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To the Graduate Council:

I am submitting herewith a thesis written by John Thomas Leeman entitled "X-Rays, Body Measurements, and Uronic Acid Excretion of Calves Heterozygous and Homozygous Normal for Snorter Dwarfism." I recommend that it be accepted for nine quarter hours of credit in partial fulfillment of the requirements for the degree of Master of Science, with a major in Animal Husbandry.

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We have read this thesis and recommend its acceptance:

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X-RAYS, BODY MEASUREMENTS, AND URONIC ACID EXCRETION OF CALVES HETEROZYGOUS AND HOMOZYGOUS NORMAL FOR SNORTER DWARFISM

A Thesis

Presented to

the Graduate Council of

The University of Tennessee

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

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by

John Thomas Leeman

August 1962

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John Thomas Leeman

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CHAPTER I

INTRODUCTION

Several types of dwarfism have been recognized in beef cattle but none has, to date proposed an economic problem to the beef cattle industry with the exception of the "Snorter" type. The potential production of Snorter dwarf calves has made the problem of prime importance to the individual breeder.

Probably the greatest loss has occurred through the decrease in value of purebred animals which are not guaranteed to be free of the dwarf gene. Purebred and commercial producers are skeptical of animals which have a possibility of being a carrier of the Snorter dwarf gene because of the monetary loss due to the production of dwarf calves. These calves often die at birth or shortly after. Those which live have a shorter life expectancy than normal. They are characterized by labored breathing, slow growth rate, protruding, bulging eyes, and uncoordinated movements. Their bodies are extremely blocky and low set on short, heavy-boned legs which are often bowed. With increasing age the Snorter dwarf develops a "potbelly" and is exceptionally susceptible to bloat.

Thus far research indicates that Snorter dwarfism is inherited in a simple Mendelian manner, with two recessive autosomal genes being responsible for the phenotypic expression of the anomaly. This being true we may expect to find cattle of three genotypes relative to Snorter

dwarfism. They are homozygous normal or clean (carrying no gene for Snorter dwarfism), heterozygous or carrier (carrying one gene for this particular type of dwarfism and being phenotypically normal), and homozygous for the dwarf gene (having two Snorter dwarf genes and being a dwarf phenotypically).

At present the most feasible method of detecting carrier animals is by progeny testing bulls. This involves mating a bull of unknown genotype to known carrier or dwarf cows or the less common practice of mating the daughters of a bull to a dwarf bull. As would be expected this is both time consuming and expensive due to the number of progeny which a bull must produce in order to be proven clean (homozygous normal). For example a bull of unknown genotype must produce either five normal calves from dwarf cows or eleven normal calves from carrier cows in order to be proven at the 95 per cent level of probability. These numbers are increased to seven normal calves from dwarf cows and sixteen normal calves from carrier cows to be proven at the 99 per cent level of probability.

As a result of the limitations of this method, the frequency of occurrence of dwarf calves is slow to subside. A Virginia report (Marlowe, 1961) covering the years 1954 through 1959 revealed that 0.35 per cent of 5944 Angus calves and 1.41 per cent of 6291 Hereford calves born in a number of herds in Virginia were dwarfs. During this six year period the percentages of dwarfs increased slightly in Angus herds and decreased slightly in Hereford herds.

Most of the work done to date has involved studying various

blood characteristics, physiological responses, body measurements and radiographs of various regions of the body. Recently a similarity has been observed between the Snorter dwarf and human patients with Hurler's syndrome, a type of dwarfism characterized by excessive accumulation of acid mucopolysaccharides in certain tissues and in the urine. The purpose of this paper is to present the body measurement and X-ray data collected at the University of Tennessee from 1959 through 1962 and to present an analysis of the nature of the mucopolysaccharides excreted in the urine of animals homozygous normal and heterozygous for Snorter dwarfism.

CHAPTER II

REVIEW OF LITERATURE

Since the recognition by Johnson et al.(1950) of the specific type of dwarfism of beef cattle termed "Snorter", intensive investigations have been waged to determine a reliable method for the detection of the heterozygous (carrier) animal.

Bovard (1960) has made an extensive review of the literature on hereditary dwarfism of beef cattle. Therefore the methods of detecting carriers which have been tried other than those used in this study, will be mentioned only briefly.

Gregory and Brown (1952), working at California, devised the profilometer for use in measuring the longitudinal head contour. Thyrotropic hormone activity has been studied by Carroll et al. (1951), Marlowe and Chambers (1954), Fransen and Andrews (1954), and by Crenshaw et al. (1957). The response of dwarfs, carriers and non-carriers to stress conditions have been studied by Foley et al. (1956) and by Deyoe et al. (1959) by observing changes in blood sugar level and in white cell count after injection of insulin. Dollahon et al. (1959), Cornelius et al. (1956), and Temple and Hazel (1961) have studied several blood characteristics including erythrocyte volume, ribonucleic acid and desoxyribonucleic acid in the plasma protein, haemoglobin and haematocrit determinations, leucocyte counts, cell fragility, globulin and albumin fractions of serum protein, hematological

characteristics, and zinc and copper levels. Cooper (1960) studied calcium, phosphorus, alkaline phosphatase, and citric acid concentrations in the blood at various intervals after injection of parathyroid hormone. Differences in the cerebrospinal fluid pressure have been studied by Fransen and Andrews (1958) and by Dollahon et al. (1959). None of the above methods has proven satisfactory as a method of detecting carriers on an industry-wide basis.

Several workers have attempted to recognize the carriers of the Snorter dwarf gene by examination of radiographs of the lumbar spinal region of calves. Emmerson and Hazel (1956) reported that the most noticeable abnormality observed in X-rays of the lumbar region was the undulations of the ventral profile of the last 4 or 5 thoracic vertebrae and all of the lumbar vertebrae. X-rays of this region of one to ten day old calves showed evidence of longitudinal compression in dwarf and carrier calves but not in clean calves.

High (1955) used the X-ray method for classification of a group of 61 calves expected to be 50 per cent carrier and 50 per cent homozygous normal. The calves were the progeny of three proven carrier sires and dams which were assumed clean on the basis of offspring produced. The probability that the X-ray classifications fit the expected genetic ratio was between .4 and .5.

Hazel et al. (1956) stated that the 40 to 50 Snorter dwarfs examined all exhibited severe longitudinal compression and irregular protrusion of the body of the vertebra below the usual epiphiseal-diaephyseal union. Many of the heterozygotes exhibited abnormalities

of a less extreme nature. Twelve normal calves having one dwarf parent had abnormal vertebrae varying from slight to extreme degrees of abnormality. All dairy calves X-rayed had only normal vertebrae with an obvious smooth, half-moon curvature of the lower body when seen in ventral profile.

Bovard et al. (1956) measured the combined length of the six lumbar vertebrae from individual radiographs of 19 Shorter dwarf and 131 normal Hereford calves at birth. The mean difference between normal and dwarf calves in lumbar length was 1.31 centimeters. However, the average difference between normal and dwarf calves in birth weight was 13.5 pounds and the regression of lumbar length on birth weight was b = 0.046 centimeters per pound which was highly significant ($P < .01$). Thus the size of the calf when born affected the length of the lumbar.

Adams (1956), using the X-ray technique for studying the lumbar vertebrae, supports other conclusions that the lumbar vertebrae of Snorter dwarfs are distinct morphologically from those of phenotypically normal calves. He further stated that the point of differentiation between homozygous normal and heterozygous normal, is not clearly defined. This is in agreement with findings of Hazel et al. (1956). Progeny tests on animals X-rayed while young, disclosed that some vertebrae classifications did not agree with genotype. Homozygous normal animals were occasionally classified as abnormal with minor variations in vertebrae development which apparently were not at all associated with the Snorter dwarf gene. Also heterozygotes were

occasionally classified as normal.

In a study of the accuracy of the X-ray method for identifying the three genotypes for Snorter dwarfism, Rankin et al. (1959) found that approximately 78 per cent of the dwarf carriers, 74 per cent of the dwarf free animals and 96 per cent of the dwarf animals were correctly classified. The greatest number of errors in predictions were found to be in the range of normal to mildly abnormal vertebrae. It was also found that more males than females had abnormal vertebrae with the abnormalities being more extreme in the males.

A further evaluation of the X-ray method of detecting animals heterozygous for Snorter dwarfism was given by High et al. (1959). This study included over 1500 purebred and grade Hereford and purebred Angus calves. Approximately 20 herds were represented for a three year period and three of these herds for four years. Results indicated that the X-ray method is highly accurate in identifying Snorter dwarf calves, but there was considerable overlap within the range of normal morphology to mild vertebrae abnormality between presumed non-carrier and carrier animals. It was concluded that radiographs of a bull's progeny may be used as a general method of predicting his dwarfism status before progeny testing him. However, this method of detecting carriers for the Snorter dwarf gene was not recommended as a basis for identification of individual animals with respect to genotype for dwarfism.

Although experimental evidence is lacking, early observations indicated that normal animals heterozygous for Snorter dwarfism may

have been favored in selection of breeding stock, due to superiority of certain body characteristics. As a result of this the use of several body measurements at different ages has been investigated as a possible indicator of the dwarfism status of cattle. Using the X-ray technique as a means of classifying animals into genotypic groups with respect to Snorter dwarfism, High et al. (1956) found that C type calves (clean) were on the average heavier, higher at the withers, slightly lower in grade, and longer in the cannon in proportion to cannon width than the B type animals (carrier) at 150 or 220 days of age.

Arthaud et al. (1957) obtained body measurements within a week after birth from 95 calves with clean pedigrees (46 males, 49 females) and 29 calves heterozygous for the Snorter dwarf gene (13 males, 16 females). Adjustments were made for differences in birth weight. Mean differences in measurements (mean for pedigree clean minus mean for carrier) by sexes were: (differences for males listed first) cannon bone length, 3.35 and 4.09 mm.; cannon width, -2.01 and -2.18 mm.; length of first five lumbar vertebrae, 2.27 and -1.56 mm.; cannon length/cannon width, 0.53 and 0.64; cannon length/cannon circumference, 0.08 and 0.07; and cannon circumference, -4.36 and -3.31 mm. All differences were highly singificant ($P < .01$) except lumbar vertebrae length ($P < .05$).

High (1955) applied a discriminant function to five body measurements including weight, loin width, head length, cannon length and body length. The calves were classified as to carrier or non-carrier by X-ray methods. The two sexes were analyzed separately.

The above functions gave significant differences between the non-carrier and the carrier type classification in both sexes.

Arthaud et al. (1957) also applied discriminant functions to birth weight, cannon length, cannon width and cephalic index. They found considerable overlap between pedigree clean and carrier calves for both males and females as did High (1955).

Recent findings indicate that there are certain paralleling characteristics between Snorter dwarfism and Hurler's syndrome of humans. According to McKusick (1960), some of the clinical manifestations of Hurler's syndrome are disproportionate dwarfism with a characteristic grotesque skeletal deformity, limitations of joint movement, cardiac abnormality, and clouding of the cornea. The head is large with a bulging forehead. The lips are large and the mouth usually remains open with an enlarged tongue. Radiologic examination usually shows a wedge-shaped deformity of the body of the vertebrae with an anterior hook-like projection. Nasal congestion, noisy mouth breathing, and frequent upper respiratory infection occurs in essentially all patients. Hurler's syndrome may be transmitted by an autosomal recessive or a sex-linked recessive gene. Inheritance by the autosomal recessive gene is most common.

Koger et al. (1960) state that the Snorter dwarf in cattle has certain characteristics analogous to Hurler's syndrome. Hurler's syndrome is characterized by an excretion of excessive acid mucopolysaccharides in the urine and by an accumulation of these compounds in certain tissues. With this in mind Koger and his associates isolated

and identified the acid mucopolysaccharides excreted in the urine of Snorter dwarfs, known carriers, and assumed non-carriers. Preliminary results showed dwarf animals to excrete high levels of acid mucopolysaccharides while non-carriers excreted little or insignificant amounts. Known carriers appeared to excrete intermediate amounts. Histological and chemical analysis of the liver and other tissue demonstrated abnormally large accumulations of acid mucopolysaccharides in the tissue of dwarf animals. With reference to the same work, Lorincz (1960) indicated that the dwarf animals excreted a 20 to 30 fold increase in acid mucopolysaccharides over normals. This increase in acid mucopolysaccharide production, storage, and excretion by persons with Hurler's syndrome has been demonstrated by Bishton et al. (1956), Brante (1951), Meyer et al. (1958) as well as others.

According to McKusick (1960), the mucopolysaccharides chondroitin sulfate B and heparitin sulfate are probably produced in excess in Hurler's syndrome. It is suggested that connective tissue cells each produce only one specific type of product, e. g., cells producing hyaluronic acid cannot produce any of the chondroitin sulfates. Tentatively, Meyer and his co-workers (1958) propose that Hurler's syndrome is the result of a genetic error in the chemical differentiation of the fibrioblast--"a chemical metaplasia." The excessive production of polysaccharides results in the accumulation of these substances, not only in the connective tissue, but also in the cells of many organs, and the excretion of large amounts in the urine.

Going on the assumption that the excessive mucopolysaccharide

excreted by those who are carriers of the recessive gene for Hurler's syndrome is in the form of chondroitin sulfate B, Teller et al. (1961) attempted to identify carriers of Hurler's syndrome. The specific extinction coefficients were determined for pure chondroitin sulfates A, B, and C in the carbazole color reaction and the naphthoresorcinol color reaction for hexuronic acids. The ratio carbazole/naphthoresorcinol (C/N) was found to be 13.6 for chondroitin sulfate A, 0.6 for chondroitin sulfate B and 14 for chondroitin sulfate C.

Urine samples were obtained from assumed homozygous normal and heterozygous individuals as well as subjects with Hurler's syndrome and subjected to the carbazole and naphthorescorcinol test. The C/N ratio fluctuated between 10 and 13 in all age groups for homozygous normal children. These values were comparable with the C/N ratios determined in crystalline chondroitin sulfates A and C.

Eleven patients with varying manifestations of Hurler's syndrome had C/N ratios of 2.6 to 6.0. This finding indicated a 58 to 85 per cent admixture of chondroitin sulfate B with the acid mucopolysaccharides isolated from the urine of these children.

Four clinically healthy sibs of these children with Hurler's syndrome excreted normal amounts of acid mucopolysaccharides, but with C/N ratios of 6.5, 7.5, 9.3, and 10.6 which are abnormally low or in the lower range of normal. Similarly, the parents of these children had a normal total excretion of acid mucopolysaccharides, but with C/N ratios of 5.0 and 2.3 which are abnormally low, suggesting that a certain proportion of their urinary acid mucopolysaccharides consist of

chondroitin sulfate B.

Koger (1960) reports that the Snorter dwarf excretes in the urine an acid mucopolysaccharide similar or identical to chondroitin sulphuric acid-B, which is concurrent with the findings cited on Hurler's syndrome. If this acid mucopolysaccharide proves to be a link between the diseases in the two different species, then we may conclude that a method for detecting the heterozygous individual in one species may contribute to the same identification in the other species.

CHAPTER III

EXPERIMENTAL PROCEDURE

Source of Animals

The animals used throughout this experiment were the progeny of a herd of 30 pedigree clean cows, three dwarf bulls, and three pedigree clean bulls. Each year approximately half of the clean cows were bred to a Snorter dwarf bull and half to a pedigree clean bull. By maintaining this breeding plan, the resulting progeny were either known clean or known carrier animals with respect to the Snorter dwarf gene. The herd of 30 pedigree clean cows and the pedigree clean bulls were Polled Herefords. Snorter dwarf bull A, used for the 1959 progeny was an Angus x Hereford crossbred. Dwarf bull B, used to produce the 1960, 1961 and part of the 1962 progeny was a Polled Hereford. Dwarf bull C which sired part of the 1962 progeny was also a Polled Hereford.

X-ray Technique

X-ray pictures of the lumbar vertebrae were made of the 1959, 1960, 1961 and 1962 progeny of the above described matings. The X-rays were usually made within one week after birth. The exposures were made with a portable X-ray machine with a 110 milliamp and a 100 kilovolt capacity. The calves were placed on their left sides and strapped to a board designed for holding during X-raying. Two exposures were made of the lumbar vertebrae using a distance of thirty inches between the X-ray tube and the film. The time of each exposure and the

kilovoltage used varied with the calf's loin width. The time usually varied from .2 to .3 of a second and the kilovoltage varied from 65 to 75. The milliamps were held constant at 100. Calipers graduated in centimeters were used to measure loin width. A one-eighth inch lead-lined shield with a lead glass window was used to provide protection to the operator of the X-ray machine. Kodak Blue Brand Medical X-ray film and a stationary grid were used. The film and the cassettes were stored in a portable lead-lined plywood box. This enabled easy transportation and protection of the film during X-raying.

A dark room containing stainless steel tanks for all solutions was used for developing the X-ray films. Light was provided by using a fifteen-watt Kodak safe lamp. Fiber top work benches, stainless steel film hangers, a drying rack, and a timer completed the equipment needed.

The manufacturer's instructions were carefully followed in the preparation of developing and fixing solutions. Five quarts of Kodak Liquid X-ray Developer and Replenisher (Solution A) were thoroughly mixed with water (one part developer to three parts water) to fill the five gallon developer tank. Hardener (Solution B) was added to each freshly prepared solution. When replenisher was needed to maintain the desired quantity, one part of Solution A was added to two parts of water. The same procedure was followed with Kodak Liquid X-ray Fixer and Replenisher (Solution A and B) in the fixer tank. During developing both of these solutions were kept at 68° F. by the use of a thermostatic valve on the master rinse bath tank in which the smaller solution tanks

were set. The 5 gallon developer tank had a capacity of 5 films at a time. The films remained in the developer tank for five minutes. They were then rinsed and transferred to the fixer tank where they remained for 12 to 15 minutes. The films were then washed for approximately one hour in running water maintained at 68° F.

Classification of X-rays

The X-rays were classified by the method first used by Dr. L. N. Hazel at Iowa State. The classification was made by two of the staff members at the University of Tennessee.[1]

The X-rays were classified on the basis of the lateral view of the ventral profile of the body of the lumbar vertebrae. If this lateral view of the ventral profile of all six lumbar vertebrae gave a smooth, half moon curvature with no protrusions within the normal line of curvature, the X-ray was classified as C or clean. If this normal curvature was interrupted by any irregular protrusions or small teat-like projections the animal was classified as B or carrier. The B classification was divided into seven sub-classes, these being B_2 through B_8. Classification progressed from B_2 through B_8 relative to the number of the lumbar vertebrae affected and the degree of abnormality. The distinction between B_2 and C type vertebrae is not clear cut indicating the possibility of overlap between the two. The dwarf or A type vertebrae deviated in an extreme manner from the normal C

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[1] Dr. H. J. Smith and Dr. R. J. Cooper, Professor and Assistant Professor of Animal Husbandry, respectively.

type vertebrae. All six lumbar vertebrae had a severe double teat-like projection which completely interrupted the normal curvature of the ventral profile when seen from a lateral view.

Body Measurements

Body measurements were made of the 1959, 1960, 1961 and 1962 progeny of the previously described matings. The body measurements were taken at three different ages, birth to 1 week of age, 120 to 140 days of age and 190 to 210 days of age. The six body measurements taken on each calf were head width, head length, wither height, body length, cannon bone circumference, cannon bone length and body weight. The first four measurements listed above were made with calipers mounted on a one meter long stick which was graduated to one-eighth of an inch. For ease of computation all measurements were converted to the nearest one-tenth of an inch. The cannon bone circumference and cannon bone length were measured with a steel tape graduated to one-tenth of an inch.

Head length was measured from the depression immediately behind the poll to the hairline of the nose. Head width was measured at the widest part of the head, which is the distance between the bony prominences behind the eyes. The body length was measured as the horizontal distance from the anterior edge of the point of the shoulder to the posterior edge of the pin bone. Wither height was measured along a line perpendicular to the ground at a point just posterior to the elbow joint. The height was measured from the ground to the top of the shoulder. Cannon length was measured on the posterior surface of the

front leg from a depression formed by the joint cavity at the back of the knee (at the proximal end of the metacarpal bone) to the hair line on the dew claw. Cannon bone circumference was measured at the smallest point which was approximately 2 to 3 inches below the knee.

All body measurements were taken from the left side of the calf by Dr. R. J. Cooper of the Animal Husbandry Department at the University of Tennessee. The calves were standing in as normal position as possible for all body measurements.

Urine Collections

Single urine collections were made on eight carrier and seven clean females which ranged in age from 97 to 148 days. These were the 1962 progeny of a pedigree clean bull and two dwarf bulls and the previously described herd of thirty clean females. Urine samples were again collected from these same calves twenty-eight days later when they had an age range of 125 to 176 days in order to determine the repeatability of the carbazole and naphthoresorcinol tests and the C/N ratio.

Urine collections were taken from the heifers by corraling them and running them into a chute. The area around the vulva was cleaned with paper towels. The heifers were induced to urinate almost without exception by massaging the area approximately four inches below the vulva. Urine samples were collected in clean large mouth pint jars. All samples were placed in a cooler maintained at 3° C. as soon as possible. They remained at this temperature until the uronic acid tests were made.

Separation of Acid Mucopolysaccharides from Urine

Acid mucopolysaccharides were separated from the urine by a modification of the method of Di Ferrante and Rich (1956). Samples of urine (30 milliliter aliquotes) were dialyzed against water kept at 3° C. for 20 to 24 hours with 2 to 3 changes of the dialyzate. The urine was allowed to reach room temperature and transferred to 40 milliliter conical centrifuge tubes. Samples were brought to pH of 5 with 2N HCl with the use of indicator paper. One milliliter of cetyl trimethyl ammonium bromide reagent (2.5 per cent solution in water) was added to each tube and mixed. The tubes were kept overnight at 3° C. The samples were then centrifuged at room temperature for 15 minutes at 2600 revolutions per minute. The supernatant fluid was carefully decanted off and the tubes inverted and allowed to drain for one minute. The precipitate was washed twice with 30 milliliters of ethyl alcohol saturated with NaCl using a glass rod to disperse the precipitate before each centrifugation. One and five tenths milliliters distilled water and two drops of 0.1N NaOH were added and the precipitate was dispersed to aid solution. The solution was then transferred quantitatively, with several washings, to a 5 milliliter volumetric flask and brought to volume with water. On 1 milliliter aliquotes of this solution, the glucuronic acid content was determined by the carbazole method of Dische (1947).

Carbazole Test

Duplicate 1 milliliter aliquotes of the solution containing 5 to 100 γ of uronic acid were mixed, with cooling, with 6 milliliters

of concentrated sulfuric acid (c.p.). The solution was heated for 20 minutes in glass stoppered tubes in a boiling water bath. The samples were removed and cooled to room temperature. Two-tenths milliliter of a 0.1 per cent alcoholic solution of pure carbazole was added to each sample with shaking. The samples stood at room temperature for two hours. The color intensity reached a maximum in about two hours and remained constant for about another hour. After this the intensity decreased. The samples were then transferred to colorimeter tubes and read on an Evelyn photoelectric colorimeter set at the wave length of 540 mu.

Naphthoresorcinol Test

The procedure of Deichman (1943) was followed for the naphthoresorcinol color test. Five-tenths milliliter of urine was transferred to a 50 milliliter glass-stoppered graduated cylinder. The urine was then diluted to 5 milliliters with water. Two-tenths milliliter of 19 per cent hydrochloric acid was added and the solution held for 45 minutes in a water bath maintained at 75° C. Five milliliters of concentrated HCl and 1 milliliter of 10 per cent naphthorescorcinol were added and the cylinder was allowed to stand for 90 minutes in a water bath maintained at 50° C. The solution was cooled under the tap and extracted with 10 milliliters of ether by shaking the cylinder vigorously for 5 seconds. After separation into 2 layers, sufficient ether was added to bring the ether volume to exactly 15 milliliters. The ether was mixed gently by rotating the cylinder. The ether was then pipetted into a glass-stoppered colorimeter tube and read on an Evelyn

photoelectric colorimeter set at the wave length of 540 mu.

The naphthoresorcinol test and the carbazole test were done simultaneously in order to eliminate possible variation due to age of the urine.

CHAPTER IV

RESULTS AND DISCUSSION

X-ray Classifications

A summary of the classifications of X-rays of the carrier and clean calves is given separately for males and females in Table I. Within each genotype calves were grouped by sex and sire in order to recognize differences which may exist.

When calves with classifications of B_2 through B_4 are grouped together and calves with classification of B_5 - B_8 are grouped together the three dwarf bulls produce progeny in each group as follows: Sire A, 50 per cent and 50 per cent; Sire B, 20 per cent and 80 per cent; Sire C, 71 per cent and 29 per cent.

In the clean genotype Sire E had 14.3 per cent of his calves classified in the B_5 through B_8 group in contrast to the other two sires in this genotype, which had no calves classified above B_4. These results support the opinion of Hazel et al. (1956), that a genetic factor or factors other then the gene controlling Snorter dwarfism has an influence on the morphology of the lumbar vertebrae.

Differences may be observed in the distribution of males and females in the B classification by noting that 77.8 per cent of the males of carrier genotype are classified between B_5 and B_8. Only 57.2 per cent of the females of the same genotype are classified from B_5 through B_8. This is in agreement with the findings of Rankin et al. (1959), that males tend to show more severe abnormalities of the lumbar vertebrae than do the females.

TABLE I

SUMMARY OF X-RAY CLASSIFICATIONS BY GENOTYPE, SEX, AND SIRE

Genotype	Classification								
	C	B_2	B_3	B_4	B_5	B_6	B_7	B_8	A
Male calves									
Dwarf									
Sire B									1
Sire G								1	1
Carrier									
Sire A			1		2	1			
Sire B			1	1	3	6	7	2	
Sire C	1			2					
Clean									
Sire D	5	4							
Sire E	3	4	2		2	1	1		
Sire F	5								
Female calves									
Dwarf									
Sire G									1
Carrier									
Sire A		2	1		1				
Sire B		1		5	6	5	1	1	
Sire C		2	1		1	1			
Clean									
Sire D	6	3		2					
Sire E	6	6	2	1					
Sire F	6	1		2					

Table II lists the number and per cent of X-rays in each classification by genotype and sex. Chi Square values were calculated to determine whether the number of X-rays in each classification were independent of genotype. Differences between the clean and carrier genotypes in the distribution of classification scores were very highly significant for both males and females ($P < .001$). This means that the observed difference between genotypes in the distribution of X-rays in the various classification, would be due to chance less than one time out of one thousand. We may then attribute this heterogeneity of classification to a real difference resulting from genotype. To further characterize the distribution of X-rays, the B classification was divided into two groups, B_2 through B_4 and B_5 through B_8. Observation reveals that more cleans than carriers are classified from B_2 through B_4 and more carriers than cleans are classified from B_5 through B_8. Considering the males, 18.5 per cent of the carriers are in the B_2 through B_4 group as compared with 37.0 per cent of the cleans. The B_5 through B_8 group contains 77.8 per cent of the carrier males and only 14.8 per cent of the clean males. All of the clean males with B_5 through B_8 classification are the progeny of Sire E. The same trend may be observed in the distribution of females. The B_2 through B_4 classification has 42.9 per cent of the carrier and 48.6 per cent of the clean females, whereas the B_5 through B_8 classification contains 57.1 per cent of the carrier females and no clean females. When the two sexes are combined 30.9 per cent of the carriers and 43.5 per cent of the cleans are classified from B_2 through B_4, and 67.3 per cent of the

TABLE II

NUMBER AND PER CENT OF X-RAYS IN EACH CLASSIFICATION BY GENOTYPE AND SEX

Genotype	Classification								
	C	B_2	B_3	B_4	B_5	B_6	B_7	B_8	B_2-B_8
Carrier males									
Number	1	--	2	3	5	7	7	2	26
Per cent	3.7	--	7.4	11.1	18.5	25.9	25.9	7.4	96.3
Clean males									
Number	13	8	2	--	2	1	1	--	14
Per cent	48.1	29.6	7.4	--	7.4	3.7	3.7	--	51.9
Carrier females									
Number	--	5	2	5	8	6	1	1	28
Per cent	--	17.9	7.1	17.9	28.6	21.4	3.6	3.6	100
Clean females									
Number	18	10	2	5	--	--	--	--	17
Per cent	51.4	28.6	5.7	14.3	--	--	--	--	48.6
All carriers									
Number	1	5	4	8	13	13	8	3	54
Per cent	1.8	9.1	7.3	14.5	23.6	23.6	14.5	5.5	98.2
All cleans									
Number	31	18	4	5	2	1	1	--	31
Per cent	50	29.0	6.5	8.1	3.2	1.6	1.6	--	50

carriers and 6.5 per cent of the cleans are classified from B_5 through B_8. Thus it is evident that a greater per cent of the carriers show abnormalities of the lumbar vertebrae than do the cleans.

Table II shows that a larger error is associated with the identification of the clean animals than with the carrier animals. The total B classification, B_2 through B_8, contained 96.3 per cent of the carrier males. Only 3.7 per cent of the carrier males were given a C classification. In contrast to this 48.1 per cent of the clean males were given a C classification and 51.9 per cent were classified in the B group. It may be noted here that the B_2 classification contained 29.6 per cent of the X-rays of clean males. If the B_2 classification had been grouped with the C classification as predicting clean genotype, the accuracy of identification of the clean males would have been increased. The accuracy of identification of carrier males would not have been lessened because no carrier males were placed in the B_2 classification. All carrier females X-rayed were classified in the B group, making the recognization of known carrier females 100 per cent accurate in this study. The clean females were less accurately identified, in that the C classification contained only 50 per cent of those X-rayed and the B classification contained 50 per cent. Again, as was true with the males, if the B_2 class had been combined with the C class the accuracy of identification of clean females would have been increased. In this case the accuracy of identification of carrier females would have been slightly decreased, but the decrease in accuracy of recognizing carriers would not have been as great as the increase in

accuracy of recognition of the clean females.

The total per cent accuracy of the X-ray method for identifying carriers and cleans of both sexes was found to be 72.6 per cent. However, this percentage can be misleading due to the high accuracy found to prevail in the identification of carrier animals and the relatively low accuracy in identification of clean animals.

Body Measurements

In order to determine variations in conformation between clean and carrier animals, six body measurements were taken. These included head width, head length, wither height, body length, cannon bone circumference, cannon bone length, and body weight. A summary of these averages by year, sex, and genotype for the ages birth to 1 week, 120 to 140 days, and 190 to 210 days is given in Tables III, IV, and V, respectively.

Clean minus carrier differences for the measurements and levels of significance are given by year and sex for the three ages in Tables VI, VII, and VIII, respectively. No test of significance was made on differences of 0.1 or less, and in this discussion a significant difference will refer to means which differ at the 5 per cent level of probability or less. Weighted averages of the differences for each year are given at the bottom of each table. The measurements for 1962 included only those taken from birth to one week of age.

Carrier males had wider heads than clean males in all four years at birth to one week of age. Differences were small in 1959 and 1962 being only 0.1 inch. However, in 1960 and 1961 the carrier males were

TABLE III

AVERAGES OF BODY MEASUREMENTS[a] TAKEN BEFORE ONE WEEK OF AGE

Year	Genotype	Sex	No.	Age, days	HW, in.	HL, in.	WH, in.	BL, in.	CC, in.	CL, in.	Wt., lb.
1959	Clean	M	8	6.6	4.9	8.7	26.2	25.0	4.3	5.3	75.9
	Carrier	M	4	4.5	5.0	8.4	23.9	24.0	4.4	4.8	68.2
	Clean	F	9	4.1	4.8	8.7	25.4	24.2	4.2	5.3	72.0
	Carrier	F	4	1.0	4.8	8.4	23.0	21.6	3.8	4.9	59.5
1960	Clean	M	10	3.0	4.8	8.8	25.2	23.8	4.4	5.2	64.0
	Carrier	M	4	3.0	5.0	8.9	25.6	24.2	4.3	5.2	72.0
	Clean	F	9	4.0	4.7	8.5	24.3	23.1	4.0	5.1	59.0
	Carrier	F	4	3.0	4.9	9.0	25.3	24.4	4.2	5.3	66.0
1961	Clean	M	3	3.4	4.7	8.0	24.2	22.3	4.1	5.1	55.3
	Carrier	M	10	4.2	5.0	8.8	25.4	24.5	4.5	5.2	75.2
	Clean	F	4	4.8	4.6	8.4	24.4	23.4	4.0	5.4	60.5
	Carrier	F	7	3.3	4.9	8.8	25.5	24.3	4.3	5.4	74.0
1962	Clean	M	6	3.0	4.9	8.7	26.1	24.6	4.3	5.7	73.8
	Carrier	M	9	3.0	5.0	8.5	25.2	23.7	4.5	5.1	74.2
	Clean	F	8	3.0	4.9	8.5	24.2	24.2	4.1	5.7	70.8
	Carrier	F	11	4.0	4.9	8.4	24.9	24.1	4.3	5.1	70.2

[a] HW = Head width
HL = Head length
WH = Wither height
BL = Body length
CC = Cannon bone circumference
CL = Cannon bone length
Wt = Weight

TABLE IV

AVERAGES OF BODY MEASUREMENTS TAKEN AT 120 TO 140 DAYS OF AGE

Year	Genotype	Sex	No.	Age, days	HW, in.	HL, in.	WH, in.	BL, in.	CC, in.	CL, in.	Wt., lb.
1959	Clean	M	7	134	6.6	12.7	35.2	39.3	6.0	6.4	349
	Carrier	M	4	133	6.6	12.3	33.1	37.9	5.9	5.8	315
	Clean	F	9	128	6.4	12.2	33.9	38.0	5.5	6.5	289
	Carrier	F	4	129	6.4	12.1	32.2	35.6	5.4	5.9	259
1960	Clean	M	10	131	6.4	12.6	33.5	36.9	5.8	6.3	283
	Carrier	M	4	129	6.6	12.6	33.8	36.4	5.9	6.1	295
	Clean	F	9	132	6.4	12.3	32.8	36.9	5.4	6.1	276
	Carrier	F	4	127	6.5	12.1	33.8	36.8	5.8	6.1	308
1961	Clean	M	3	134	6.5	12.4	33.2	37.0	5.7	6.0	275
	Carrier	M	10	136	6.5	12.4	33.1	37.2	5.8	6.5	300
	Clean	F	4	127	6.3	11.8	32.4	36.2	5.5	6.3	260
	Carrier	F	7	134	6.5	12.4	34.0	37.8	5.5	6.7	306

TABLE V

AVERAGES OF BODY MEASUREMENTS TAKEN AT 190 TO 210 DAYS OF AGE

Year	Genotype	Sex	No.	Age, days	HW, in.	HL, in.	WH, in.	BL, in.	CC, in.	CL, in.	Wt., lb.
1959	Clean	M	7	202	7.3	13.8	38.3	44.3	6.4	6.7	463
	Carrier	M	4	202	7.2	13.5	37.2	42.2	6.4	6.2	436
	Clean	F	9	197	6.9	13.3	37.4	42.4	5.9	6.7	417
	Carrier	F	4	198	6.9	13.3	35.4	41.0	5.8	6.2	389
1960	Clean	M	10	192	7.0	13.8	37.0	42.3	6.2	6.6	424
	Carrier	M	4	200	7.1	14.0	37.4	41.4	6.4	6.4	452
	Clean	F	9	202	6.8	13.2	35.7	41.1	5.8	6.4	397
	Carrier	F	4	198	7.0	13.5	37.0	42.0	6.2	6.5	428
1961	Clean	M	3	201	7.0	13.8	36.9	41.3	6.2	6.5	400
	Carrier	M	10	206	7.3	13.9	37.3	41.6	6.4	6.4	442
	Clean	F	3	194	6.9	13.3	35.6	40.8	5.7	6.6	378
	Carrier	F	7	204	7.2	13.7	37.5	42.0	6.1	6.5	444

TABLE VI

CLEAN MINUS CARRIER DIFFERENCES FOR BODY MEASUREMENTS TAKEN BEFORE ONE WEEK OF AGE

Year Sex	HW, in.	HL in.	WH, in.	BL, in.	CC, in.	CL, in.	Wt., lb.
1959							
Males	-0.1	0.3	2.3[a]	0.6	-0.1	0.5[a]	7.7
Females	0.0	0.3[b]	2.4[c]	2.6[c]	0.4	0.4	12.5[c]
1960							
Males	-0.2[b]	-0.1	-0.4	0.4	0.1	0.0	-8.0[b]
Females	-0.2	-0.5[b]	-1.0[a]	-1.3[a]	-0.2	-0.2	-7.0
1961							
Males	-0.3[c]	-0.8[c]	-1.2[b]	-2.2[c]	-0.4[c]	-0.1	-19.9[c]
Females	-0.3[c]	-0.4[c]	-1.1[b]	-0.1	-0.3	0.0	-13.5[c]
1962							
Males	-0.1	0.2	0.9[a]	0.9	-0.2	0.6[c]	0.4
Females	0.0	0.1	-0.7[b]	0.1	-0.2[b]	0.6[c]	0.4
Four years combined							
Males	-0.17	-0.05	0.48	-0.12	-0.14[b]	0.29[c]	-3.97
Females	-0.11	-0.13	-0.21[c]	0.07	-0.14[b]	-0.23	-1.14

[a]Significant at $P \leq .10$.

[b]Significant at $P \leq .05$.

[c]Significant at $P \leq .01$.

TABLE VII

CLEAN MINUS CARRIER DIFFERENCES FOR BODY MEASUREMENTS TAKEN AT 120 TO 140 DAYS OF AGE

Year Sex	HW, in.	HL, in.	WH, in.	BL, in.	CC, in.	CL, in.	Wt., lb.
1959							
Males	0.0	0.4	2.1[b]	1.4	0.1	0.6[b]	34[b]
Females	0.0	0.1	1.7[c]	2.4[b]	0.1	0.6[c]	30
1960							
Males	-0.2[a]	0.0	-0.3	0.5	-0.1	0.2	-12
Females	-0.1	0.2	-1.0[b]	0.1	-0.4[c]	0.0	-22
1961							
Males	0.0	0.0	0.1	-0.2	-0.1	-0.5	-25[c]
Females	-0.2[b]	-0.6[b]	-1.6[b]	-1.6	0.0	-0.4	-46[b]
Three years combined							
Males	-0.07	0.13	0.61[b]	0.59[a]	-0.03	0.12	-00.70
Females	-0.10	-0.09	-0.26	0.35	-0.10	0.08	-11.72

[a]Significant at $P \leq .10$.

[b]Significant at $P \leq .05$.

[c]Significant at $P \leq .01$.

TABLE VIII

CLEAN MINUS CARRIER DIFFERENCES FOR BODY MEASUREMENTS, TAKEN AT 190 TO 210 DAYS OF AGE

Year sex	HW, in.	HL, in.	WH, in.	BL, in.	CC, in.	CL, in.	Wt., lb.
1959							
Males	0.1	0.3	1.1	2.1[a]	0.0	0.5	27.0
Females	0.0	0.0	2.0[c]	1.4	0.1	0.5[c]	28.0
1960							
Males	-0.1	-0.2	-0.4	0.9	-0.2	0.2	-28.0
Females	-0.2[a]	-0.3	-1.3[a]	-0.9	-0.4[a]	-0.1	-31.0[a]
1961							
Males	-0.3[b]	-0.1	-0.4	-0.3	-0.2	-0.1	-42.0
Females	-0.3[c]	-0.4	-1.9[b]	-1.2	-0.3[c]	0.0	-66.0[b]
Three years combined							
Males	-0.09	-0.03	0.10	0.94[a]	-0.13	0.29	-11.58
Females	-0.15[b]	-0.22	-0.27	-0.15	-0.19[b]	0.15[b]	-19.20[a]

[a]Significant at $P \leq .10$.

[b]Significant at $P \leq .05$.

[c]Significant at $P \leq .01$.

0.2 inch and 0.3 inch wider in the head respectively ($P < 0.5$). At 120 to 140 days of age carrier males had wider heads than clean males only in 1960. This difference was 0.2 inch ($P < .10$). By the age of 190 to 210 days in 1959 clean males were found to have slightly wider heads than the carrier males. In 1960 and 1961 carrier males maintained wider heads than clean males at 190 to 210 days of age. Carrier and clean females followed much the same pattern as the males in head width, with fewer differences being noticed in most instances. Significant differences were observed between the carrier and clean females in 1961 at all three ages, the carrier females having wider heads than the clean females.

Clean males and females had longer heads than the carrier males and females at all three ages in 1959, with the exception of females at 190 to 210 days of age for which no difference existed. Clean males and females ranged from 0.3 to 0.4 inches and 0.1 to 0.3 inches longer in the heads than the carrier animals, respectively. The 1960 head length measurements did not follow the 1959 pattern, in that carrier calves of both sexes tended to have longer heads. The exception to this was at 120 to 140 days of age where no differences were observed in the males and the clean females were slightly longer headed (0.2 inch) than the carrier females, though the difference was not significant.

The difference in wither height between carriers and clean animals was fairly consistent within years. In 1959 clean males and females were taller at the withers than carriers at all three ages.

All wither height differences were significant in 1959 with the exception of males at 190 to 210 days of age ($P < .10$). In 1960 and 1961, carriers of both sexes were, in general, taller at the withers. The only case in which clean calves were taller than carriers was in 1961 at 120 to 140 days of age when clean males were 0.1 inch taller than carrier males. In 1962 at birth to one week of age the clean males were 0.9 inch taller than carrier males and the carrier females were 0.7 inch taller than clean females.

Clean males and females ranged from 0.6 to 2.6 inches longer in the body than the carrier calves in 1959, however, the only differences which were significant were for females from birth to one week and 120 to 140 days of age. In 1960 clean males ranged from 0.4 to 0.9 inches longer than carrier males, with no consistent difference being noticed for the females. Carrier males and females were from 0.1 to 2.2 inches longer in the body than clean calves in 1961. In 1962 the clean males and females were 0.9 and 0.1 inches longer respectively at birth to one week of age. However, these differences were not significant.

Differences in cannon circumference were relatively small in 1959, with clean animals tending to have slightly larger cannons. In 1960, 1961, and 1962, where differences existed, carrier males and females had a larger cannon bone circumference than the clean calves. The clean minus carrier difference during this three year period ranged from 0.0 to 0.4 inches.

Males and females of clean genotype had longer cannon bones than carrier males and females in 1959. Differences were from 0.4

to 0.6 inches with the difference remaining fairly consistent between ages. No definite trend and no significant differences in cannon length were observed in 1960. In 1961 carrier animals had slightly longer cannon bones. This difference was not large and was not significant. Clean males and females had 0.6 inch longer cannons than carriers of the same sex in 1961 ($P < .01$).

Clean males ranged from 7.7 to 34.0 pounds heavier than carrier males in 1959 with the last difference being significant. Females followed the same pattern with cleans ranging from 12.5 to 30.0 pounds heavier than the carriers. The 12.5 pound difference was the only one of significance here. The following two years carrier males and females were heavier at all ages than the clean calves. Some of the differences were not significant. Differences before one week of age in 1962 were negligible.

When considering the overall means of the measurements taken before one week of age for the four years combined, carrier males were larger in all body measurements and body weight, with the exception of wither height and cannon length. Arthaud et al. (1957) also found carriers to have a larger cannon circumference at this age. At 120 to 140 days of age carrier males were only very slightly larger in head width, cannon circumference and weight, .07 inch, .03 inch, and .70 pounds respectively. None of these differences was significant. At 190 to 210 days of age carrier males had slightly wider and longer heads with a larger cannon circumference, .09 inch, .03 inch, and .13 inch respectively. Carrier males also averaged 11.58 pounds heavier

than clean males at 190 to 210 days of age. Clean males were consistently higher at the withers at this age. High et al. (1956) also found clean (C type) calves to be taller at the withers.

Carrier females were on the whole larger for all body measurements and body weight than the clean females. Exceptions were the differences in cannon length at 120 to 140 days of age and 190 to 210 days of age and body length at 120 to 140 days. Some of the differences were small and non-significant but they fit into a consistent pattern.

Throughout the body measurement data the number of significant differences between genotypes tended to decrease with increase in age. This is probably due to increased variations in environment within a genotype as age progresses. Also it may be noted that there is considerable variation between years within the same genotype. This variation is particularly evident between the 1959 calves and the 1960 and 1961 calves. As has already been stated three dwarf and three clean bulls were used. The 1960 and 1961 progeny had common sires which were a different set than those used for the 1959 progeny. These results indicate that the genetic make up of a dwarf bull or a clean bull, independent of the Snorter dwarf gene, has a considerable effect on the various body measurements taken in this study. Table IX lists the mean differences in body measurements, taken before one week of age, of the progeny of the two dwarf sires used in 1962. Here it may be seen that the male progeny of Sire B were significantly larger ($P < .10$) in head length, wither height, and cannon length. Also the female progeny of Sire B were significantly taller at the withers

TABLE IX

DIFFERENCES BETWEEN 1962 PROGENY OF TWO DWARF BULLS IN BODY MEASUREMENTS TAKEN BEFORE ONE WEEK OF AGE (PROGENY OF SIRE B--PROGENY OF SIRE C)

Sex	HW, in.	HL, in.	WH, in.	BL, in.	CC, in.	CL, in.	Wt., lb.
Males	0.2	0.5[a]	1.2[a]	1.7	0.1	0.3[a]	11.3
Females	0.1	0.2	0.7[a]	1.0	0.0	0.2	7.7

[a]Significant at $P \leq .10$.

($P < .10$). This lends further to the indication that the effects of the Snorter dwarf gene may be overshadowed by other genes controlling growth and conformation.

Urine Analysis

A summary of the results of the uronic acid determination on urine samples from carrier and clean heifer calves are listed in Table X. No clear cut trend was observed between individual animals of the clean and carrier genotype. However, when considering the average of the two collections carrier animals had slightly higher concentrations of uronic acid in the urine, as determined by the naphthoresorcinol test, than did the clean animals. Uronic acid as determined by the naphthoresorcinol test showed calves of the clean genotype to have an average of .090 milligrams per milliliter of urine as compared to .095 milligrams per milliliter of urine in carrier calves. The carbazole test showed calves of carrier genotype to have slightly lower concentrations of uronic acid present in the urine on the average than the clean calves. Carriers had .0034 milligrams per milliliter and cleans had .0040 milligrams per milliliter. The C/N ratio (milligrams of uronic acid by the carbazole test to milligrams of uronic acid by the naphthoresorcinol test) was higher on the first collection but lower on the second and on the average for calves of the clean genotype. These results on clean and carrier animals of the Snorter dwarf gene are not in agreement with those reported by Teller et al. (1961), who were working with Hurler's syndrome. They found that people who were homozygous normal, for the gene controlling Hurler's syndrome, had

TABLE X

MILLIGRAMS OF URONIC ACID PER MILLILITER OF URINE FROM CLEAN AND CARRIER CALVES AS DETERMINED BY THE CARBAZOLE AND NAPTHORESORCINOL TESTS

Calf no.	Genotype	Carbazole test			Naphthoresorcinol test			C/N ratio		
		1[a]	2[b]	Av.	1	2	Av.	1	2	Av.
26	Clean	.0172	.0019	.0096	.112	.164	.138	.153	.012	.082
155	Clean	.0018	.0048	.0033	.088	.171	.130	.020	.028	.024
27	Clean	.0041	.0017	.0029	.082	.091	.050	.050	.018	.034
256	Clean	---	.0070	---	---	.097	---	---	.072	---
85	Clean	.0017	.0015	.0016	.045	.089	.067	.037	.017	.027
143	Clean	.0042	.0012	.0027	.050	.089	.070	.083	.013	.048
202	Clean	.0032	.0051	.0042	.034	.137	.086	.093	.037	.065
Av.		.0054	.0033	.0040	.068	.120	.090	.727	.028	.047
214	Carrier	.0023	.0012	.0018	.048	.097	.072	.049	.012	.030
17	Carrier	.0033	.0072	.0052	.032	.089	.060	.104	.082	.093
15	Carrier	.0048	---	---	.064	---	---	.074	---	---
235	Carrier	.0048	.0048	.0048	.146	.320	.233	.033	.015	.024
182	Carrier	.0038	.0030	.0034	.048	.090	.069	.080	.033	.056
5	Carrier	.0012	.0044	.0028	.062	.133	.098	.019	.033	.026
168	Carrier	.0054	.0013	.0034	.034	.081	.058	.159	.016	.088
4	Carrier	.0013	.0032	.0022	.008	.138	.073	.167	.023	.095
Av.		.0034	.0036	.0034	.056	.135	.095	.086	.031	.059

[a] 1 = Collection made on June 27.

[b] 2 = Collection made on July 25.

distinctly higher C/N ratios than carriers of the gene.

The correlation between the determination on samples from the same calf taken at different times was calculated for each test and the C/N ratio. Repeatability was found to be very low for the C/N ratio ($r = .034$). Repeatability was somewhat higher for determinations by the carbazole test but were still considered to be low ($r = .24$). The naphthoresorcinol test was found to be more highly repeatable ($r = .76$) than either the C/N ratio or the carbazole test. In view of the results obtained it is suggested that aliquotes of urine taken from a cumulative collection may provide a more uniform and representative method of sampling than the single collections.

CHAPTER V

SUMMARY

Calves which were the 1959, 1960, 1961, and 1962 progeny of three clean and three Snorter dwarf bulls and pedigree clean cows were used in this study. The lumbar vertebrae of 117 calves were X-rayed and six body measurements and body weight were taken before one week of age of 110 of these calves and at two later ages of 75 of the calves. Excretion of uronic acid in the urine of 15 of the 1962 progeny was determined by the carbazole and the naphthoresorcinol color reactions.

The calves X-rayed were classified as clean, carrier, or dwarf by the method first used by Dr. L. N. Hazel of Iowa State College. In this study the X-ray technique was highly accurate in recognizing the animals of known carrier genotype. Ninety-six per cent of the carrier males and 100 per cent of the carrier females were correctly classified. However, only 48 per cent of the clean males and 51 per cent of the clean females were correctly classified. Males of the carrier genotype were found to have more severe abnormalities of the lumbar vertebrae than did females of the same genotype. Also, differences between sire progeny groups were observed in the degree of abnormality in the lumbar vertebrae.

Six body measurements (head width, head length, wither height, body length, cannon bone circumference and cannon bone length) and

body weight were obtained at three ages. No consistent significant differences were observed from year to year. However, differences were observed between the clean and carrier animals within the same year. Differences were also found to vary with the sires used.

The concentration of uronic acid present in urine samples from 7 clean and 8 carrier females was determined. Tests were made for uronic acid by the carbazole and naphthoresorcinol color reactions. Single collections were taken from the same calves at a 28 day interval. No consistent difference was observed between individual animals of the clean and carrier genotypes. However, on the average the carrier animals had a slightly higher concentration of uronic acid present, as determined by the naphthoresorcinol test, than did the clean animals. Carrier calves had .95 milligrams of uronic acid per milliliter of urine as compared to .90 milligrams per milliliter in clean calves. The repeatability of determinations on collections taken at different times was low for both the carbazole and the C/N ratio. The naphthoresorcinol test was more highly repeatable than either the C/N ratio or the carbazole test. It is suggested that cumulative samples may provide a more representative method of collecting the urine.

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