlation coefficients among the 21 observations made on each animal are presented in Table IX. This table served as the basis for various relationship charts and graphs which are presented in the discussion section.

Table III. Means of nicotinamide nucleotide coenzymes per gram of frozen liver tissue by lines and line crosses. (Microgram/gram)

Genetic Groups	1	2	3	4	1x2	1x3	2x1	2x3	3x1	3x2	All Groups
Number of animals	5	7	5	13	6	3	3	5	7	1	55
Total Coenzymes	529.06	542.43	731.80	546.87	518.91	571.57	535.58	602.66	555.52	490.30	564.32 ± 12.28
NAD	333.70	300.51	400.79	290.91	319.03	309.71	307.27	387.14	309.55	216.44	320.77 ± 10.92
NADP	195.09	241.92	330.35	255.17	200.24	261.51	228.44	215.55	228.07	273.86	241.04 ± 7.59
NAD^+	258.48	240.22	295.89	228.49	242.06	214.72	249.35	303.01	236.62	180.66	247.64 ± 8.55
$NADP^+$	73.86	83.32	86.16	95.26	49.95	94.36	78.76	77.31	83.97	49.77	81.18 ± 5.03
NADH	75.29	60.29	104.90	62.40	76.96	95.32	57.78	81.22	72.93	35.58	72.86 ± 4.01
NADPH	121.43	158.60	242.86	160.21	150.29	167.15	149.67	141.19	144.10	244.09	160.46 ± 6.25
$\frac{NAD^+}{NADP^+}$	332.35	323.54	382.05	323.75	292.01	309.09	328.11	380.32	320.59	230.43	328.83 ± 8.86
$\frac{NADH}{NADPH}$	196.72	218.89	347.77	222.62	227.25	262.48	207.46	222.41	217.04	279.67	233.31 ± 7.35
$\frac{(NAD^+)+(NADP^+)}{(NADH)+(NADPH)}$	1.72	1.47	1.09	1.49	1.30	1.18	1.61	1.85	1.54	0.82	1.47 ± 0.57
$\frac{NAD^+}{NADH}$	3.85	4.99	2.87	4.21	3.19	2.23	4.59	4.42	4.44	5.07	4.02 ± 0.26
$\frac{NADP^+}{NADPH}$	0.61	0.52	0.35	0.66	0.34	0.59	0.54	0.71	0.65	0.20	0.56 ± 0.47

Table IV. Mean values of endocrine weights, age, wither height, inbreeding coefficients and production traits by lines and line crosses.

Genetic Groups	1	2	3	4	1x2	1x3	2x1	2x3	3x1	3x2	All Groups
Number of animals	5	7	5	13	6	3	3	5	7	1	55
Gain per day lbs.	2.84	2.96	2.82	2.71	3.02	2.98	3.10	2.84	2.77	3.18	2.87 ± 0.38
Feed per unit gain lbs.	697.40	637.86	652.40	746.31	645.83	646.66	575.00	669.60	726.14	650.00	682.49 ±11.69
% Fat	25.19	26.95	31.85	29.13	30.46	22.89	29.89	28.19	29.31	26.43	28.48 ± 0.63
% Lean	54.05	53.85	51.23	53.13	53.77	58.03	54.64	54.99	52.19	53.30	53.60 ± 0.52
Thyroid Wt. (GM)	28.53	16.20	15.23	23.93	20.64	20.89	20.39	18.59	20.23	16.70	20.77 ± 1.21
Pituitary Wt. (GM)	1.82	1.67	1.74	2.03	1.82	1.97	1.62	1.91	1.67	1.83	1.83 ± 0.42
Age in days at 1000 lbs. wt.	400	410	436	400	410	433	387	428	407	397	410 ± 4.36
Wither Height in inches	43.30	43.36	43.60	43.31	42.67	45.17	42.83	43.10	44.00	44.00	43.43 ± 0.13
Inbreeding Coefficients	0.23	0.16	0.27	0.11	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Table V. Means of certain coenzymes in microgram per gram of frozen liver tissue, wither height in inches, and production traits.

Genetic groups	1+2 / 2	1+3 / 2	2+3 / 2	1x2	2x1	2x3	1x3	3x1	1x2+2+1 / 2	1x3+3+1 / 2	Inbreds	Line Crosses	Inbred Herefords
Number of animals	12	10	10	6	3	5	3	7	9	10	30	25	17
Total coenzymes	536.86	630.44	621.34	518.72	635.58	602.66	571.57	555.52	524.50	560.34	573.72	553.08	594.20
NADP	222.41	262.72	278.76	200.24	228.44	215.55	261.51	228.07	209.64	238.11	254.60	224.78	254.12
NADPH	143.11	182.15	193.71	150.29	149.67	141.19	167.15	144.10	150.08	151.02	167.15	152.44	172.45
NADH + NADPH	209.66	272.25	272.59	227.25	207.56	222.41	262.48	217.04	220.66	230.72	238.29	227.37	250.28
Wither height	43.333	43.450	43.458	42.667	42.833	43.100	45.167	44.000	42.722	43.700	43.367	43.500	43.110
Gain per day-lbs.	2.9130	2.8320	2.9050	3.0200	3.1000	2.8400	2.9800	2.7700	3.1180	2.8321	2.8091	2.9505	2.8920
Feed per unit gain lbs.	662.66	674.90	643.91	645.83	575.00	669.60	646.66	726.14	622.22	602.30	697.20	664.84	659.68

Table VI. "t" values for between lines, lines vs. line crosses, inbreds vs. line crosses and parents vs. offspring.

Genetic groups	1 vs 2	1 vs 3	1 vs 4	2 vs 3	2 vs 4	3 vs 4	$\frac{1+2}{2}$ vs 1x2	$\frac{1+2}{2}$ vs 2x1	$\frac{1+2}{2}$ vs $\frac{1x2+2x1}{2}$	$\frac{1+3}{2}$ vs 1x3	$\frac{1+3}{2}$ vs 3x1	$\frac{1+3}{2}$ vs $\frac{1x3+3x1}{2}$	$\frac{2+3}{2}$ vs 2x3	Inbreds(16.76%) vs Line Crosses	Inbreds(21.16%) vs Line Crosses
Number of animals	12	10	18	12	20	18	18	15	21	13	17	20	17	55	42
Tot al Coenzymes	0. 2971	4. 1710**	0. 4408	4. 1944**	0. 1299	4. 0891**	0. 4635	0. 2771	0. 3340	1. 1634	1. 9762*	1. 7831*	0. 4658	0. 4003	0. 7256
NADP	1. 6834*	4. 5389**	2. 4360**	3. 1809**	0. 5972	2. 7021**	0. 9276	0. 2913	0. 5576	0. 0395	2. 0112*	1. 0855	2. 4980**	0. 9392	0. 8382
NADPH	1. 6909*	5. 1702**	1. 9695*	3. 8318**	0. 0914	3. 7636**	0. 3819	0. 3347	0. 3872	0. 6092	2. 1619*	1. 7687*	2. 6260**	0. 6284	0. 7250
(NADH) + (NADPH)	0. 9202	5. 7869**	0. 7322	5. 3250**	0. 1588	5. 1771**	1. 4955	0. 0824	0. 5549	0. 4429	1. 7570*	0. 5810	0. 3759	0. 6211	0. 7536
Wither height	0. 1090	0. 6890	0. 0540	0. 4673	0. 3712	0. 5615	0. 8497	0. 8695	1. 3521	2. 9400**	1. 3221	2. 1518*	2. 2705*	0. 2235	0. 5958
Gain per day	0. 8271	0. 1026	1. 0151	0. 8271	2. 1694*	0. 8603	0. 8492	2. 8500**	1. 6942*	0. 8960	0. 4838	0. 0007	0. 8550	1. 2020	0. 3182
Feed per unit gain	1. 3019	0. 9146	1. 1950	0. 3210	2. 9820**	2. 2932*	0. 4304	1. 7427*	1. 0784	0. 5320	1. 4076	1. 1431	0. 3759	0. 8890	0. 2846

*P< 0. 05 = 1. 6775
**P< 0. 01 = 2. 4065

Table VII. Mean squares for the analysis of variance for four inbred lines and line crosses.

Source	d. f.	Total Coenzymes	NAD	NADP	NAD^+	$NADP^+$	NADH	NADPH	Gain per day (lbs)	Feed per unit gain (lbs)
Treatment	9	20240. 22**	9116. 89	7806. 63**	4609. 61	1164. 50	1302. 73	5935. 92**	0. 1492*	14925. 35*
Error	45	5915. 65	6069. 96	2246. 75	3914. 91	1440. 87	803. 46	1401. 73	0. 0634	6049. 39

Source	Percent fat	Percent lean	Thyroid gland (wt)	Pituitary gland (wt)	Wither height	Age at 1000 lbs. (body wt)	$NAD^+ + NADP^+$	NADH + NADPH	$\frac{(NAD^+)+(NADP^+)}{(NADH)+(NADPH)}$	$\frac{NAD^+}{NADH}$	$\frac{NADP^+}{NADPH}$
Treatment	29. 49	13. 19	86. 04	0. 1274	1. 91*	1142. 53	5274. 72	9389. 97**	0. 2977	3. 52	0. 11
Error	20. 44	14. 97	79. 59	0. 0898	0. 79	1027. 19	4135. 71	1696. 81	0. 1560	3. 87	0. 13

*P< 0. 05
**P< 0. 01

Table VIII. Mean squares for the diallel analysis.

Source	d. f.	Total Coenzymes	NAD	NADP	NAD^+	$NADP^+$	NADH	NADPH	Gain per day (lbs)	Feed per unit gain (lbs)	Percent Fat	Percent Lean	Thyroid gland wt. (GM)	Pituitary gland wt. (GM)	Wither height (inches)	Age at 1000 lbs (body wt)	$\frac{NAD^+}{NADP^+}$	$\frac{NADH}{NADPH}$	$\frac{(NAD^+)+(NADP^+)}{(NADH)+(NADPH)}$	$\frac{NAD^+}{NADH}$	$\frac{NADP^+}{NADPH}$
General combining ability	2	1099.75	2909.96	1331.34	1822.85	151.42	350.41	2929.39	0.029805	638.00	3.9529	5.4565	16.5932	0.001384	0.6273	46.6206	1525.18	1280.54	0.08694	1.0032	0.01635
F. Prime		3.00	3.00	3.47	3.45	4.00	3.00	3.00	4.00	9.00	3.41	4.71	4.00	4.00	3.00	3.45	3.00	3.00	3.00	4.00	4.0
Specific combining ability	3	9575.66**	5539.67*	1366.94*	2510.44*	340.42	720.61*	1502.52**	0.016810	356.55	14.25	2.7275	24.9632	0.02685	0.7730*	653.5635	4343.49*	2508.92**	0.1289*	1.0235	0.0358
Reciprocal effect	3	2938.03	89.03	2485.81*	35.98	203.15	177.99	1739.74*	0.04925*	3867.28	4.3482	3.3736	5.1915	0.0092	0.3479	72.5682	175.62	2646.04**	0.1006	0.9480	0.02845
Error	45	1653.99	1697.14	628.18	1094.59	402.86	224.64	391.92	0.017719	1691.39	5.7164	4.1881	22.2525	0.02512	0.2205	287.1998	1156.33	474.42	0.0436	1.0814	0.03607

*$P \leq 0.05$

**$P < 0.01$

Concentration of Oxidized and Reduced Nicotinamide Nucleotide Coenzymes

NAD was largely present in the oxidized form (NAD^+) compared to the reduced form (NADH) (Table III) and the data show further that the $NADP^+$ content of the tissue was lower than the content of NADPH. This pattern of occurrences of the coenzymes in this study is in general agreement with the findings in rats by Glock and McLean (1955a); Jacobson and Kaplan (1957); Bassham et al. (1959); Pande et al. (1964); and in sheep by Caiger et al. (1962); and Filsell et al. (1963). Some of the unexplained differences in the concentrations of reduced and oxidized forms may be attributed to the rapid freezing of tissue, methods of extraction and the interval between extraction and enzymatic reaction for assay (Burch and Dippe, 1964; Bassham et al. 1959; and Pande et al. 1964).

Nicotinamide Nucleotide Coenzymes and Age

There is a positive significant correlation between age and total coenzymes, NAD, NADP, NAD^+ (Table IX). This relation between age and these coenzymes was also indicated by Branster and Morton (1956) with tumor tissue; Nemeth and Dickerman (1960), Raiha (1961) with guinea pigs; Raux et al. (1962) with rabbits; Burch and Dippe (1964) with rats; and Caiger et al. (1962) and

Table IX. Simple correlation coefficients of the twenty-one observations.

	Total Coenzymes	NAD	NADP	NAD^+	$NADP^+$	NADH	NADPH	Gain per day	Feed per unit gain	% Fat	% Lean	Thyroid gland wt.	Pituitary gland wt.	Inbreeding Coefficient	Age at 1000 lbs.	Wither height	$\frac{(NAD^+)}{(NADP^+)}$	$\frac{(NADH)}{(NADPH)}$	$\frac{(NAD^+)+(NADP^+)}{(NADH)+(NADPH)}$	$\frac{NAD^+}{NADH}$	$\frac{NADP^+}{NADPH}$
Total Coenzymes																					
NAD	+0.001																				
NADP	+0.502	-0.867																			
NAD^+	+0.759	+0.945	-0.087																		
$NADP^+$	+0.157	-0.196	+0.561	-0.232																	
NADH	+0.562	+0.707	-0.046	+0.435	-0.053																
NADPH	+0.462	+0.044	+0.747	+0.075	-0.127	-0.024															
Gain per day	-0.101	+0.102	-0.268	+0.136	-0.258	-0.022	-0.098														
Feed per unit gain	-0.008	-0.232	+0.168	-0.259	+0.357	-0.077	-0.086	-0.781													
% Fat	+0.462	+0.365	+0.222	+0.369	-0.046	+0.219	+0.285	-0.127	-0.154												
% Lean	-0.324	-0.264	-0.171	-0.265	+0.056	-0.167	-0.234	+0.173	-0.249	-0.810											
Thyroid gland wt.	-0.247	-0.116	-0.214	-0.145	+0.158	-0.002	-0.378	-0.072	+0.211	-0.252	+0.085										
Pituitary gland wt.	-0.012	+0.053	-0.050	+0.040	+0.114	+0.065	-0.118	+0.046	+0.077	-0.101	+0.048	+0.114									
Inbreeding Coefficient	+0.288	+0.168	+0.255	+0.183	+0.065	+0.076	+0.241	-0.115	+0.039	+0.104	-0.275	-0.077	+0.043								
Age at 1000 lbs.	+0.515	+0.412	+0.256	+0.386	+0.031	+0.300	+0.276	-0.034	-0.114	+0.228	-0.177	-0.222	+0.074	-0.008							
Wither height	+0.149	+0.051	+0.162	-0.050	+0.178	+0.266	+0.045	-0.109	+0.101	-0.098	+0.008	+0.114	+0.172	-0.104	-0.035						
$\frac{(NAD^+)}{(NADP^+)}$	+0.822	+0.799	+0.236	+0.834	+0.349	+0.389	+0.000	-0.150	-0.048	+0.330	-0.225	-0.050	+0.103	+0.214	+0.390	+0.052					
$\frac{(NADH)}{(NADPH)}$	+0.702	+0.423	+0.611	+0.301	-0.137	+0.525	+0.838	-0.096	-0.115	+0.362	-0.290	-0.323	-0.065	+0.246	+0.398	+0.183	+0.213				
$\frac{(NAD^+)+(NADP^+)}{(NADH)+(NADPH)}$	+0.047	+0.047	-0.317	+0.360	+0.382	-0.140	-0.681	+0.052	+0.085	-0.083	+0.114	+0.213	+0.098	-0.055	-0 008	-0.149	+0.562	-0.656			
$\frac{NAD^+}{NADH}$	-0.197	-0.265	+0.006	-0.037	-0.116	-0.799	+0.105	+0.086	-0.093	-0.099	+0.094	-0.122	-0.111	-0.004	-0.014	-0.237	-0.030	-0.347	+0.259		
$\frac{NADP^+}{NADPH}$	-0.070	-0.115	+0.044	-0.162	+0.814	-0.004	-0.583	-0.133	+0.346	-0.204	+0.185	+0.341	+0.146	-0.099	-0.094	+0.039	+0.306	-0.499	+0.694	-0.139	

*P <0.05 = 0.2615
**P <0.01 = 0.3950

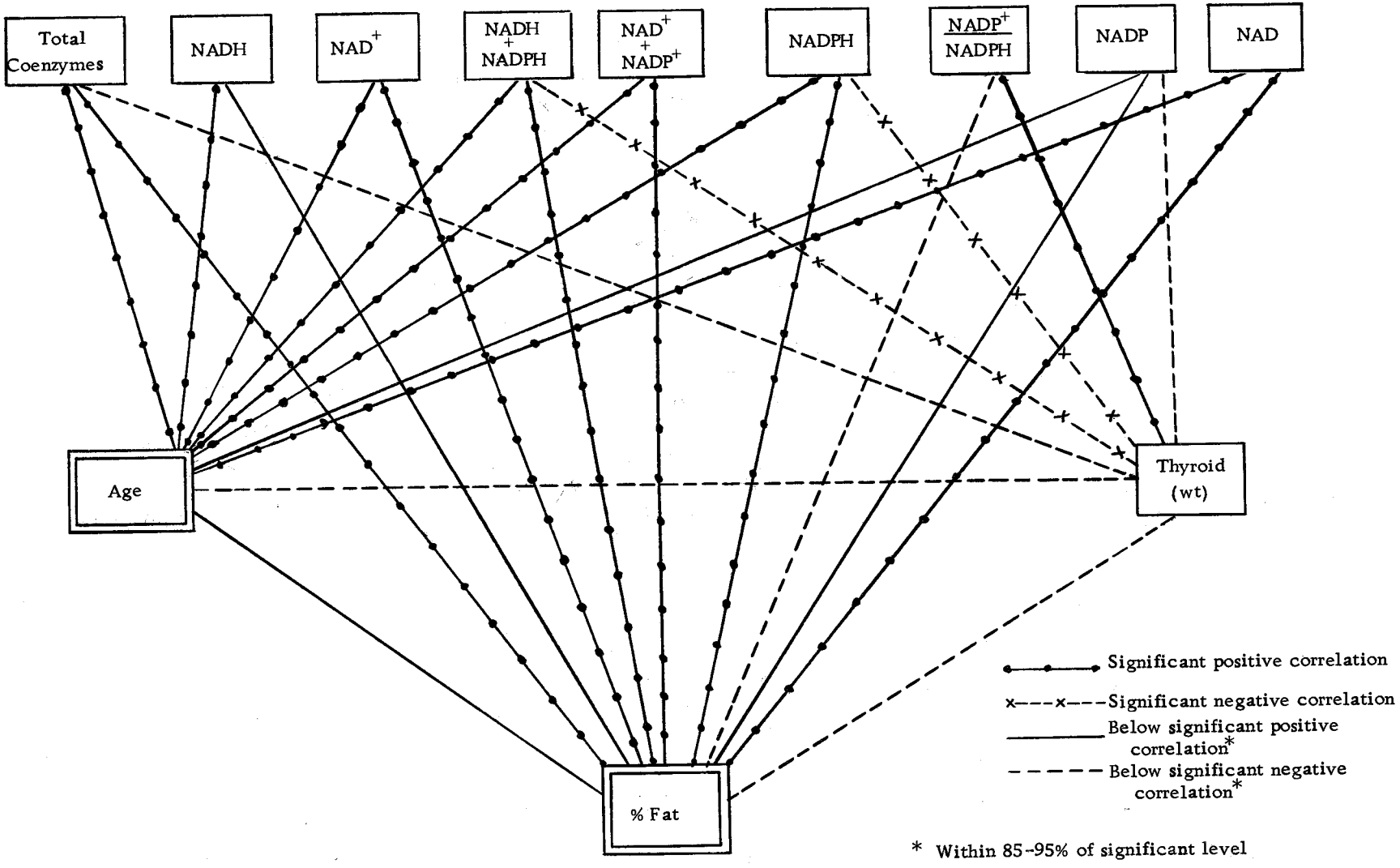


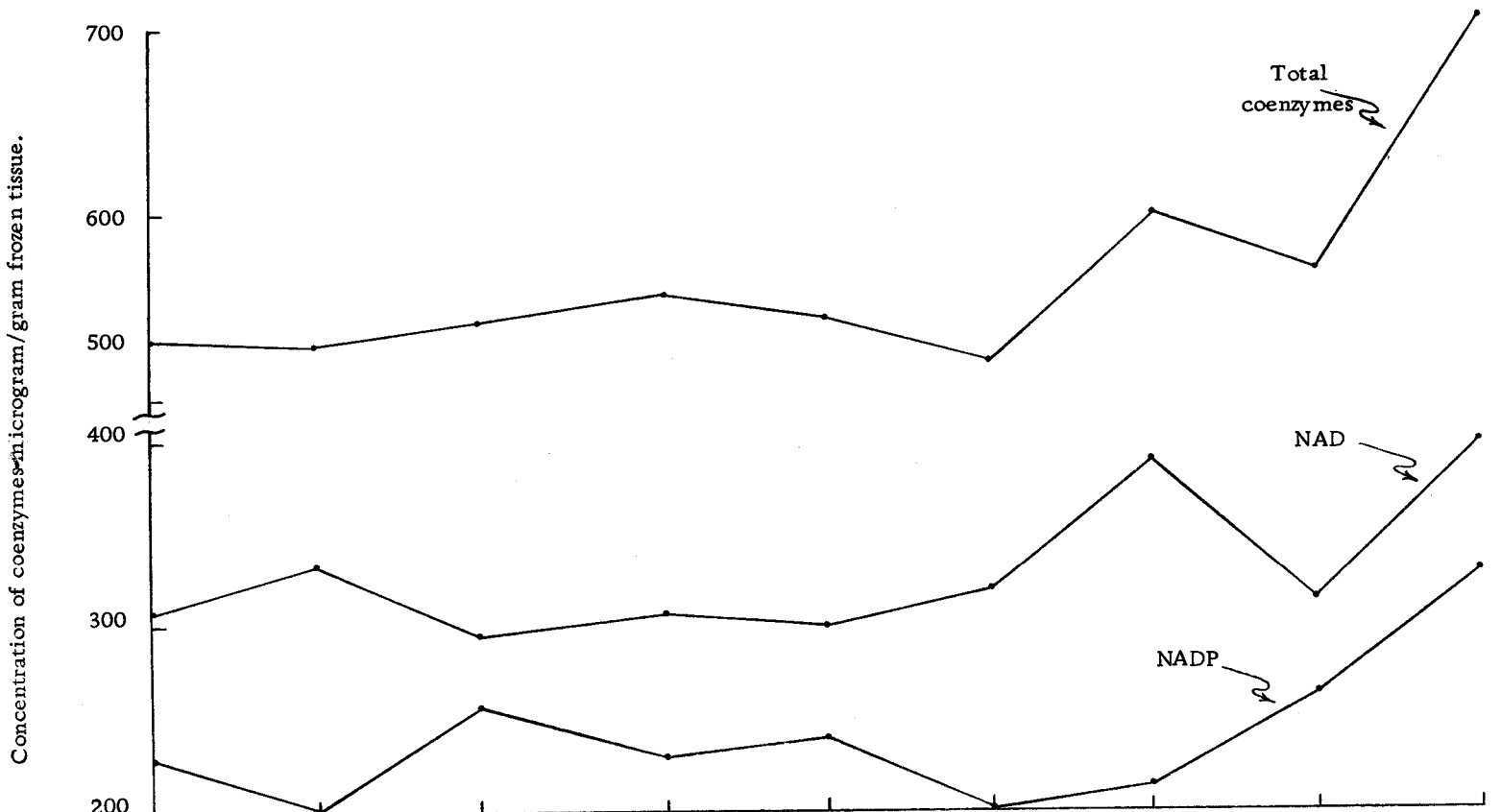
Figure 1. Relationship between age, percent fat, coenzymes and thyroid gland.

Filsell et al. , (1963) with rats and sheep. These changes in the levels of coenzymes are associated with the pattern of metabolism from the embryonic and prenatal stages to maturity and therefore are reflected in growth rate and efficiency of production. It seems appropriate to postpone the discussion of the relationship of these coenzymes to endocrine glands and their relationship to production traits and carcass composition at this time and to discuss each subsequently under its related topic.

Nicotinamide Nucleotide Coenzymes, Age, Percent Fat, Percent Lean and Thyroid Weight Interrelationships

Both coenzymes, NAD and NADP, were positively associated with age at 1000 lbs. (Table IX). The relationships of the various forms of these coenzymes with age, fat and thyroid are presented in Figure 1.

Age at 1000 lbs. and fat percent in the carcass are positively correlated (Figure 1). Age at 1000 lbs. is positively correlated with all the coenzymes which positively affected fat deposit. These relations are demonstrated (Figures 2 and 3) with mean values of inbred and line crosses and parents compared with their line cross offspring. The ratio of $\frac{NADP^+}{NADPH}$ has a negative correlation with fat. Thyroid gland weight shows a negative correlation with age at 1000 lbs. and fat percent in the carcass which can be interpreted as



Age (days at slaughter)	387	400	400	407	410	410	428	433	436
Lines	2x1	1	4	3x1	2	1x2	2x3	1x3	3
Number	3	5	13	7	7	6	5	3	5

Figure 2. Means of coenzymes and age (days at slaughter) for lines and line crosses.

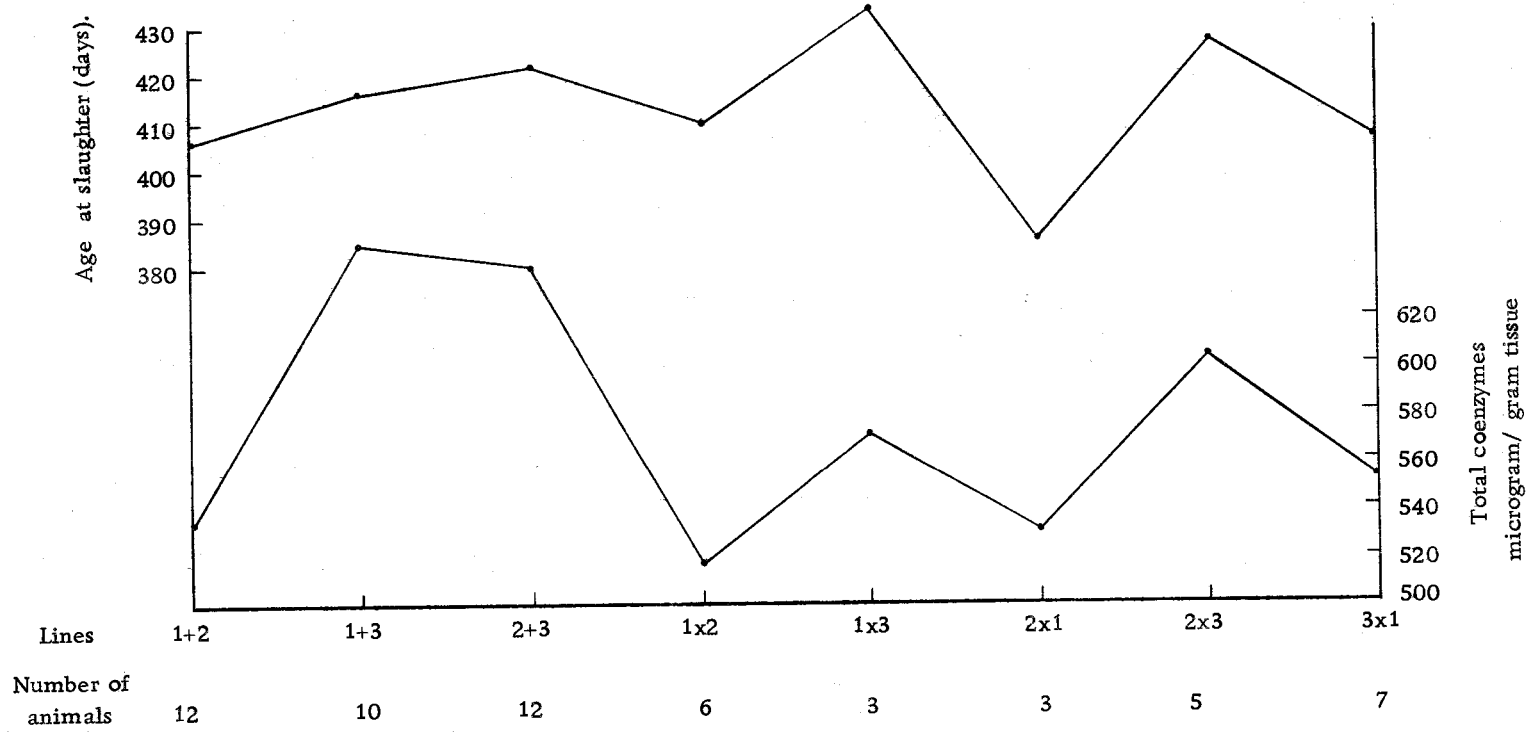


Figure 3. Means of coenzymes and age at slaughter for the average of parents and offspring.

follows: as thyroid weight increases, percent fat in the carcass decreases and as age at 1000 lbs. increases, the amount of thyroid tissue at 1000 lbs. body weight decreases. This is shown in the section on nicotinamide nucleotide coenzymes, thyroid weight and production traits. With the decrease in the thyroid weight, the percent fat in the body increases as age increases. Fat percent in the carcass is highly negatively correlated with percent lean. The thyroid appears to depress lipogenesis as it is positively correlated with $\frac{NADP^+}{NADPH}$ ratio and all the coenzymes which positively affect fatty acid synthesis and percent fat in the carcass. Relation of thyroid to $\frac{NADP^+}{NADPH}$ and the percent fat with mean values for lines and line crosses is shown in Figure 4. Thyroid, coenzymes and production traits relationships are shown in Figure 5. Thyroid gland is positively correlated with the $\frac{NADP^+}{NADPH}$ ratio which positively affects percent lean. In the meantime thyroid gland is negatively correlated with those coenzymes which in turn negatively affect percent lean in the carcass. If the weight of the thyroid is assumed to be an indication of thyroxine production and metabolic activity of the animal as can be inferred from evidence then a large thyroid would indicate a more active metabolic state and the younger animal would be more active than at more mature stages of life, since the younger animal would have a larger thyroid per unit body weight. On the cellular level these findings appear to agree

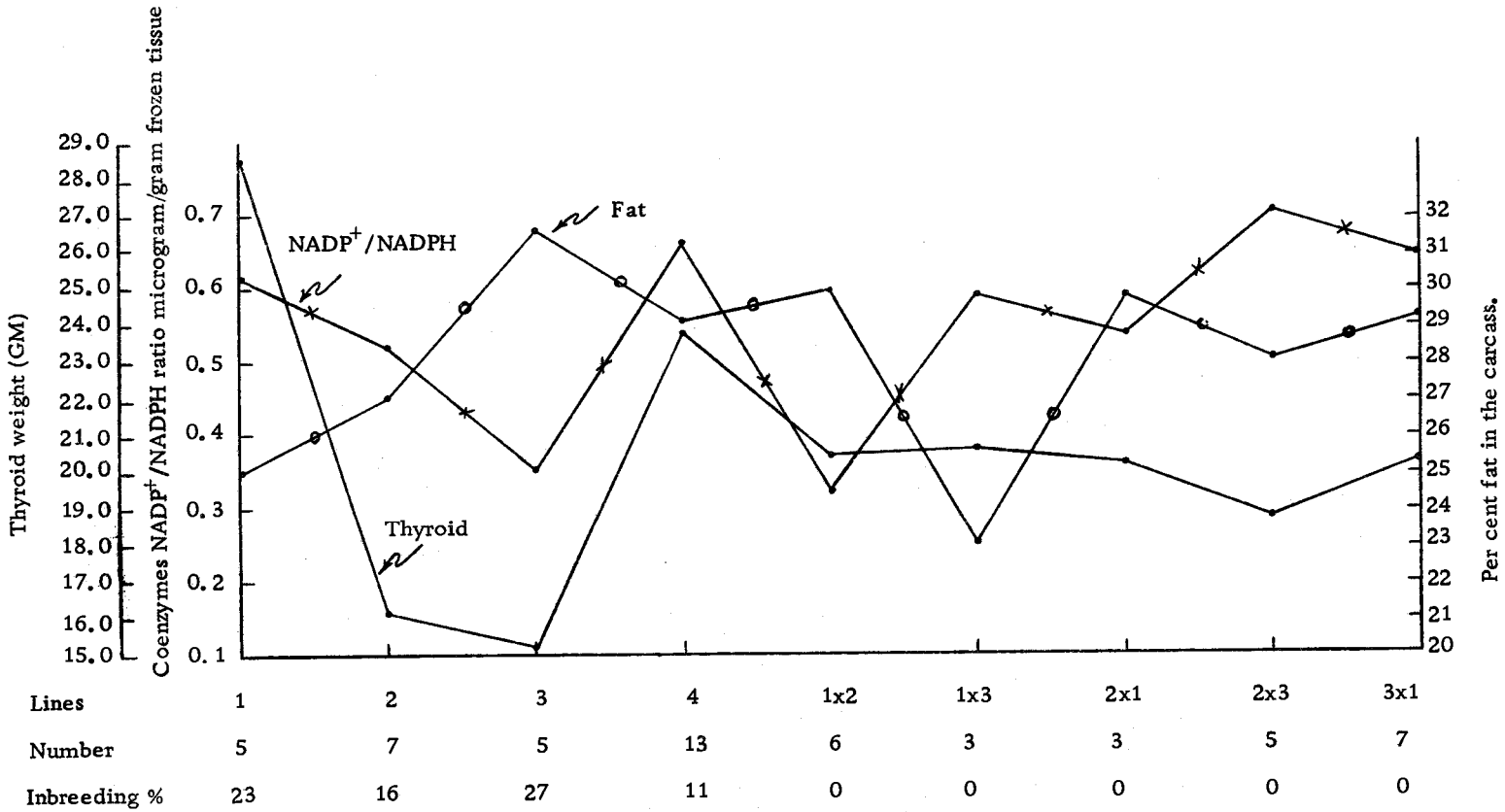


Figure 4. Means of coenzyme ratio, percent fat and thyroid weight for lines and line crosses.

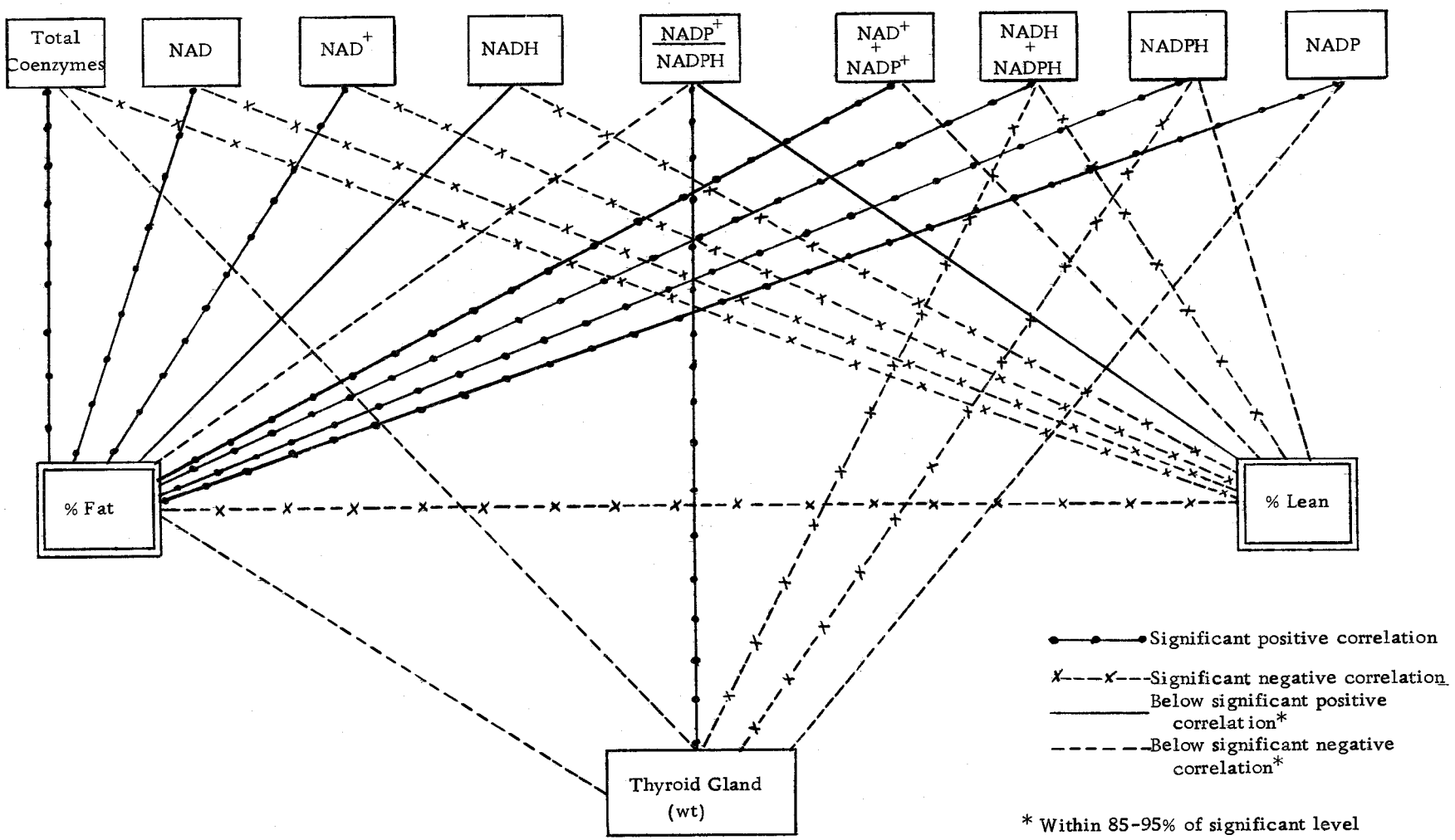


Figure 5. Relationship between percent fat, percent lean, coenzymes and thyroid gland weight.

with the following reports by various authors with in vitro studies dealing with coenzymes, endocrine and fat synthesis. Longdon (1957) for example with rat liver homogenate demonstrated that fatty acids are synthesized in the cytoplasm and that NADPH serves as the hydrogen donor for the reduction synthesis of fatty acids. McLean, (1958a) showed that the soluble portion of the lactating rat mammary gland had a high NADP and a high $\frac{NADPH}{NADP^+}$ ratio which favors fatty acid synthesis. She showed that this coenzyme increases from late gestation and reaches its maximum at the end of lactation. The large concentration of glucose-6-phosphate dehydrogenase and 6-phosphogluconic dehydrogenase as were found by the above author at these stages of lactation indicate an active oxidative pentose phosphate shunt providing a large supply of NADPH for fat synthesis.

The process of lipogenesis requires NADPH for the malonyl CoA route and NADPH and NADH for the chain elongation pathway. The major source of NADPH comes through the operation of oxidative pentose phosphate shunt; while NADH may be supplied through the action of many dehydrogenases. Although NADPH may also be synthesized through the transhydrogenase system, the major cytoplasmic source of NADPH comes from the pentose phosphate shunt. Lipogenesis therefore is coupled with the glucose 6-phosphate oxidation. The oxidation of the latter require $NADP^+$ to provide NADPH

for the fatty acid synthesis. The oxidation of acetyl CoA through the Krebs cycle requires oxaloacetate which arises from the carboxylation of pyruvate, derived from glycolysis. Thus, lipogenesis is coupled with the pentose phosphate shunt, while fatty acid oxidation depends on glycolysis (see Dickens, 1959, 1961; White et al., 1964). The thyroid is shown in the present study to depress lipogenesis which is in agreement with the following authors: Pastan et al., (1961) showed that the ratio of $\frac{NADP^+}{NADPH}$ was increased in thyroid slices from the TSH treated animal. This ratio in this present study is negatively correlated with fat percent in the carcass. Field et al., (1960, 1961, and 1963) present evidence which indicates that thyroid glands from TSH treated dogs and thyroid slices with added thyroxine increase glucose and acetate oxidation by increasing the $NADP^+$ concentration, since the rate of oxidation was the function of $NADP^+$ concentration in the thyroid slices. As is clearly shown in Figure 5, total coenzymes, NAD, NAD^+, NADH, NADPH, are negatively correlated with percent lean. The positive correlation between NAD^+ and fat synthesis is difficult to interpret. Although NADH is required for chain elongation of fatty acids (White et al., 1964; Dickens 1959), the oxidized form of NAD seems to be responsible for the oxidation of food substances through glycolysis and the Krebs cycle and the production of ATP through the NADH and oxidative phosphorylation system. The percent lean and fat in

the body as is shown by a negative correlation coefficient (Table IX) indicates a balance between the two, that the coenzymes which depress percent lean would indirectly result in high percent fat and thus its positive correlation with fat is apparent. This would be the case with a negative correlation of NAD and NAD^+ with percent lean in the carcass. If the percent lean in the carcass is assumed to be a measure of growth, this agrees well with the postulation by Morton (1958, 1961), that these coenzymes regulate cell division and that a low level would somehow trigger mitosis. This postulation is supported by Caiger et al., (1962) and Filsell et al., (1963). The work of these three authors will be elaborated upon in the section related to growth. In conclusion to this section it may be said that the younger animal appears to have a different metabolic pattern compared to mature animals as had been indicated earlier by Bogart et al., (1963). These differences seem to be associated with age and the differences in the concentration of various enzymes which decrease with age as studied in various animals, (ruminants and monogastric) by Jarrett and Filsell (1958 and 1960), Gallagher and Buttery (1959), Burch et al., (1963), Dennis (1966), and Bartley et al., (1966). Having these changes in mind and the low thyroid weight at 1000 lbs., body weight with increasing age, high levels of coenzymes related to fat synthesis which depress mitosis would therefore favor fat deposition and decrease lean meat in the carcass.

These developmental changes in the animal would be reflected in feed per unit gain and growth.

Nicotinamide Nucleotide Coenzymes, Feed per Unit Gain, Growth, Thyroid Weight and Percent Lean

Positive associations of $NADP^+$, $\frac{NADP^+}{NADPH}$ and thyroid weight with feed per unit gain are indicated in Table IX. Feed per unit of gain is, in the meantime, negatively correlated with NAD and NAD^+, percent lean, and gain per day. Percent lean is negatively correlated with fat and with all the coenzymes which increase feed per unit of gain. Thyroid weight is correlated negatively with all forms of coenzymes which negatively affect percent lean. Thyroid is also positively correlated with feed per unit of gain and NADPH, the oxidized form ($NADP^+$) which is positively correlated with feed per unit gain. Thyroid weight is also positively correlated with $\frac{NADP^+}{NADPH}$. This ratio also positively affects feed per unit of gain. Negative correlation exists between gain per day and $NADP^+$; the latter is also positively correlated with feed per unit of gain. These correlations are demonstrated in Figures 6 and 7. These figures explain the relationship between coenzymes, feed per unit of gain, gain per day, thyroid weight and percent lean; fat is included merely to help interrelate the relations for the traits in question. Although these relationship charts are self-explanatory, in words, however it can be interpreted as follows:

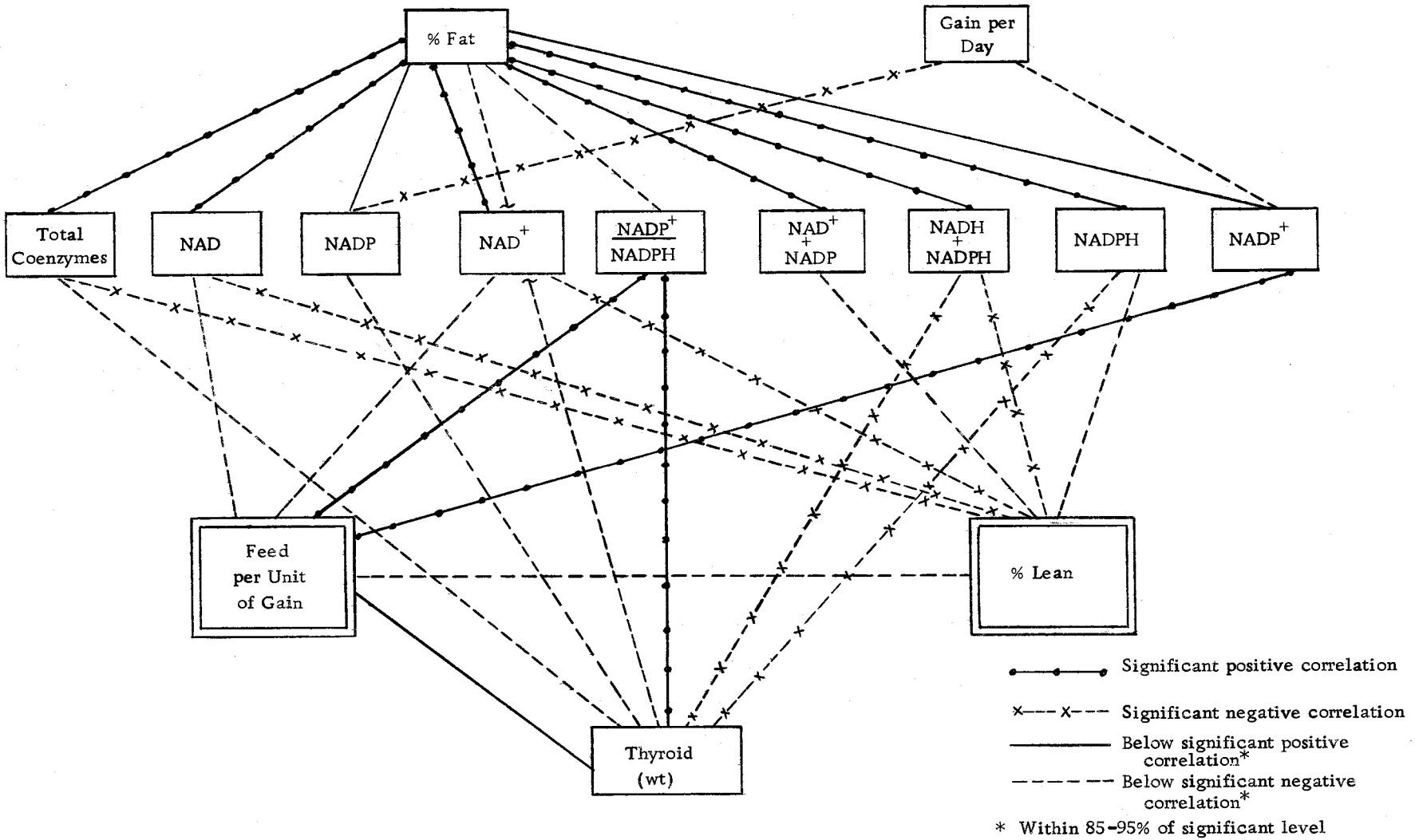


Figure 6. Relationship between feed per unit gain, percent lean, gain per day, fat, throid gland weight and coenzymes.

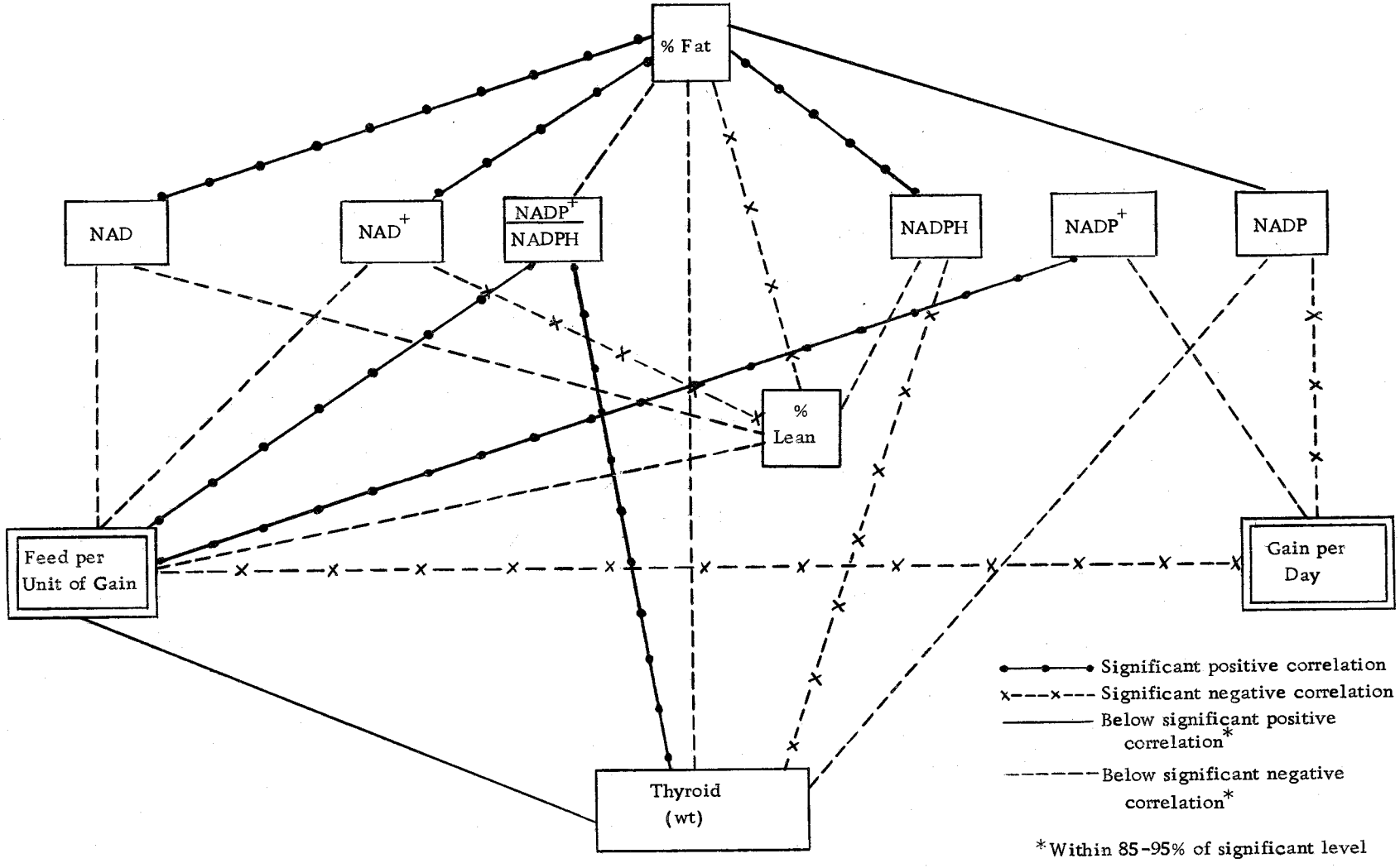


Figure 7. Relationship between feed per unit gain, gain per day, fat percent, thyroid gland and coenzymes.

As the concentration of $NADP^+$ and ratio of the $\frac{NADP^+}{NADPH}$ increases the feed per unit of gain increases. In the meantime the thyroid gland decrease the coenzymes needed for fat synthesis, which would increase the percent lean in the carcass. Reference to Figure 6 shows that those forms of coenzymes which favor fat deposit reduce percent lean and thus, increase feed per unit gain indirectly. The only exceptions are NAD^+ and NAD which are negatively correlated with lean and with feed per unit gain. Although positive correlations exist between these latter coenzymes and fat percent, their negative correlation with feed per unit gain and percent lean indicates a complicated role that NAD may be playing in metabolism. Although no positive interpretation can be offered, a simple speculation may be that NAD (NAD^+ and NADH) is maintaining normal regulatory balance between feed per unit gain, percent lean and fat and gain per day as long as the concentration is of proper order. Changes in the critical concentration of these coenzymes might disrupt the balance between the above mentioned. Reference to Figure 7 would indicate that as the concentration of $NADP^+$ and the ratio of $\frac{NADP^+}{NADPH}$ increases feed required per unit gain increases. These relationships can be clearly seen in Figure 8, with means of parents vs. offspring. This figure shows the positive relation that exists between $NADP^+$, feed per unit gain and thyroid. The increased feed needed per unit gain automatically

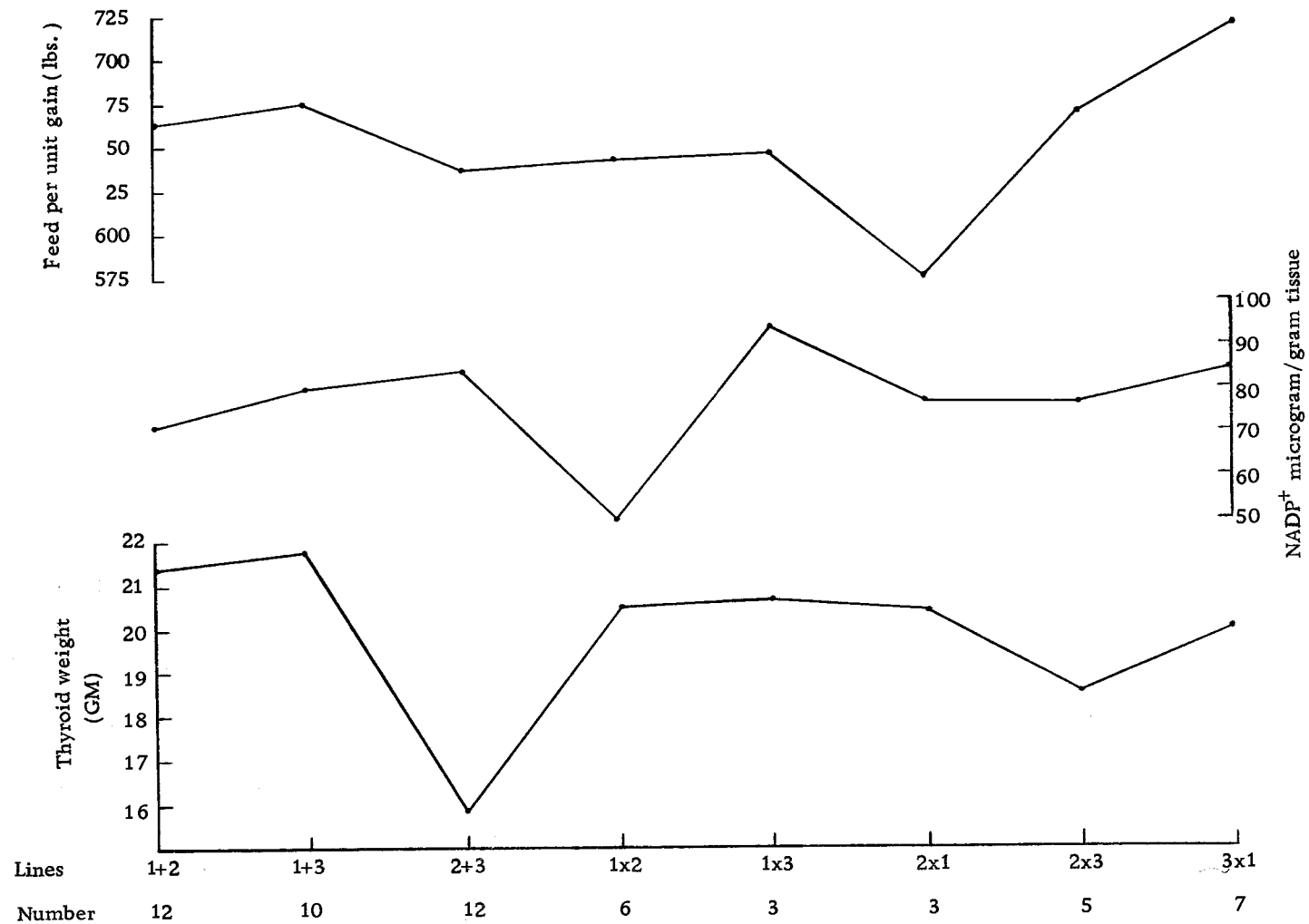


Figure 8. Means of coenzymes and feed per unit gain for the average of parents and offspring.

decreases the gain per day as the two are highly negatively correlated (-0.781). This decrease in gain per day also results from the negative correlation that exists between gain per day and content of NADP and $NADP^+$. This is shown in Figure 9 with mean values of parents vs. offspring. Thyroid appears to be playing a double role in maintaining the relationships between gain per day and feed per unit gain. It can be seen (Figure 7) that as the weight of the thyroid increases, the ratio of $\frac{NADP^+}{NADPH}$ increases. This latter ratio increases feed per unit of gain as was mentioned earlier. The net result would be the indirect effect of thyroid to increase feed per unit gain and thus would decrease gain per day. On the other hand, an increase in thyroid weight due to its negative correlation with NADPH and NADP will decrease the supply of these coenzymes which are required for fatty acid synthesis. The consequence is a decrease in fatty acid synthesis and an indirect increase in the percent lean since fat and lean are highly negatively (-0.809) correlated. The decrease in the NADP due to large thyroid size also increases gain per day. In summary, the thyroid appears to have the strange ability to increase both feed per unit gain and gain per day. The latter two as mentioned above are negatively correlated. These functions of thyroid cannot be explained with the present experimental evidence. It can only be speculated that there may be a limit to the weight of thyroid which can induce the changes in the coenzyme

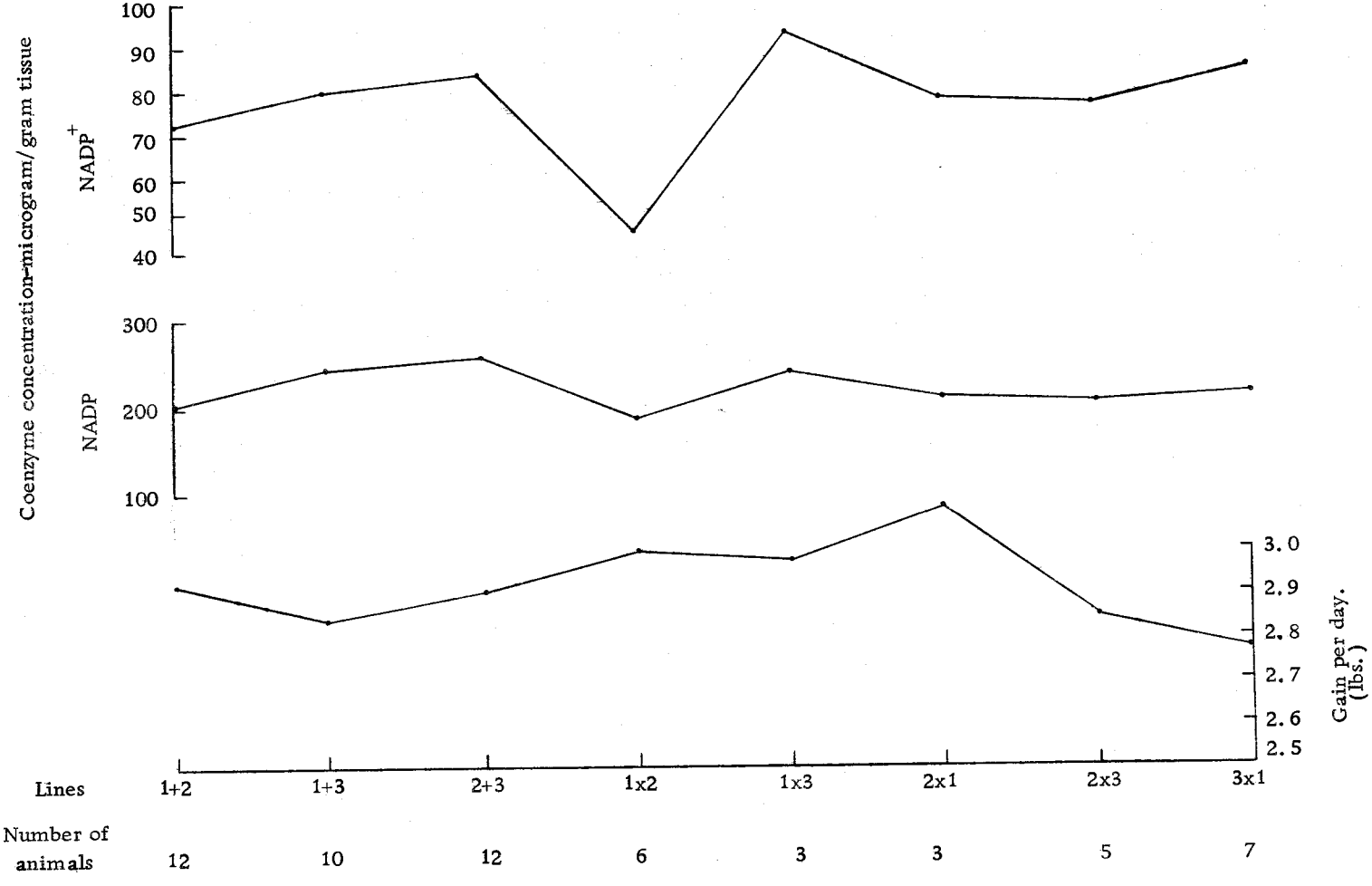


Figure 9. Means of coenzymes and gain per day for the average of parents and offspring.

level, thus affecting feed efficiency and gain per day. It is clear if the weight of thyroid was taken as function of its activity then extremely large thyroid weights might be indicative of highly hyperactivity. This, however, was not true in the experiment (Mason et al., 1956), where intermediate thyroids were associated with highest rate of gain and large thyroid were shown to be associated with low oxygen consumption. It is possible that the present work may be involved with the thyroid gland weight which is still within the limit of normal weight range. The data from the present study do not seem to agree in general, in regard to thyroid weight, with that of Mason et al., (1956), but further evidence is required to determine the proper thyroid gland weight that induces the proper coenzyme concentration for efficient growth and rate of gain. Although, as known in general, and is indicated (Price et al., 1956; Bogart et al., 1963; and Clark et al., 1963) in various experiments that as age increases, feed efficiency and rate of gain decreases; no direct correlations of any kind were observed for the above traits with age with present work. The present experiment, however, provided evidence to support the work of the above authors on the basis of coenzyme concentration which changes with age. Reference again to Figures 1, 2, and 3, also section on nicotinamide nucleotide coenzymes, thyroid weight, and production traits would indicate that all forms of coenzymes are positively correlated with

age and as can be seen, these coenzymes favor fat deposition, and reduce lean (Figure 5) and thus reflect slow growth and low efficiency of production.

The relationship of age and nicotinamide nucleotide coenzymes is also demonstrated by various workers (Nemeth and Dickerman, 1960; Raihra, 1961; Raux, et al., 1962; Burch and Dippe, 1964; Caiger, et al., 1962; and Filsell, et al., 1963). Branster and Morton (1956), indicated that rate of synthesis of NAD in the tumor tissue was much lower than in the normal tissue. These authors presented evidence which shows that the concentration of the coenzyme is related to the concentration of the nuclear enzyme, nicotinamide mono-nucleotide pyrophosphorylase, which is lower in the embryonic, regenerating and tumor tissue than in the corresponding normal adult tissue. Morton,(1958, 1961) thus, postulated an interesting mechanism through which the nicotinamide nucleotide coenzyme is shown to regulate cell division. According to this author, the nucleus is the only source of NAD. After being synthesized in the nucleus along with DNA and RNA, the coenzyme is sent out to the cytoplasm to help carry out the biochemical reaction dictated by DNA and RNA. The level of NAD in the cytoplasm, therefore, drops due to its involvement in various reactions and due to the increased size of the cytoplasm. In addition NAD is attacked by the NAD'ase which is normally present in the cytoplasm. As the concentration

decreases below a critical level in the cytoplasm, the nucleus is forced to meet the demand for NAD by the cytoplasm. Consequently the nucleus increases its size by duplicating its content and consequently cell division results. In the cases where a genetic defect exists in the nucleus, which cannot synthesize adequate amounts of NAD, the nucleus keeps on dividing at a much faster rate to meet the demand for this coenzyme by the cytoplasm. The mechanism is operated as the basis of feedback inhibition. These types of cells are represented by the tumor tissues. Embryonic tissue, regenerating and tumor tissues compared to adult tissues appear to have lower levels of nicotinamide mono-nucleotide pyrophosphorylase enzymes which is located in the nucleus and is responsible for the synthesis of NAD. The tumor tissue therefore resembles closely the embryonic tissue. This postulation certainly is attractive and is supported with experiments on rats and sheep by Caiger et al., (1962) and Filsell, et al., (1963) who also indicated that low levels of the coenzymes were associated with rapidly growing animals.

The results of the present study seems to support this postulation to the extent that positive correlations of coenzymes and age were obtained (Figures 1, 2, and 3, and Table IX). Objection to this theory is that the interrelations of endocrine (thyroid) is not being considered. The present study indicates that thyroid gland weight is negatively correlated with all forms of coenzymes. In

the meantime thyroid weight is negatively correlated with age at 1000 lbs. (Table IX and Figures 1 and 5). As a consequence at the embryonic, prenatal, neonatal stages when large thyroid gland weights exist, the level of the coenzymes are at their minimum. As thyroid weight decreases with age, the level of inhibition is released and as a result the levels of coenzymes increase. On the basis of the above evidence, the author would suggest further studies in which the postulation presented by Morton (1958, 1961) and supported by Caiger et al., (1962) and Filsell, et al., (1963) is to be studied in relation to endocrine glands (thyroid) since the relationship of the thyroid to coenzymes (see Figures 1, 2, 4, 5, 6, 7, 8, 17 and Figure 19), now is obvious as a result of the present study with intact bulls of various lines and line crosses raised and killed at standard conditions.

Nicotinamide Nucleotide Coenzymes, Inbreeding and Growth

Experimental evidence supports the fact that a high degree of inbreeding is associated with loss of vigor. Alexander and Bogart for example, (1961) working with the same lines used in this study reported that inbreeding significantly depressed suckling gain and that post weaning rate was not affected. Hoornbeek, (1964) working with same lines supported earlier work of Alexander and Bogart, (1961) indicating that cattle with zero and low percentages of

inbreeding performed at a higher level than those with higher inbreeding for suckling gain. He pointed out that the performance in cattle with low inbreeding was at a lower level for post weaning rate of gain. Although suckling gain was not studied in the present investigation, the results of study with post-weaning rate of gain for the 55 bulls in general agrees with that of Alexander and Bogart, 1961 since no significant differences were found for the "t" values, (Table VI) when all inbreds were tested against line crosses for post weaning rate of gain, and feed per unit gain. With the exception of the 2 x 1 line cross which was significantly superior to $\frac{1 + 2}{2}$ for feed per unit gain, and $\frac{1 \times 2 + 2 \times 1}{2}$ which was also superior to $\frac{1+2}{2}$, no other significant difference was found for the "t" values when the mean of inbred parents were tested against their line cross progenies. Lines compared among themselves using the "t" test showed that line 2 was superior to line 4, both in gain per day and feed per unit gain. Line 3 also showed superiority to line 4 in feed per unit gain. Lines 1, 2, and 3 did not show any statistically significant difference in gain per day or feed per unit gain. The "t" values for these tests will be elaborated on later in the statistical section.

As of yet, no firm basis on the cellular level have been presented to show how fixation of loci brings about various degrees of loss of vigor. This study, too, is no exception, although evidence

obtained through the present study provides ground for speculation which might form a basis for further work in this line of investigation. A significant negative correlation between inbreeding and percent lean in the carcass is seen in Table IX. Inbreeding in the meantime also shows a positive correlation with total coenzymes, NADP, NADPH, [(NAD^+) + $(NADP^+)$] and [(NADH) + (NADPH)] . All of these coenzymes in the meantime are negatively correlated with percent lean in the carcass. The relationships are demonstrated in Figure 10. Briefly it can be interpreted as follows: As the inbreeding increases, percent lean in the carcass decreases. As inbreeding increases the levels of those coenzymes that are negatively correlated with lean also increase in the liver. As a consequence with an increased concentration of coenzymes as a result of inbreeding, the percent lean in the carcass of inbred animals decreases. This is clearly demonstrated in Figure 11, where mean values of percent lean for lines with various degrees of inbreeding and line crosses are plotted. The mean values of those traits (gain per day, feed per unit gain and certain coenzymes) which showed statistically significant "F" values as well as percent fat, lean and thyroid gland weight are plotted in Figures 12 and 13 for straight lines and line crosses, the "t" values for which is presented in Table VI. As can be seen from Figures 12 and 13, which are to be read together, differences in the levels of the coenzymes are

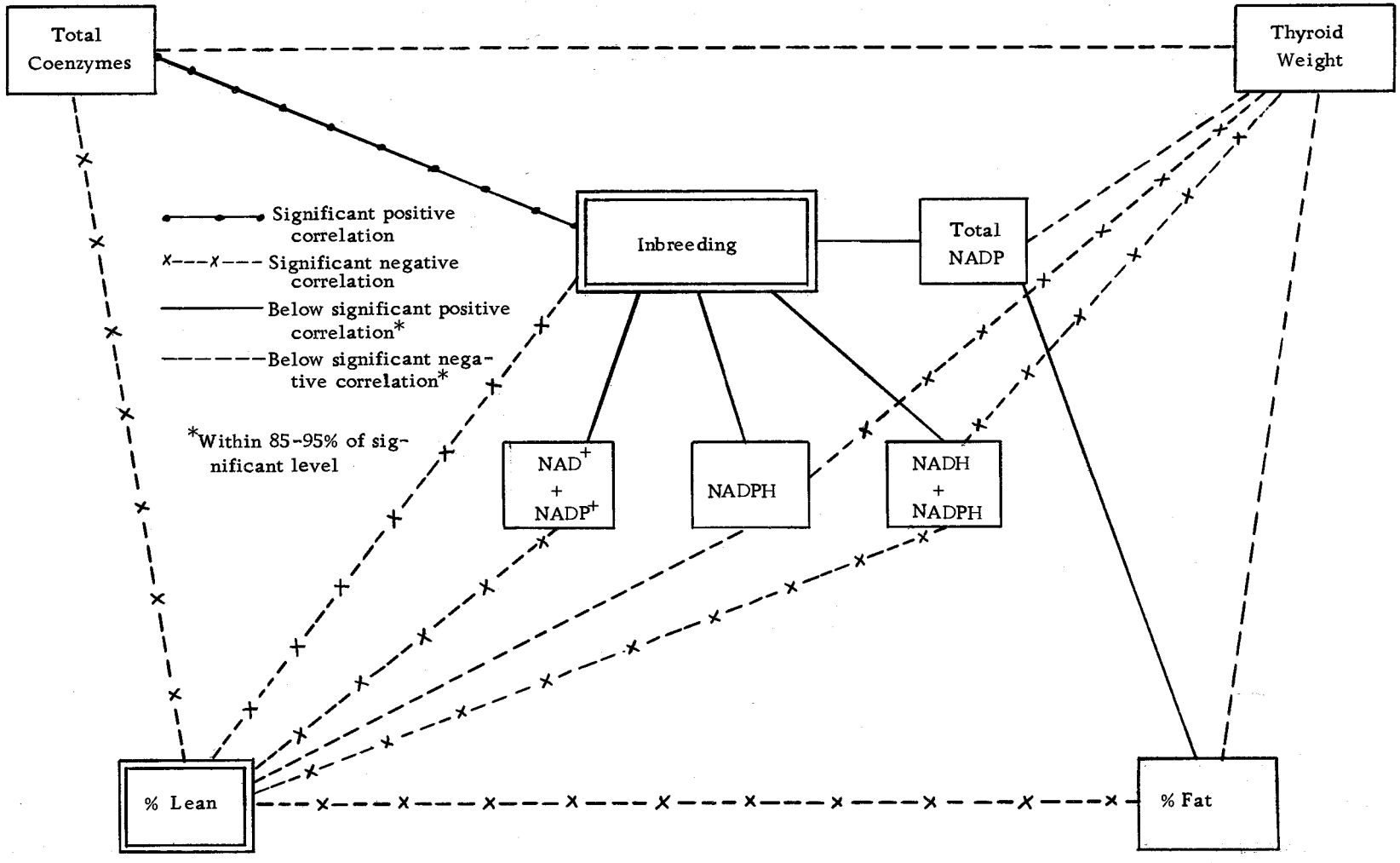


Figure 10. Relationship between inbreeding, percent lean, coenzymes, percent fat and thyroid gland weight.

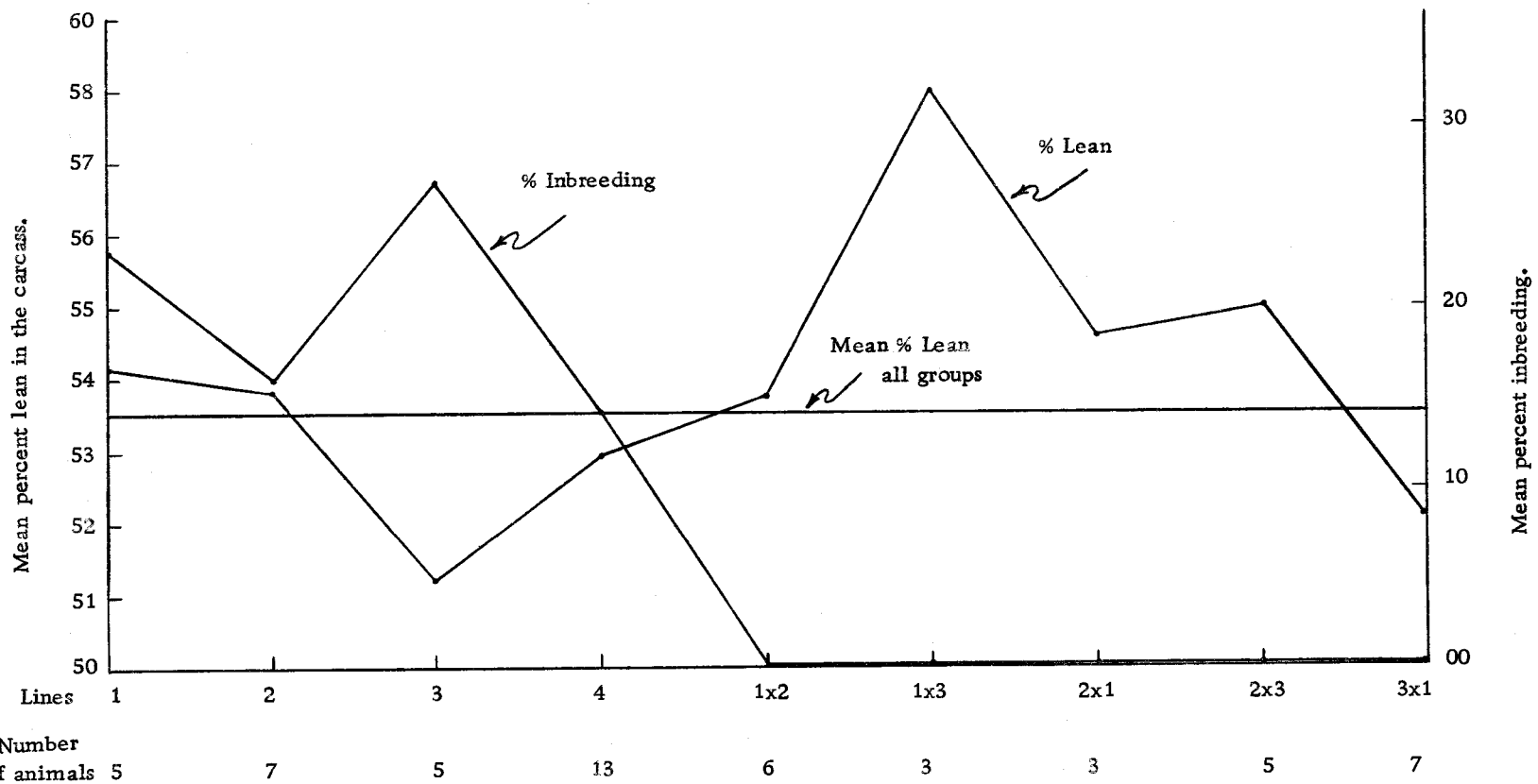


Figure 11. Means of percent lean and percent inbreeding for lines and line crosses.

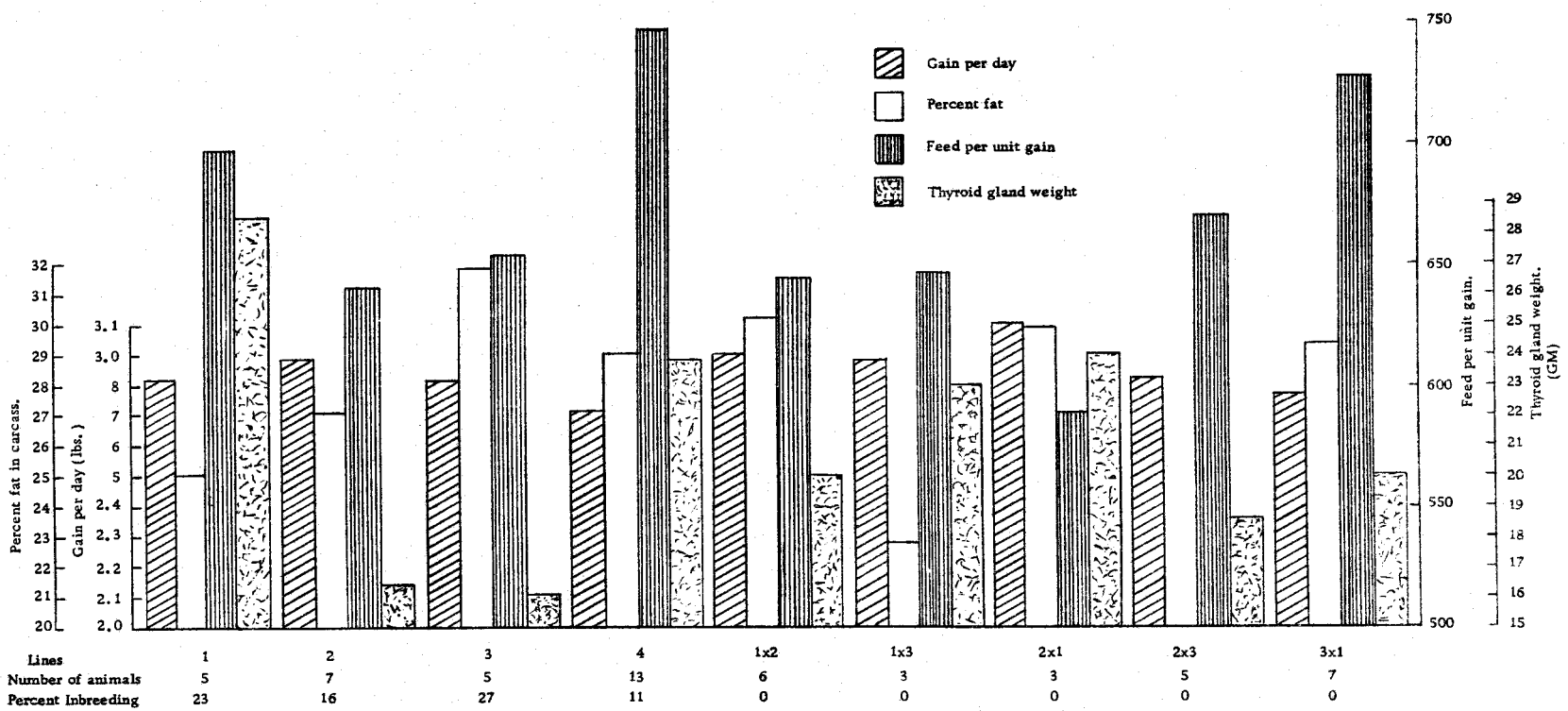


Figure 12. Means of thyroid gland weight and production traits for lines and line crosses.

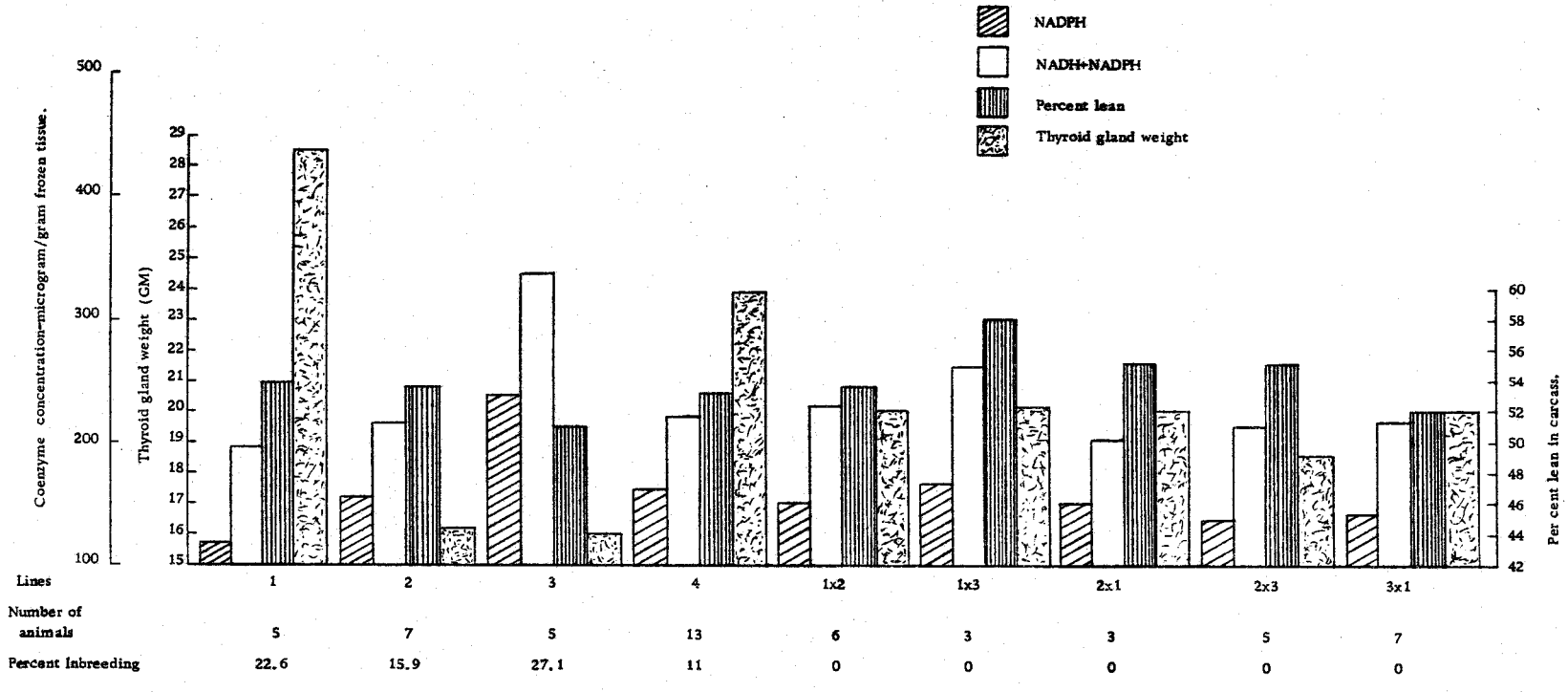


Figure 13. Means of coenzymes, percent lean and thyroid gland weight for lines and line crosses.

reflected in the values of those traits to which they are correlated. In particular, differences between percent lean in the carcass is pointed out by Figure 13 and it can be seen that the inbred lines have a percent lean which is below that of the line crosses. There is a negative correlation between thyroid weight and level of NADPH (Figure 13) and a positive correlation of the latter with fat (Figure 12). The NADH+ NADPH level in Figure 13 reflects the positive correlation with percent fat in Table 12. Total coenzymes, $NADP^+$, $\frac{NADP^+}{NADPH}$ ratio, percent fat, gain per day and feed per unit of gain are plotted in Figure 14 for inbred lines and line crosses. Reference to this figure demonstrates the negative correlation that exists between the $\frac{NADP^+}{NADPH}$ ratio and percent fat in the body. A further demonstration of the $\frac{NADP^+}{NADPH}$ ratio which is negatively correlated with percent fat is presented with mean values of straight lines and line crosses (Figure 4). The negative correlation between feed per unit gain and gain per day is also easily seen in Figure 14. Lower total coenzymes and lower $NADP^+$ levels in this figure and in Figure 9, seem to be associated with higher gain per day. The coenzyme, $NADP^+$, also seems to positively increase feed per unit gain (see Figures 8 and 14), as these two are also significantly positively correlated. Some of the variations that exist in these correlations, as applied to the means of various lines (Figures 12, 13 and 14) can be attributed to small and unequal sample sizes representing some of the

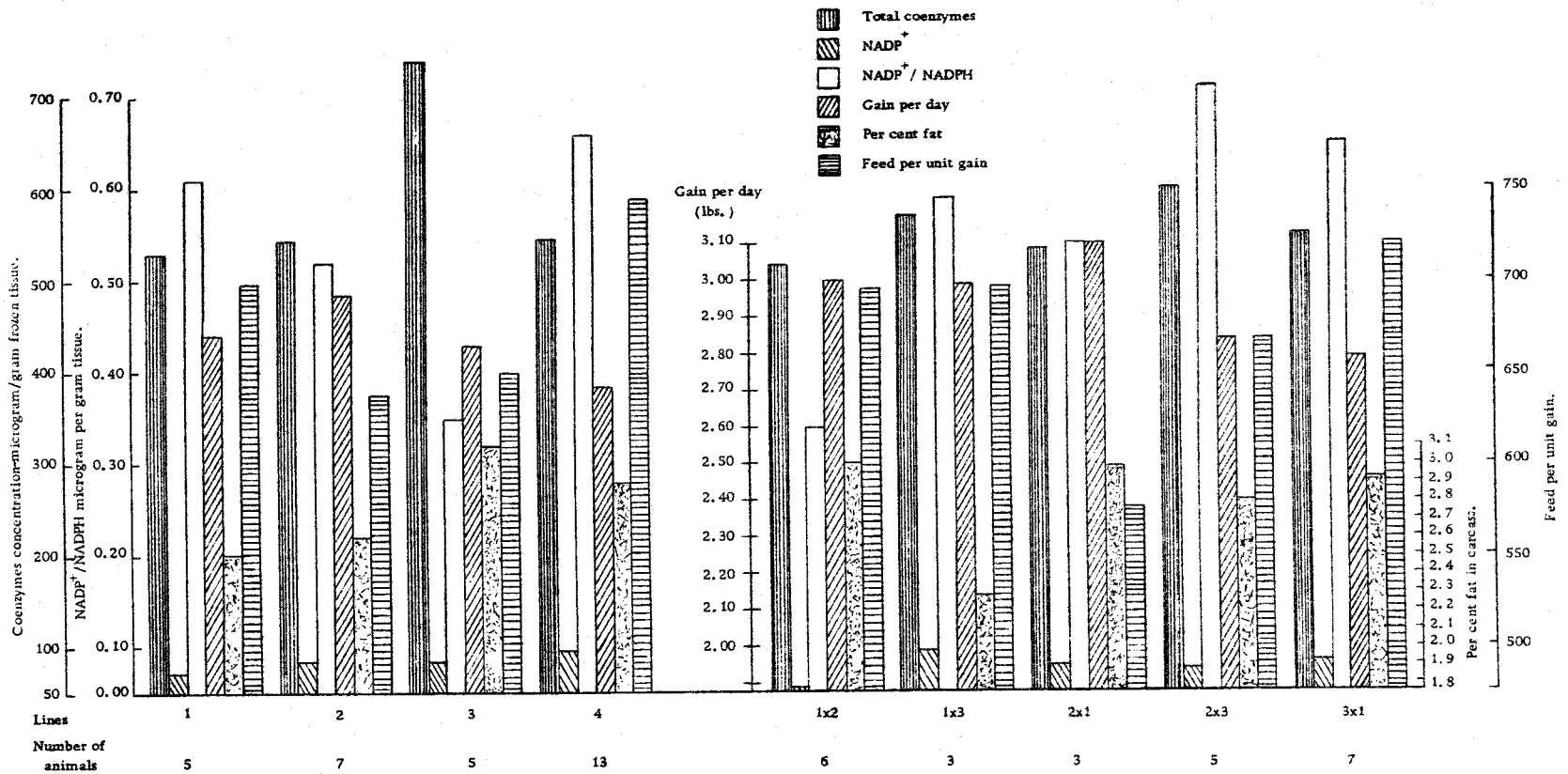
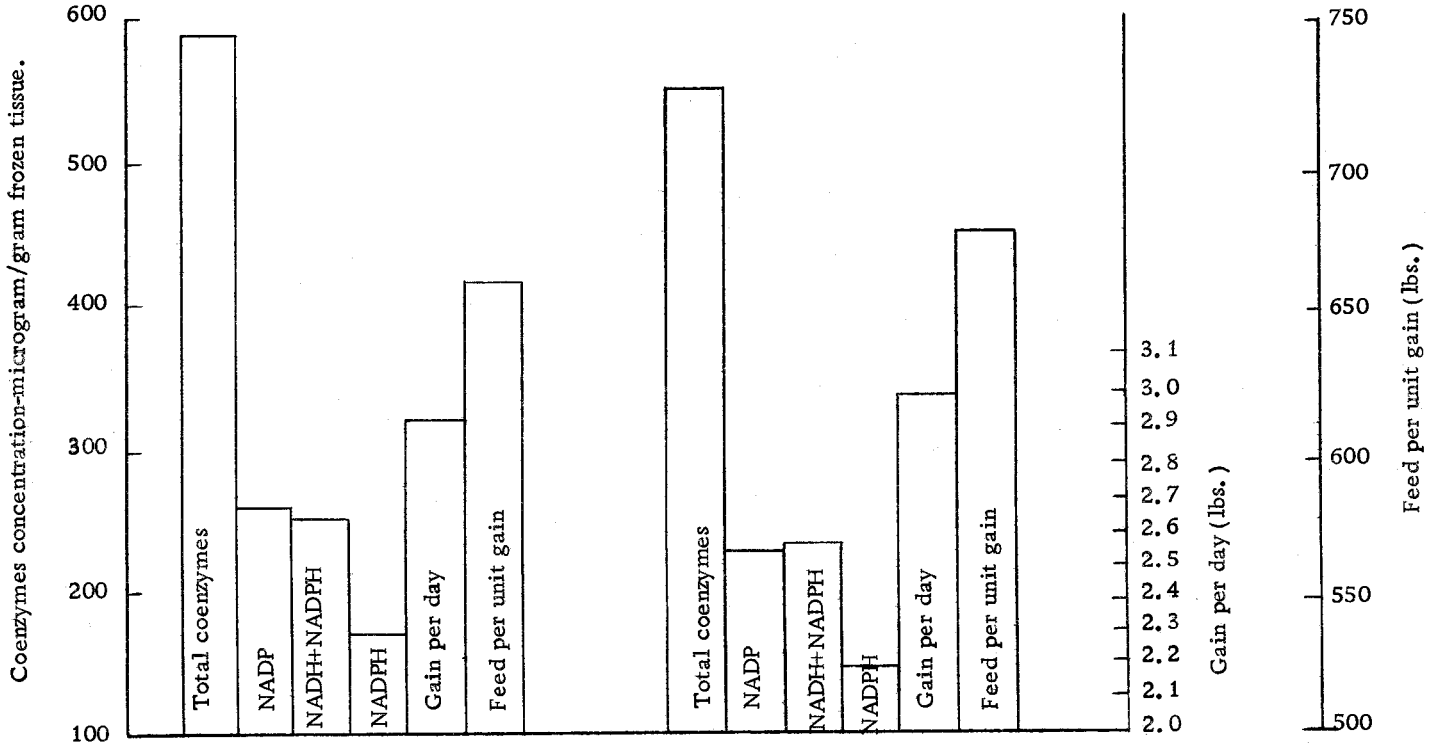


Figure 14. Means of coenzymes and production traits for lines and line crosses.

lines. The data were further broken down to study the difference between inbred lines and line crosses. Reference to Table IX will indicate that when all inbred lines, including Angus, with an average of 16.76 percent inbreeding were tested against the line cross, no significant "t" values were obtained for any of the coenzymes or production traits studied. In the second test of inbreds vs. line crosses, the angus group was removed to increase the inbreeding level to 21.0 percent and since the Angus group was not represented by any line cross. Again no significant "t" values were obtained for either the coenzymes or the production traits (see Table IX). It can, therefore, be said that in cattle with 17 and 21 percent inbreeding compared with line crosses performed similarly when all line crosses were compared with all inbreds. Significant superiority in rate of gain and feed per unit of gain as indicated earlier were shown by the "t" test for 2 x 1 compared with $\frac{1+2}{2}$ and $\frac{1x2 + 2x1}{2}$ compared with $\frac{1 + 2}{2}$. The results obtained in this study seem to confirm the earlier report by Alexander and Bogart (1961) who indicated that although inbreeding caused a depression in the suckling gain, post weaning rate of gain was not affected. The results obtained here, however, are not in agreement with those reported by Hoornbeek (1964) who indicated that the line crosses performed superiorly in suckling gain but were inferior in post weaning rate of gain. It might be pointed out that, although generally not

statistically significant, the higher gain per day, lower feed per unit gain, and higher percent lean in the carcass were trends which were associated with line cross animals. This superiority of line cross bulls was also associated with low levels of all four coenzymes studied. These are shown in Figures 15 and 16 with mean values. If these coenzymes are to be correlated with production traits as in this case, this observation between the inbred lines and the line crosses seems to be logical since no variation in the coenzymes exist to be reflected in production traits between these two groups of animals. It now appears from the study of correlations, (Table IX and from Figures 12, 13, 14, 15 and 16) that lower levels of coenzymes are associated with a higher rate of growth. As indicated earlier, Branster and Morton (1956), Morton (1958, 1961), Caiger et al., (1962) and Filsell et al., (1963) also obtained information indicating that lower levels of coenzymes are characteristic of rapidly growing animals. Morton (1958, 1961), therefore, proposed his interesting theory, already described earlier that lower levels of coenzymes trigger cell division and growth. The mechanism was discussed earlier. No report from the literature is available in which actual weight in the carcass is correlated with nicotinamide coenzyme levels in the tissue of various lines of inbreds and line cross animals. Such was considered in this present study and the evidence as discussed indicates that a low level of



Lines	Inbred lines 1, 2, 3.	Line cross Herefords (1x2, 1x3, 1x2, 2x1, 3x2, 3x1)
Mean percent Inbreeding	(21.16)	0
Number of animals	17	25

Figure 15. Means of coenzymes and production traits for inbreds and line crosses.

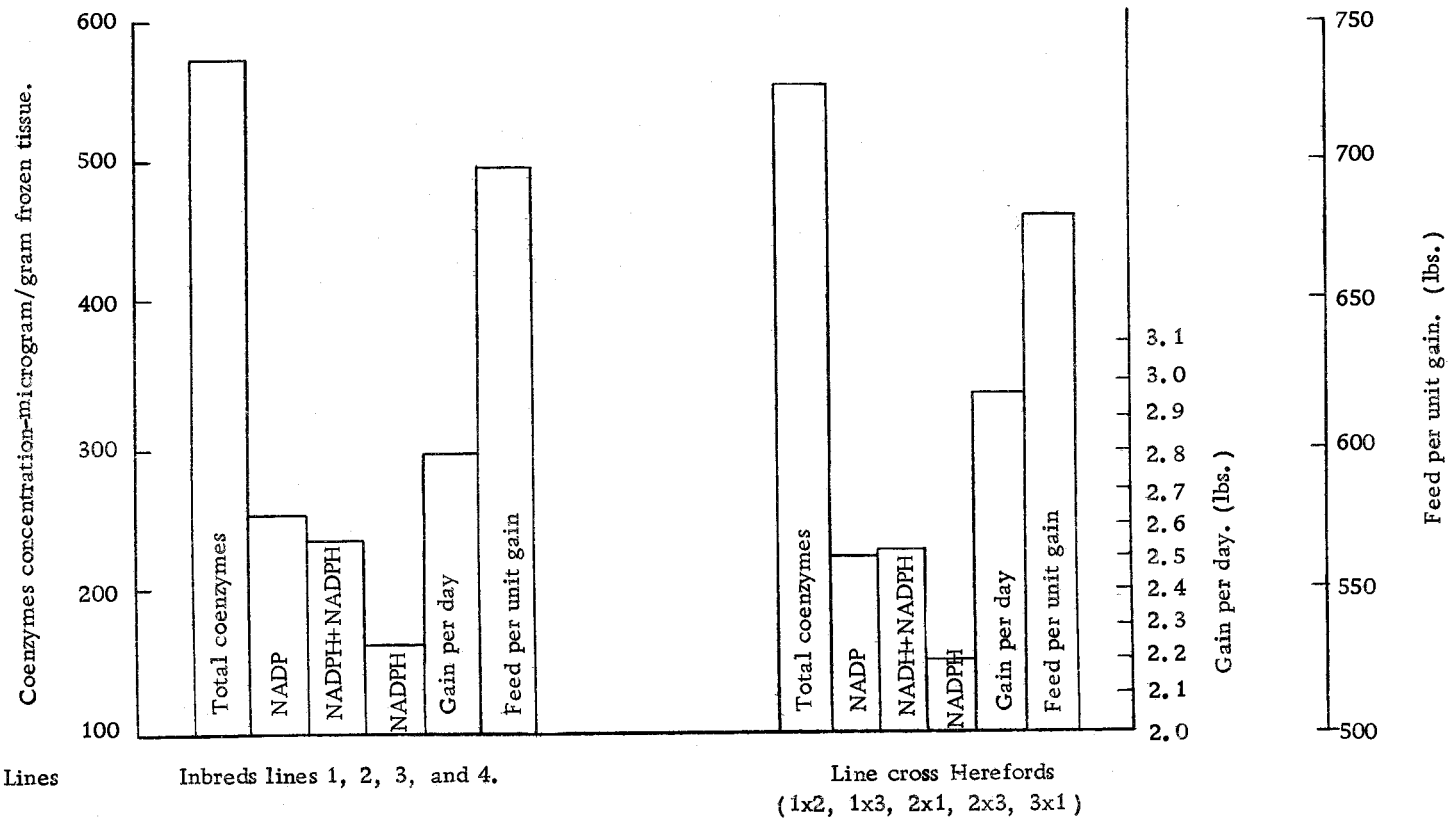


Figure 16. Means of coenzymes and production traits for inbreds and line crosses.

coenzymes is associated with a high percent of lean in the carcass and higher gain per day for lines and line crosses (see Figures 11, 13, 14, 15, and 16).

The fact that inbreeding is positively correlated with coenzymes and that lean is negatively correlated with coenzymes and inbreeding (Table IV, and Figures 10 and 11), leads one to present the idea on a theoretical basis that line cross animals have a tendency to have lower levels of coenzymes and thus a higher percent of lean in their carcass, indicating a more rapid growth compared to their inbred parents. More work is needed to investigate this as a possibility in an answer to the mechanism through which hetrosis is being brought about. In this respect, inbreeding seems to have a tendency to increase the concentration of the coenzymes similar to the changes in coenzymes with age in animals (see Figures 1, 2, 3 and 5).

Nicotinamide Nucleotide Coenzymes, Thyroid Weight and Production Traits

Although the relationship of thyroid weight to various traits (gain per day, feed per unit gain, percent fat, percent lean, age and inbreeding) has already been discussed earlier under related topics, its overall relationships to these traits is further demonstrated in Figure 17. Thyroid weight is negatively correlated with

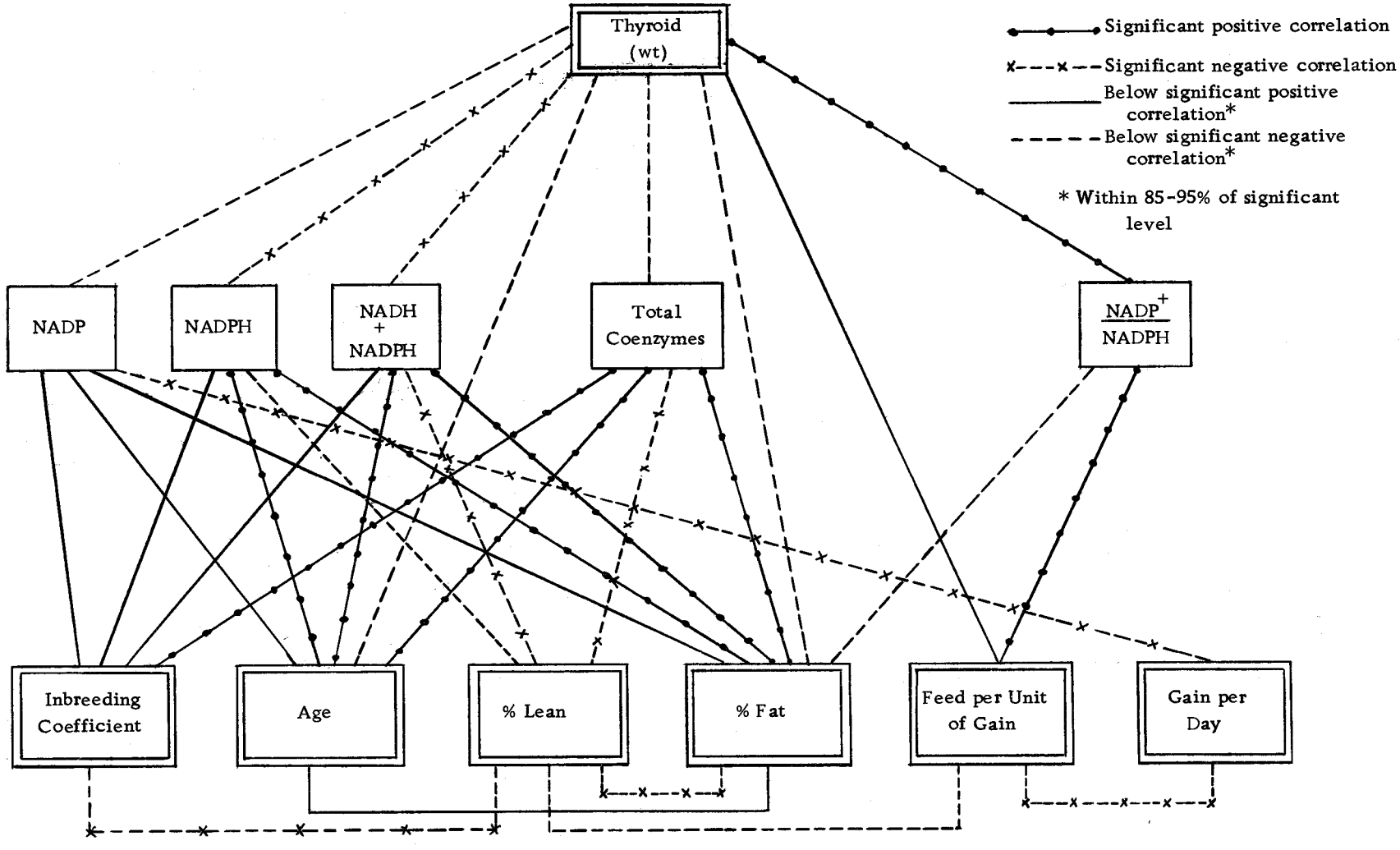


Figure 17. Relationship between thyroid gland weight, coenzymes and production traits.

total coenzymes and a high negative correlation of thyroid weight with NADPH,[(NADH + NADPH)] as shown in Table IX and Figure 17. A negative correlation exists also between thyroid weight and fat percent. A positive correlation exists between thyroid weight and $NADP^+$, $\frac{NADP^+}{NADPH}$ feed per unit gain and percent lean in the carcass (Figures 1, 4, 5, 6, 7, 8, 10, 12, 13 and 17, and Table IX). Although no further explanation on how the thyroid affects each of these traits seems necessary, a brief comment might help to explain Figure 17. As is shown the thyroid seems to increase the $\frac{NADP^+}{NADPH}$ ratio. This ratio in the meantime increases feed per unit gain, while it decreases percent fat in the body. The thyroid may indirectly increase gain per day by decreasing NAD which is negatively correlated with percent lean. The decrease of fat by the thyroid is mediated through the significant decreasing action of the thyroid on the level of NADPH since the latter is known to be responsible for fatty acid synthesis and its significant positive correlation with fat is indicated in this study. Further, the decrease in fat percent by the thyroid is brought about by the decreasing action of the thyroid on total coenzymes which are also positively (0.462) correlated with fat percent. The over-all decrease in fat by thyroid action may be due to the fact that the thyroid decrease those coenzymes (total coenzymes, NAD^+, NADPH), which are negatively correlated with percent lean and thus would increase percent lean and the latter will

automatically decrease the percent fat since the two are negatively correlated (-0.809). The negative correlation of thyroid weight with age (Figures 18 and 19) can be interpreted that as age increases the amount of thyroid tissue at 1000 lbs. body weight decreases. Furthermore, as age increases the reduced thyroid weight will release its decreasing mechanism over the $\frac{NADP^+}{NADPH}$ ratio and this causes more fatty acid synthesis. In the meantime, the decreased thyroid weight with age increases NADPH, [(NADH + NADPH)] and total coenzymes. These latter coenzymes are required for fatty acid synthesis and are positively correlated with percent fat in the carcass and are negatively correlated with percent lean. This seems logical since percent fat in the body has a significant positive correlation with age. The relationship seems simple if the influence of the thyroid gland can be overlooked. This is shown in Figure 20, where percent lean, percent fat, feed per unit gain and gain per day are related to coenzymes.

The findings in this study that thyroid weight decrease NADPH is in complete agreement with the work of Glock and McClean (1955) and they also agree in general with the studies of Paston et al., (1961, 1963), Field et al., (1960, 1961, 1963), who found that the $NADP^+$ concentration in the thyroid from TSH treated dogs was increased at the cost of NAD. These authors, however, indicated that the NADPH concentration was not affected.

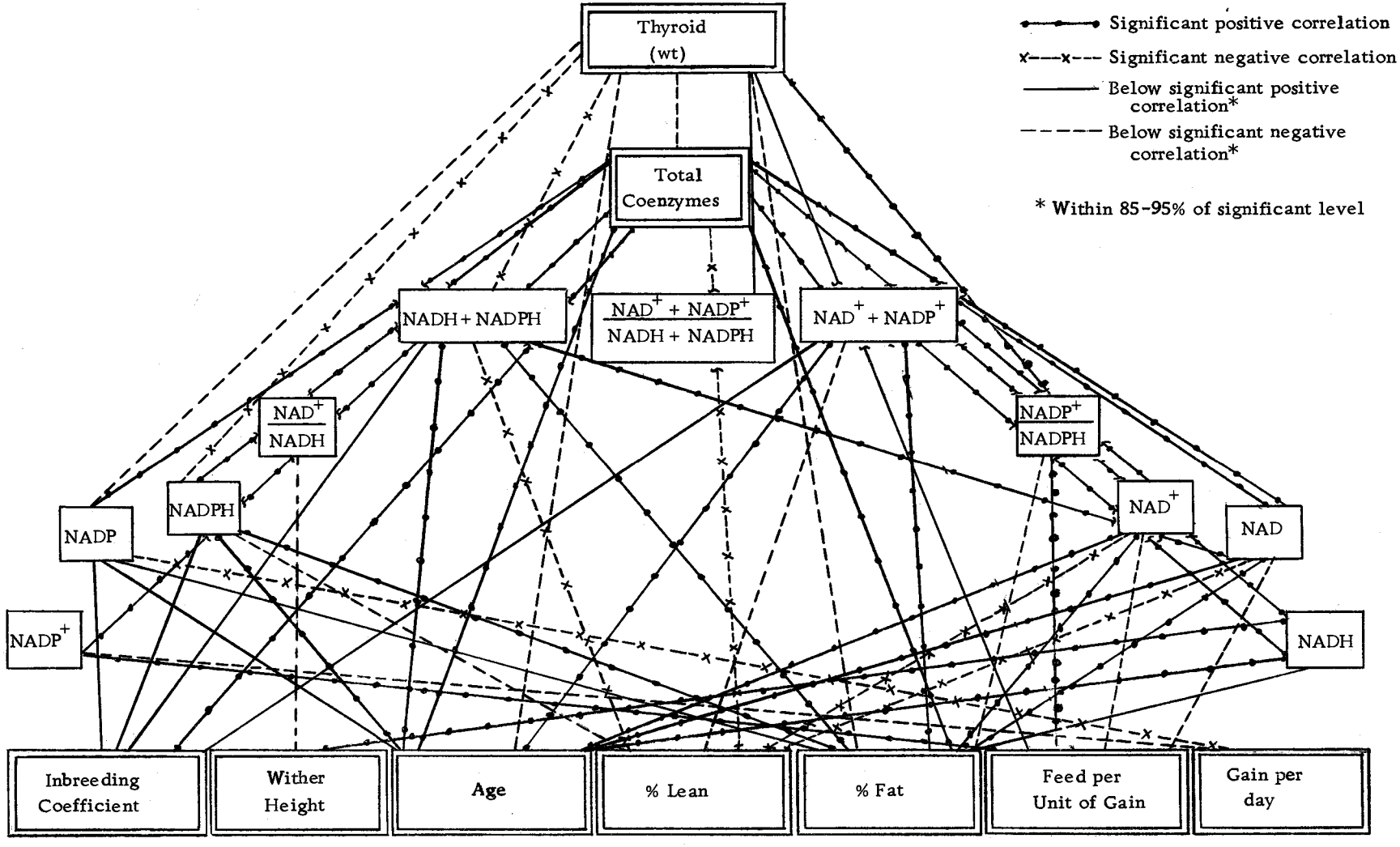


Figure 18. Relationship between thyroid gland weight coenzymes and production traits and inbreeding.

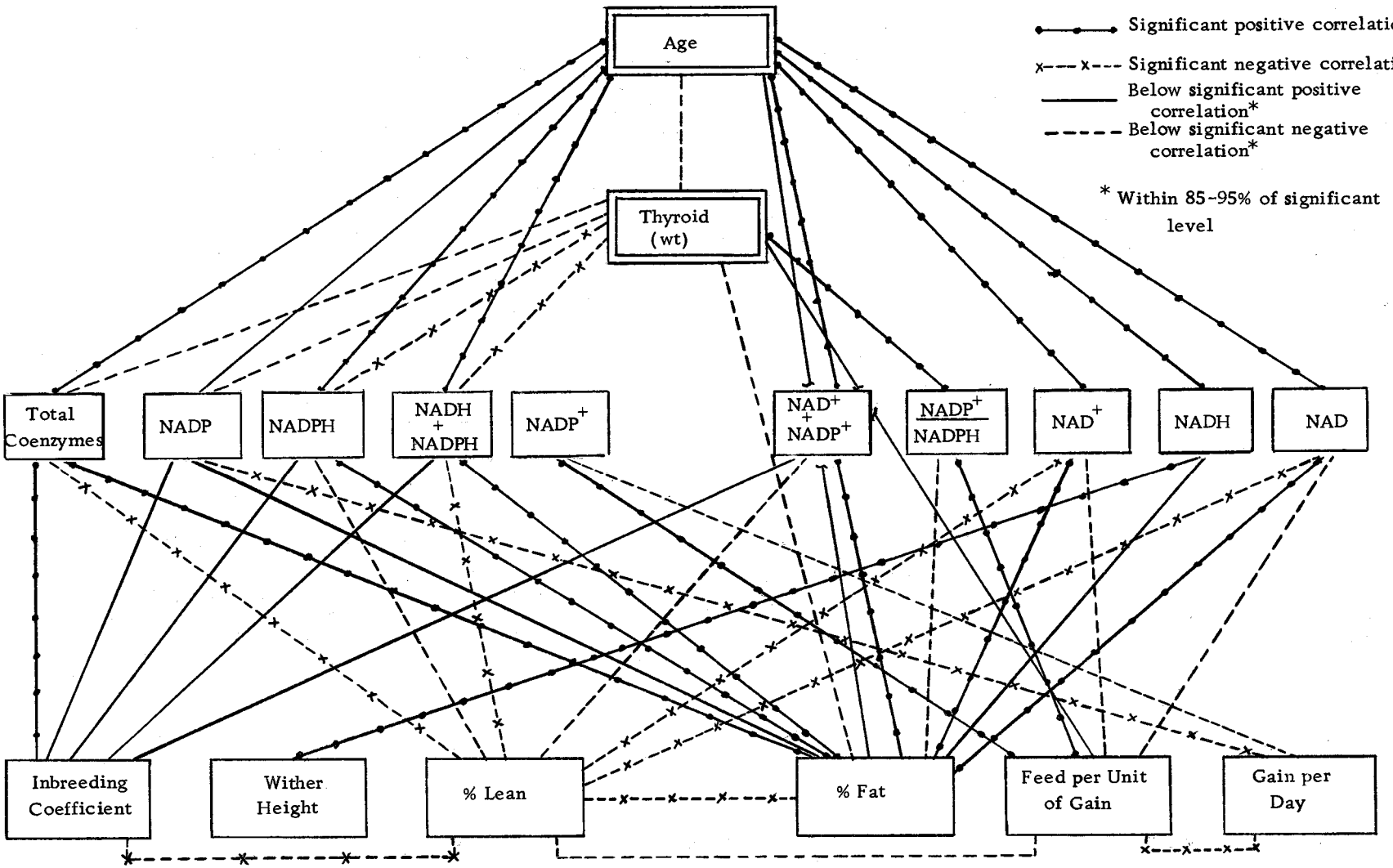


Figure 19. Relationship of age, thyroid gland weight, coenzymes to production traits.

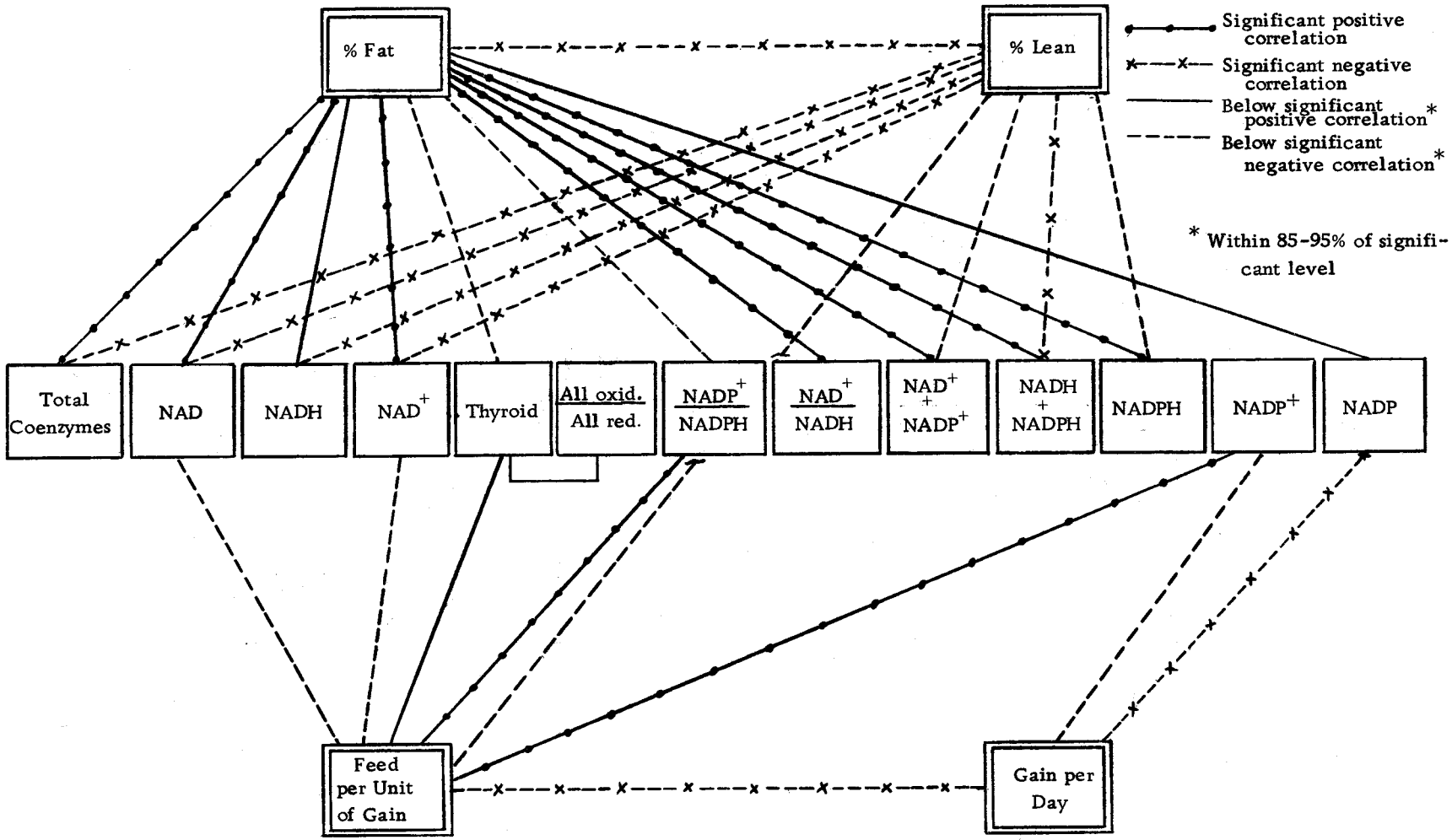


Figure 20. Relationship between production traits and coenzymes.

The present study differs from the ones by the above authors due to the fact that in present study only thyroid weight shows a significant negative correlation with NADPH and this is confirmed by the fact that thyroid weight in the present study also shows a significant negative correlation with [(NADH + NADPH)] and a significant positive correlation with the $\frac{NADP^+}{NADPH}$ ratio (see Table IX and Figures 17 and 18).

Pastan et al., (1963) indicated that the increase in $NADP^+$ was associated with a decrease in NAD. Since the present study did not utilize TSH as such, only the indirect effect of TSH through the thyroid which was obtained in the present study with intact bulls is presented. The fact that the present study used thyroid weight as an indication of both TSH and thyroxine, while the above author worked with TSH as such and also to the fact that the present study utilized liver tissues while the above authors used thyroid slices the present study cannot present a complete picture of TSH. The present study supports the work by Field et al., (1961), who found that the addition of thyroid hormones to thyroid slices increased $NADP^+$ concentration. The results from the work of Field et al., (1963) who shows that thyroid from TSH treated dogs had a high $NADP^+$ content also agree with the present work. The author in comparing these experiments with the present study has assumed that TSH ultimately means thyroid activities.

The complexity that might arise by using thyroid weight as a measure of its activity was posed and discussed earlier in this thesis. Its relationships to the nicotinamide nucleotide coenzymes and production traits were presented and its relationships to percent fat, percent lean, feed per unit gain, and age were established to be mediated through these coenzymes. The thyroid-nicotinamide nucleotides coenzyme relation was also observed by Pastan et al., (1961, 1963); Field et al., (1960, 1961, and 1963), who point out the fact that high thyroid activity is associated with higher glucose oxidation. These authors also pointed out that the rate of oxidation of glucose was a function of the concentration of $NADP^+$ in the thyroid preparation. Lardy and Fildott (1951) showed that incubation of thyroid gland tissue from hyper thyroid rats inhibited oxidative phosphorylation. Martius and Hess (1951) indicated that isolated mitochondria from rats treated with thyroxine showed much lower oxidative phosphorylation compared to nontreated rats. Lehninger, et al., (1955), Dickens, et al., (1956) presented the results of their studies with mitochondria isolated from rats treated with a source of thyroxine and showed that oxidative phosphorylation in the mitochondria was inhibited by uncoupling the electron transfer system from the phosphorylation system. These authors also indicated that thyroxine caused a swelling of the mitochondria in these studies. Kaplan (1959) indicates that nicotinamide nucleotides are

bound in the mitochondria. Spirtes and Eichel (1954) showed that loss of nicotinamide nucleotide from the mitochondria is due to the loss of the oxidized forms. Kaufman and Kaplan (1959) indicate that the swelling of the mitochondria was associated with the loss of oxidized forms of nicotinamide nucleotide coenzymes and the subsequent destruction of the oxidized forms. Kaufman and Kaplan (1960) further presented evidence to the fact that the swelling of mitochondria was correlated with the loss of nicotinamide nucleotide coenzyme and that the loss was due to the oxidized form since the oxidized form is easily escapable from the mitochondria while the reduced form is not directly lost from the mitochondria. The oxidized form after escaping the mitochondria according to these authors is destroyed by NAD'ase.

The relationships of the thyroid to the nicotinamide nucleotide coenzymes and its important role in metabolism affecting production was shown in the present study. The availability of literature related to various aspects of this endocrine gland and its relationship to oxidative phosphorylation tempts the author to present, although theoretical a model for the action of thyroid: Increased thyroid weight is taken (within the limits of this study) to be an indication of more thyroxine and thus a more active metabolic state. Increase in weight of thyroid decreases all forms of nicotinamide nucleotide coenzymes but it increases the $\frac{NADP^+}{NADPH}$ ratio (see Table IX). This

is supported by Pastan, et al., (1961, 1963) and Field, et al., (1961, 1963). After oxidation, the coenzymes escape the mitochondria and are subsequently destroyed by the NAD'ase. The swelling of the mitochondria is associated with loss of the nicotinamide nucleotide coenzymes (see Spirtes and Echil, 1954; Glock and McLean, 1955b, Lehninger, et al., 1955; Dickens, et al., 1956; Kaplan, 1959; Kaufman and Kaplan, 1959; Kaufman and Kaplan, 1960). The oxidative phosphorylation due to the thyroid activity can, therefore, be suspected to be due to: (1) The thyroid speeds up the conversion of the reduced form to the oxidized forms which later escaped from the mitochondria and destroyed by NAD'ase. (2) The thyroid causes swelling of the mitochondria, and this causes an increase in the size and surface of mitochondria. This increase in surface size of mitochondria by swelling which is associated with the loss of the nicotinamide nucleotide coenzymes would tend to decrease the concentration of the mitochondrial enzymes and coenzymes and also would mechanically increase the distance between the mitochondrial enzymes in the system, and distrub mitochondrial particles, thus slowing down the process of oxidative phosphorylation. As low ATP synthesis is the characteristic of this condition, animals suffering from higher thyroxine level would tend to oxidize more food to keep up the ATP requirement and consequently the energy released from this oxidation of food is lost as SDA. Further work is suggested on

this to bring out the mode in which thyroid mediates its action through nicotinamide nucleotide coenzymes in metabolism.

Nicotinamide Nucleotide Coenzymes, Pituitary Weight, Wither Height and Production Traits

Various reports regarding the role of pituitary hormones in metabolism and their relationship to nicotinamide nucleotide coenzymes with in vitro study have been presented. Burris and Bogart, (1953); Burris et al., (1954); Krueger, et al., (1954); Bogart, et al., (1963), indicated that calves growing at a rapid rate had higher TSH in their pituitary glands. However Pastan (1961, 1963) and Field (1961, 1963) showed that the concentration of NADP (mainly $NADP^+$) in the thyroid from TSH treated animals was increased. This increase was associated with a fall in the NAD concentration. The correlations obtained in this present study show that NADP has a significant negative correlation with gain per day and that $NADP^+$ has significant positive correlation with feed per unit gain, whereas NAD, although not significant, has a negative correlation with feed per unit gain and the latter coenzyme also has a significant positive correlation with percent fat and a significant negative correlation with percent lean, (see Table IX and related figures). The evidence obtained with the work of Pastan (1961, 1963) and Fields, (1960, 1961, 1963) demonstrates that TSH increases the concentration of

$NADP^+$ while it decreases the levels of NAD. Work in regard to the growth hormone shows that treatment with growth hormone causes an increase in nitrogen or protein and water content in the body and increases fat oxidation, thus depressing lipogenesis (see Lee and Shaffer, 1934; Greenbaum, 1953; Greenbaum and MacLean, 1953; Greenbaum and Glascock, 1957). Greenbaum, et al., (1965) showed that the nicotinamide nucleotide level in the liver of rats treated with growth hormone from the pituitary increased in concentration of NAD^+ and NADH 35% over the control group and there was a depression in lipogenesis. According to these authors this increase in NAD^+ and NADH was balanced by a decrease in the $NADP^+$ and NADPH. The latter are required for the operation of the pentose phosphate shunt to facilitate G-6-phosphate and phosphogluconic oxidation and supply NADPH for fat synthesis.

Regarding the relationship of the pituitary gland as such to the nicotinamide coenzymes, Greengard et al., (1961, 1964) indicates that NAD^+ concentration in the liver increased with hypophosectomy. This finding seems to be attractive and goes well with the postulation that lower levels of NAD are characteristic of rapidly growing animals. The pituitary gland, apparently through the action of its hormones, induces changes in the coenzymes as can be seen from the work by Greenbaum et al., (1965; Pastan, 1961, 1963; Field, 1960, 1961, 1963; and Greengard 1961, 1964). Although all three groups being in controversy among themselves in regards to the

action of the pituitary on these coenzymes, one point definitely seems clear to the author that growth hormones increase NAD at the expense of NADP while TSH acts on the contrary to growth hormone by increasing the concentration of NADP at the expense of NAD. In both cases the total coenzymes are not affected since these hormones might be merely interconverting the coenzymes or they may be making certain substrate as precursor available. This fact is important as each of these coenzymes will be described by the author with the results of the present study associated with different aspects of production traits.

The results obtained in present study indicates a significant positive correlation between NADPH and percent fat and a negative correlation of the NADPH with percent lean. Although other than pituitary weight as such which was used, the present study has no experimental support in regards to the action of growth hormone and thyroid stimulating hormone. The author, putting together the evidences in this study (see Table IX), and those of Mason et al., (1956); Bogart et al., (1963); Pastan (1961, 1963); Field (1960, 1961, 1963); Lee and Shaffer (1934); Greenbaum (1953); Greenbaum and McLean (1953); Greenbaum and Glascock (1957); Greenbaum (1965); Greengard et al., (1961, 1964); Dickens (1959, 1961), would like to present a theoretical mechanism through which both growth hormone and thyroid-stimulating hormone, although through different

mechanisms, could result in more lean, more moisture, less fat, and a higher gain per day.

Growth hormone. Growth hormone increases NAD^+ at the cost of NADP. This effect of growth hormone on the one hand will decrease lipogenesis through decreased NADP, while on the other hand it will decrease feed per unit gain through its increasing action on NAD^+. As a consequence the decreased NADP and $NADP^+$, reduced feed per unit gain, reduced fat deposit will all result in increase gain per day (see Figure 7).

Thyroid-stimulating Hormone. TSH increases NADP and $NADP^+$ but decreases NADPH due to low levels of enzymes associated with the pentose phosphate pathway. Consequence of this is reduced fat, reduced cortical steroid hormone of the adrenal cortex since NADPH serves to synthesize fatty acids and cortical steroids. Reduced fat would mechanically allow for lean build-up and reduced cortical steroids will help cut down on the amino acid catabolism allowing the deposition of amino acid as muscle tissue. The results of these actions would lead to higher gain per day, more lean, more moisture and less fat. The affect of TSH as described above is supported by the work of Burris and Bogart (1953) and Burris, et al., (1954) who, as a result of testosterone treatment, obtained higher activity of TSH in the pituitary of the treated calves to be associated with higher gain per day, more protein, higher moisture,

less fat in the carcass of the treated group compared to the control group. Further indirect evidence also comes from the work by Krueger, et al., (1954) who as a result of treatment with methyl testosterone obtained higher TSH content in the pituitary of the treated calves compared to the control group. Krueger, et al., (1954), however, showed lower gain per day and higher TDN per unit gain to be associated with the treated group receiving methyl tesosterone. Although the increase moisture content in the carcass of the treated animals in this study can be assumed to be associated with more protein and less fat the reduced gain may be attributed to some side ffect of methyl-testosterone.

An interpretation of the use of the pituitary gland as an indication of its hormonal activity in the present study is difficult. If weight can be taken as a measure of increased activity of a hormone, then, in an organ as complex as the pituitary with its ability to produce various hormones each of which has definite action in metabolism and development, the increase of weight of pituitary can be attributed to any one of these hormones. The antagonistic action of growth hormone and TSH and ACTH as far as the induction of coenzymes and metabolism is concerned would definitely make interpretation on the basis of gland weight impossible. The author in the present study used pituitary gland weight for the correlation study with various forms of nicotinamide nucleotide coenzymes, thyroid

gland weight, inbreeding coefficients, various production traits and and wither height. Wither height was included mainly as a measure of long bone growth due to the action of the growth hormone. There was no significant correlation between pituitary gland weight and the twenty traits studied (Table IX). A positive but not significant correlation was noted between pituitary gland weight and wither height, thyroid, $NADP^+$ and $NADP^+$/NADPH and $[(NAD^+ + NADP^+)]$. Of these the NADP, $NADP^+$ were positively but not significantly correlated with wither height. Negative but insignificant correlations also existed between pituitary gland weight and percent fat, NADPH, NAD^+/NADH. Of these the NAD^+/NADH ratio had a statistically non-significant negative correlation with wither height. This type of correlation with wither height also was noted between inbreeding and gain per day. Feed per unit gain also showed a non-significant positive correlation with wither height. In view of the complexity of the pituitary gland weight as a measure of its hormonal activity on the one hand and the non-significant correlations of the weight of this gland with various traits studied (Table IX) on the other hand, the author deems it appropriate not to forward any conclusion on this subject.

Statistical Interpretation. The mean squares for the analysis of variance for comparison of the inbred and line crosses presented in Table III showed that of the twenty-one traits studied only seven

(namely, total coenzymes, NADP, NADPH, [(NADH) + (NADPH)], gain per day, feed per unit gain and wither height had significant "F" values. "t" tests were applied to various genetic combinations and were presented in Table VI. As can be seen from this table, significant differences existed between lines and line crosses for nicotinamide nucleotide coenzymes, gain per day, feed per unit gain and wither height. Line 2 was higher than line 1 in mean NADP and NADPH. Line 3 also was higher than line 1 in total coenzymes, NADP, NADPH, [(NADH + NADPH)] levels. Line 1 also was lower than line 4 in levels of[NADP and NADPH.] Line 3 was higher than line 2 in total coenzymes, NADP, NADPH, and [(NADH) + (NADPH)]. Line 2 was superior to line 4 in gain per day and feed per unit gain. Line 3 was higher than line 4 in mean values of total coenzymes, NADP, NADPH, and [(NADH + NADPH)]. Line 4 was inferior to line 3 in feed per unit gain. In comparing the midpoint values of the parents vs. offspring, Table VI indicates a superiority in gain per day and feed per unit gain for the 2 x 1 cross over the average of both of its parents. The fact that there was no difference between 1 x 2 cross and the average of its parents indicates that the superiority of the 2 x 1 cross is attributable to the reciprocal effect. This is further shown since the mean value of $\frac{1x2 + 2x1}{2}$ was superior in comparison to the average of their parents. In lines 1 and 3 crosses "t" values show that the mean of the $\frac{1+3}{2}$ parents was higher than

than the 3 x 1 cross in the total coenzymes, NADP, NADPH, [(NADH + NADPH)]. A reciprocal effect for total coenzymes and wither height was shown by comparing 1 x 3 with the average of its parents and the mean values of 1 x 3 and $\frac{1x3 + 3x1}{2}$ when compared with $\frac{1+3}{2}$. A trend showing superiority in gain per day feed per unit gain was observed for 1 x 3 compared to the 3 x 1 cross. In the comparison between $\frac{2+3}{2}$ vs. 3 x 2 it was found that 3 x 2 was lower than the mean of $\frac{2+3}{2}$ in the levels of NADP, NADPH and wither height. In comparison between the inbreds (with 17 and 21 percent inbreeding) and line crosses, no significant difference was found for any of the traits tested (see Figures 15, 16 and Table VI). Although not statistically significant the lower coenzymes, higher rate of gain and lower feed per unit gain were characteristics of line crosses when compared with inbreds.

The mean square values for the analysis of variance based on diallel crossing system are presented in Table VIII. Reference to this table would reveal that there was no significant value for general combining ability for any of the twenty-one traits. Significant "F" values are shown for total coenzymes NAD, NADP, NAD^+, NADH, NADPH, [(NADH + NADPH)], [$(NAD^+ + NADP^+)$], $\frac{[(NAD^+)+(NADP^+)]}{[(NADH)+(NADPH)]}$ and wither height to exist for specific combining abilities.

The reciprocal effect is shown to exist also for NADP,

NADPH, [(NADH + NADPH)] and gain per day. No further attempt was made in this thesis to determine the specific combining ability and reciprocal effect for the lines involved. The mean square values in Table VIII may be interpreted wherever significant differences exist between lines as far as their ability for specific genetic combination and reciprocal effect is concerned. Specific combining ability is assumed to indicate non-additive gene action while the reciprocal effect is meant to designate the maternal effect.

SUMMARY AND CONCLUSIONS

1. Liver tissues from 55 inbred and diallel crossed beef bulls that were raised under standard conditions and slaughtered at 1000 lbs. body weight were analyzed for all four forms of oxidized and reduced nicotinamide nucleotide coenzymes. The coenzymes were used as the basis for calculations of all the ratios and combinations of the nicotinamide nucleotide coenzymes. The observations thus obtained as well as various production traits and certain endocrine gland weights which were also obtained under standard condition formed the basis of the present investigations.

2. Results obtained indicate that NADPH was present in excess over $NADP^+$, while NADH was present in lower concentration compared to NAD^+.

3. Age at slaughter was positively correlated with percent fat in the carcass and all the nicotinamide nucleotide coenzymes; while it was negatively correlated with thyroid gland weight.

4. Thyroid gland weight was negatively correlated with fat percent and positively correlated with $NADP^+$ and $NADP^+$/NADPH ratio. The latter were negatively correlated with fat percent and positively correlated with feed per unit gain. Thyroid weight was also negatively correlated with the coenzymes which were positively correlated with fat deposit. Fat of the carcass was negatively

correlated with those coenzymes which showed a negative correlation with percent lean in the carcass.

5. Percent fat and percent lean were negatively correlated. Those coenzymes which favored fat deposit all showed a negative correlation with percent lean in the carcass.

6. Total coenzymes, NAD, NADP, NAD^{+}, and NADPH showed a negative correlation with percent lean in the carcass.

7. NADP and $NADP^{+}$ are negatively correlated with gain per day. While NADP in the meantime showed a positive correlation with percent fat, $NADP^{+}$ showed a positive association with feed per unit gain. The feed per unit gain and gain per day were negatively correlated.

8. Wither height was negatively correlated with the NAD^{+}/ NADH ratio and was positively correlated with NADH.

9. Inbreeding was negatively correlated with percent lean in the carcass, while it was positively correlated with total coenzymes, NAD^{+} + $NADP^{+}$, NADPH, and NADH + NADPH. These coenzymes were also negatively correlated with percent lean in the carcass and positively correlated with percent fat. Inbred animals showed a lower percent of lean in the carcass compared with line crosses.

10. Lines showed statistically significant differences in production traits and coenzymes. Line crosses appeared to show better

gain per day, lower feed per unit gain and lower levels of coenzymes than the inbreds but when the inbreds were compared with line crosses, no statistically significant differences were found for production traits and coenzymes level. Significant differences were observed for gain per day, feed per unit gain, and coenzymes level for the average of parents compared with their offspring.

11. The diallel analysis showed no general combining ability for any of the twenty-one observations studied. Specific combining ability and reciprocal effects, however, were shown to exist for production traits and various coenzymes.

12. Pituitary gland weight did not show any statistically significant correlation with any of the traits studied. This was attributed to the involvement of the pituitary gland with synthesizing various hormones having antagonistic action as far as the induction of the coenzymes by pituitary hormones is concerned.

13. Based on the present work and available literature, models are presented for the action of thyroid hormones, TSH and growth hormone.

14. Interrelationships of the various forms and ratios of the nicotinamide nucleotide coenzymes, and endocrine glands, in their role in intermediary metabolism and how these influence production traits are discussed.

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