SOME HEMATOLOGICAL STUDIES OF DWARF AND NON-DWARF BEEF CATTLE

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INTRODUCTION

Although sporadic dwarfism had been reported for many years in nearly all breeds of cattle, it was not until the late 1940's and early 1950's that dwarfism became a problem in the beef industry. With the increasing occurrence of the "shortheaded," "brachycephalic," or "snorter" dwarf described by Johnson et al. (1950), dwarfism became recognized as an important economic problem in the Hereford and Angus breeds.

It is difficult to put a monetary value on the loss to the livestock industry resulting from the dwarfism problem. A survey conducted by the American National Cattleman's Association (1955), in which questionnaires were sent to selected breeders, reported a frequency of approximately 0.2% dwarf calves in the major beef producing states. This limited data would indicate that the beef industry as a whole suffered only a small monetary loss from the production of dwarf calves. However in individual herds, considerable loss occurred since many reported a frequency of dwarf calves approaching or exceeding 10%. It is probable that the greatest monetary loss occurred to the livestock industry through pedigree discrimination, due to the reduced price of breeding animals with known carriers in their ancestry.

The observation by breeders that "snorter" dwarfism existed only in certain lines of breeding suggested the abnormality might be inherited. Subsequent research and data from controlled breeding tests indicated this to be true. The mode of inheritance was generally recognized as a recessive trait, probably controlled by one pair of genes.

The problem which faced the livestock breeder was one of how to eliminate carriers of the dwarf gene from his herd. The easiest and most widely used method of selecting against carriers has been pedigree analysis. By this method, animals having known carriers of the dwarf gene in their ancestry are culled from further use in the breeding herd. This procedure seems to have been effective, as evidenced by the marked decrease in the number of dwarfs produced in the last few years. A serious objection to pedigree analysis is that it will tend to cull complete lines of breeding which will include many outstanding individuals that are free of the dwarf gene. The continued effectiveness of this method will depend on the accuracy of pedigree information, and the thoroughness of pedigree discrimination against lines of breeding having known carriers. Whether lines considered to be clean are actually free of the dwarf gene and the possibility of dwarf mutations occurring in clean lines must also be considered in predicting the continued effectiveness of this method.

A more efficient method of eliminating carriers is a progeny test program in which prospective herd sires are bred to known carrier females. This is the most effective method available to breeders, but is time consuming and expensive, and will become progressively less practical as the frequency of the dwarf gene decreases.

Research workers have tried to develop an accurate method to distinguish between homozygous cleans and heterozygotes or carriers of the dwarf gene. With an accurate method of detecting the dwarf carriers, breeders would not have to resort to costly progeny testing or to pedigree analysis with the possible loss of valuable animals free of the dwarf gene. Several methods have been proposed, but as

of yet none are accurate enough to be relied on as the sole means of detecting carrier animals.

The purposes of this study are to investigate the basic hematology of dwarfs and non-dwarf beef cattle, to carry out extensive studies on those aspects which are highly related to dwarfism, and to attempt to find an accurate diagnostic test for detection of animals which are carriers of the dwarf gene.

REVIEW OF LITERATURE

Dwarfism in Cattle

The earliest report of dwarfism in cattle concerned the Dexter "bull-dog" condition. Seligmann (1904) observed that as high as 25% of the calves of Dexter herds were monsters with round heads, depressed nostrils, and projecting lower mandibles combined with extreme shortness of the limbs. He also reported that many of the cows aborted during the sixth, seventh, or eighth months.

Wilson (1909) stated the Dexter breed originated from crossing two breeds of cattle, Irish Kerry and Red Devon. When Dexter X Dexter matings occurred, 25-50% of the calves were monsters.

Crew (1923) reported a detailed study on the "bull-dog" condition in Dexter cattle. His data agreed with the earlier work in that Dexter X Dexter matings produced about one-quarter monstrous "bull-dog" calves. Crew described these calves as follows: "The abnormalities which these still-born calves exhibited are so constant, and characteristic, that the fetus is known as a "bull-dog" calf. The cranium is bulging, the nose marked by depression, the lower jaw protruding, the upper lip is slit baring the teeth, while the swollen tongue, thrust far out, curls over the nose. Owing to the disproportionate development of the buttocks, the tail seems to have its origin far on the back; usually there is a gapping deficiency of the abdominal wall through which the intestines pass to form a large umbilical hernia.

The limbs are short and the digits usually separated." Crew indicated that malfunctioning of the pituitary may be responsible.

In order to account for the varying degrees in which the "bull-dog" appeared, Crew (1923) suggested that a dominant low-grade achondroplasia factor expresses itself in the typical Dexter type. This factor becomes lethal only in conjunction with two other complementary factors. However, the general basis of inheritance considered to be correct was suggested by Hutt (1934) and Punnett (1936). The "bull-dog" condition is controlled by a simple lethal factor which is partially dominant in the heterozygous condition, causing the short legs and brachycephaly of the Dexter type. The long legged animals of Kerry type, produced from Dexter X Dexter matings are free of the lethal gene.

A dwarfism similar to the "bull-dog" has been reported in other breeds: Nyanda cattle indigenous to Africa (Carmichael, 1933), in Guernsey cattle (Brandt, 1941), and in a British Friesian herd (Berger and Innes, 1948). Mead et al. (1946) described a dominant achondroplasia in a Jersey herd which resembled closely the Dexter type of Dexter-Kerry cattle in Ireland, although no "bull-dog" calves had been produced.

Monster calves of a type similar to the "bull-dog" were reported by Wriedt (1930) in herds of Telemark breeding in Norwegian cattle. The data indicated a lethal factor, but the Telemark condition differs in two important respects from the "bull-dog" malformation; the heterozygous animal does not differ visibly from homozygous animals free of the lethal, and generally the Telemark dwarf is born alive while the "bull-dog" dwarf is dead at birth. Punnett (1936) reported the

results of breeding experiments between Dexter and Telemark cattle. The mating of a heterozygous Telemark bull to eight Dexter cows over a period of years resulted in 24 calves, all of which were normal. The normal calves were about equally divided between calves with normal legs and those with Dexter type legs. The conclusion that the conditions in the two breeds were caused by two separate single autosomal genes was confirmed by segregation in the F_2 progeny which included one Telemark and three "bull-dog" dwarfs.

Gregory et al. (1942) reported another form of achondroplasia found in the Jersey herd at the California Experiment Station. It was inherited as a simple autosomal recessive, but was more variable in its expression than the dwarf of Telemark type. The anomaly had its greatest effect on the development of the bones of the skull and maxilla and little if any on the length of the appendicular skeletal structure. Characteristic defects noted were a short broad head, cleft palate and other deformities of the mouth and a slight but not marked reduction in length of leg.

Craft and Orr (1924) described a dwarf Hereford heifer which was a different type of dwarfism from any previously reported. The general appearance was a dwarf condition, with short and irregularly curved legs, abnormally large joints, short and thickened face, and a nervous disposition. An autopsy revealed the thyroid and pituitary glands were markedly smaller than normal, and the authors indicated the lack of secretion by these glands to be possible causes of the dwarfism.

Lush (1930) reported a "duck-legged" condition in Hereford cattle on several Texas ranches which he felt might be related to the Dexter type of Ireland. The cattle were normal in every respect except they

were four to six inches closer to the ground. The pituitary was smaller in size but no other abnormalities were found in any of the glands. The history of the trait suggested it was inherited as a simple autosomal dominant.

Mead et al. (1942) discovered a new type of proportionate dwarfism in the progeny of one sire of Jersey breeding. At birth the dwarfs were indistinguisable by appearance from normal calves, and, although they grew slowly, the dwarfs could not be definitely identified until 12 months of age. At maturity they were about 200 pounds lighter than were normal cattle. Their data indicated this dwarf condition was controlled by a simple autosomal recessive.

A "compact" type was reported in the Shorthorn breed by Stonaker and Tom (1944). This type can be identified at birth and is distinct throughout the life of the animal. The "compact" type appeared shorter in the head, neck, body, and legs than the "standard" type of Shorthorn. Because of the extreme conformation, the "compact" often placed high in the show ring and several breeders at that time favored this type.

A very similar condition known as "comprest" was described in the Hereford breed by Forbes (1946) and was likewise favored by some breeders. Stonaker (1954) reported the results of comprest x comprest and comprest x normal matings in Hereford cattle. Comprest x normal matings resulted in 50 comprest and 55 normal offspring, which closely approximated a 1:1 ratio. Each of the five comprest bulls mated to comprest cows sired dwarfs that were generally crippled or "crooked-legged." Stonaker hypothesized this type of dwarfing was due to a single partially dominant gene with CC dwarf, Cc comprest, cc normal.

Chambers et al. (1954b) reported the results of comprest matings. From 45 comprest x comprest matings, 37 calves were dropped, 11 of which were definitely dwarfs. Three of the dwarfs were of the "crooked-legged" type; five were straight legged and three dwarfs were very extreme. The three extreme dwarfs were not further classified. Two non-comprest Hereford bulls and one Angus bull, all known carriers of the "snorter" dwarf gene, were mated to 24 "comprest" cows and seven yearling heifers. From 27 calves which were dropped, six were typical dwarfs of the "snorter" type. The results indicated that either the genes responsible for the "comprest" dwarf and the "snorter" dwarf were allelic or, if not allelic, that "comprest" cattle in this test carried the "snorter" dwarf gene in high frequency.

An achrondroplasia similar to the "comprest" in having a partial dwarfing effect on the heterozygote was observed by Johansson (1953) in Sweden. One normal bull of the Swedish Red and White breed was mated to unrelated cows of mixed dairy breeding producing 28 normal and 25 malformed calves. The dwarf calves had a moderately bulging forehead, the upper jaw and the legs below the knees and hock were reduced in length, and flexed pasterns occurred, usually on the hind feet. The malformations in general were more extreme in males than in the females. It was assumed the defective animals were heterozygous, and the gene had arisen by mutation in the sire of the malformed calves. Arrillaga (1949) stated dwarfs occurred in the native tropical Puerto Rican cattle which were of a blocky, beefy appearance similar to the "comprest" type of the Hereford breed.

Baker et al. (1950) reported the occurrence in Shorthorn herds of Central Nebraska of a recessive achondroplasia which he called "stumpy."

The dwarfs were distinguished by their small size, curly coat, and enlarged knees, with the cannon bones twisted laterally making animals appear slightly bow-legged. The "stumpy" condition differed from most reported dwarfs in having a normal appearing head. Most of the animals were thin indicating some type of metabolic disturbance. Matings involving the syndrome indicated control by an autosomal recessive gene.

Baker et al. (1951) reported a slightly different dwarf syndrome found in the Angus breed. The dwarf is characterized by a long narrow head. The "longheaded" dwarf showed considerable phenotypic variation, indicating several genes were involved, or differences arising because of different degrees of expression of one pair of genes.

"Snorter" Dwarfism in Beef Cattle

The first report of the most prevalent type of dwarfism in beef cattle was made by Johnson et al. (1950). The so-called "snorter" dwarf was also reported by Lindley (1951) and Gregory et al. (1951). In describing the general anatomy of this gross syndrome, wide variations in phenotype have been found in "snorter" dwarfism. Some show obvious abnormalities at all ages, others appear almost normal at birth and develop characteristic defects later. Those animals that look normal at birth may develop width, depth, compactness, thickness of fleshing, and a mature appearance before the more obvious evidences of dwarfism appears. The following is a general visible description of "snorter" dwarfs. The dwarfs are in general reduced in body size and reduced in leg length (Pahnish et al., 1955c; Gregory et al., 1951). Heads that are wide in relation to length with bulging foreheads were observed by Gregory et al. (1951) and Gregory et al. (1953). The jaws are usually

undershot with the teeth being too far forward to mesh with the dental pad (Lindley, 1951; Gregory et al., 1953; Pahnish et al., 1955c). A protrusion of the tongue and eyes, accompanied by a glassy stare are characteristics of dwarf calves (Lindley, 1951 and Pahnish et al., 1955c). Dwarf calves at birth may show apparent muscular weakness with incoordinated locomotion combined with faulty equilibruim (Lindley, 1951 and Pahnish et al., 1955c). As the dwarfs mature a "pot bellied" condition develops with an increasing occurrence of bloat (Pahnish et al. 1955c). Pahnish et al. (1955c) have noted an increase of dyspnea of both respiratory and expiratory nature with age, and an impaired sexual activity in dwarfs which reach breeding age.

Johnson et al. (1950) suggested the frequency and the distribution of the defect indicated inheritance as a monofactoral autosomal recessive. Lush and Hazel (1952) reported a postprobant summary of 266 offspring from known carrier parents. Of this total there were 197 normal and 69 dwarf calves, which was very close to the expected 3:1 ratio if the trait is inherited as a simple recessive. A postprobant analysis by Gregory et al. (1953) also suggested a recessive gene with complete penetrance.

Pahnish et al. (1955b) conducted a series of breeding tests which confirmed that "snorter" dwarfism was caused by an autosomal recessive. From carrier x carrier matings the progeny agreed closely with the expected 3:1 ratio. Five dwarf x dwarf matings produced all dwarfs. No dwarfs were produced from matings between assumed pedigree clean and carrier animals.

Data of crosses between Angus and Hereford "snorter" dwarfs were reported by Gregory and Carroll (1956), and Dollahon et al. (1957). All

crosses produced crossbred dwarfs, indicating the same gene to be present in both breeds. Chambers et al. (1954b) also produced a crossbred "snorter" dwarf from matings between a known carrier Angus bull and "comprest" Hereford cows. Dollahon et al. (1957) reported a "guinea" condition in Florida crossbred cattle which appears to have descended from the Dexter breed. Both "guinea" x "guinea" and "snorter" dwarfs x "guinea" matings produced "guinea" offspring.

Workers at the California station have reported numerous crosses between dwarfs of many types. Gregory (1955), Gregory (1956), and Gregory et al. (1957) have described results of crosses between "short-headed," "longheaded," "stumpy" dwarfs, and "comprest" Shorthorns characterized by heavy body with short legs. They interpret their results to indicate bovine dwarfism is a complex of several components rather than a single entity, that each component is homozygous for the same autosomal recessive dwarf conditioning gene. Specific modifying genes or combination of modifiers, are responsible for differentiating dwarf types. They suggested that progeny testing for the major dwarf gene may prove invalid if progeny are classified only into brachycephalic dwarfs and normals. They feel that their tests support the hypothesis that other genes present in cattle of normal or near-normal size modify the expression of the major dwarf conditioning gene and confuse progeny tests.

Since "snorter" dwarfism is inherited, and dwarfs in general fail to reach breeding age or to reproduce, automatic selection should occur against the dwarf gene. This would tend to maintain the frequency of the dwarf gene at a low level. However, the frequency of the dwarf gene increased rapidly during the early 1950's and became an economic

problem. Two general reasons have been suggested as the causes (Warwick, 1958). First, mutations to the dwarf gene might have occurred accidently in a few popular sires. Extensive selection for these sires and their offspring could have increased markedly the frequency of the dwarf gene. The second possibility is that the gene is not completely recessive, but partially dominant and thereby having an effect on the heterozygote or carrier animal. If this effect on the carrier increased the desirability of the phenotype, the breeders would select the heterozygote and thereby increase the frequency of the dwarf gene. Many authorities feel that the heterozygotes have a more desirable phenotype. This feeling is based on the experiences of breeders, since only limited data are available to support this belief.

Johnson et al. (1950) observed that known carriers tended to be somewhat of an intermediate type. Chambers et al. (1954a) and Lush and Hazel (1952) have also suggested that the carrier animal may be intermediate between the normal and the dwarfs and more nearly approach the ideal beef type. Recent work by Arthaud et al. (1957) with measurements of calves at one week of age has supported the hypothesis that carriers are intermediate. Data from 95 pedigree clean calves and 29 known carriers adjusted for birth weight were used. The authors found the pedigree clean calves to be significantly longer in cannon bone, while the cannon circumference and cannon width were significantly smaller for pedigree clean animals than known carriers. Ratios of cannon length to cannon width, cannon length to cannon circumference, and head length to head width were significantly greater for the clean animals than known carriers of the dwarf gene. When discriminate functions, determined from the data, were applied to the same population

the overlap between pedigree clean and carriers was 37% for males and 38% for females. The large overlap which exists between pedigree clean and carrier animals for measurements of length, width, and circumference of cannon bone prevent the use of these measurements as a diagnostic test.

Since many kinds of dwarfs in other species are known to result from an endocrine malfunction, numerous studies have been made to determine whether there was a deficiency of one or more hormones in the "snorter" dwarf. Most of these studies have been concerned with assays of the pituitary for the thyrotropic, growth, gonadotropic, and adrenocorticotropic hormones. Assay tests indicate the presence of the hormone and not whether the hormone is being released to have its normal effect on the target organ. However, it is generally accepted that a high correlation exists between pituitary levels and amounts of the hormone in the blood.

Hypothyroidism results in cretinism, which is a form of dwarfism in humans. The similarities between cretinism in humans and those expressed in "snorter" dwarfs have led some workers to believe that dwarfism in cattle is a result of a hypothyroid condition. Carroll et al. (1951) used day-old white Leghorn cockrels to assay the pituitaries of dwarf and normal Hereford calves for the thyrotropic hormone. The results showed pituitary tissue from both dwarfs and normal beef cattle increased the thyroid gland weight of the cockrels. However, the pituitary of normal beef cattle caused a larger increase in thyroid weight, indicating the dwarf pituitary was deficient in thyrotropic hormone. To the contrary of this report, no other workers have found a thyroid deficiency in dwarf cattle. Marlowe and Chambers (1954)

injected pituitary tissue suspensions from 25 dwarfs and 23 normal calves into 410 baby chicks. They found no significant differences in the thyrotropic hormone potency of the pituitaries of dwarf and normal calves. The anterior pituitary glands of seven control and 23 dwarf were assayed for thyrotropic hormone content by Fransen (1955). Analysis of data indicated that chicks injected with control and dwarf pituitaries did not have significantly different thyroid weights and both groups were significantly greater than uninjected controls. Experiments were conducted on dwarfs by Crenshaw and Turner (1954) using I^{131} as a tracer to study thyroid function. Counts of I^{131} over the thyroid gland indicated that thyroids of dwarfs had the ability to collect iodine. When thyroprotein was fed or thyroxine injected preceeding the injection of I^{131}, the collection of iodine by thyroid glands was reduced. Graded doses of thyrotropin injected in dwarfs increased the uptake of I^{131} in relation to the dosage of thyrotropin. They concluded that pituitary of the dwarf secretes thyrotropin, that the secretion can be inhibited by exogenous thyroxine, and that the thyroid of the dwarf reacts normally to thyrotropin. Fransen (1955) reported that radioactive iodine uptake by the thyroid follows a similar pattern in dwarfs and control animals. He further reported that serum protein-bound-iodine and blood cholesterol, both indicators of thyroid activity, were within the normal range for dwarfs.

The reduced size of dwarfs have led workers to suspect a deficiency of growth hormone as a possible cause of dwarfism. Marlowe and Chambers (1954) measured the level of growth hormone in the anterior pituitary of 21 dwarfs and 16 normal cattle by increases in body weight, tail length, and width of epiphyseal cartilage of the tibi of hypophysectomized

female rats. Their findings indicated the growth hormone content of dwarfs pituitaries to be significantly greater than that of non-dwarfs.

Other hormones of the pituitary gland which have been assayed are the gonadotropic hormones. Pituitary suspensions from six dwarfs and six normal calves were injected into 42 hypophysectomized immature female rats by Marlowe and Chambers (1954). They reported that rats injected with the pituitaries of dwarfs had significantly greater uteri weight than those injected with the pituitaries of normal beef cattle. Fransen (1955) reported results of a chick assay for gonadotropic hormone of the anterior pituitary of 23 dwarfs and seven control cattle. No significant difference was found between dwarf and normal cattle as measured by treated chick testes weights. Carroll et al. (1951) found that dwarf calf pituitaries caused as much gonad stimulation in chicks as pituitaries from normal cattle.

Although data are limited on the adrenal function of dwarfs, Fransen and Andrews (1954) reported a fairly high incidence of cystic adrenals in dwarfs. Assays of the ACTH potency of 25 dwarf and 21 normal calf pituitaries using both baby chicks and hypophysectomized immature female rats failed to reveal any significant difference between them in adrenal weights (Marlowe and Chambers, 1954).

As "snorter" dwarfs increased in number and became a major economic problem, a number of Experiment Stations initiated research to devise an accurate method which would identify carriers of the dwarf gene. The first method to receive wide publicity was proposed by Gregory and Brown (1952). Since Gregory et al. (1951) had reported that "snorter" dwarfs have a brachycephalic head with a marked midforehead prominence at birth which persists throughout life, Gregory and Brown

(1952) postulated a similar effect in the heterozygote. To measure this they devised an instrument called a profilometer which would trace the contour of the bovine face. The mid line contour of the face combined with measurements of the head were used in diagnosing genotype. A second method proposed by Gregory et al. (1952) was a statistical treatment which yields a discriminant function that can be used to differentiate heterozygotes from homozygous cleans.

Reports by Gregory et al. (1953) and Klussendorf (1953) indicated the profilometer was a very effective method of detecting carrier animals. However, data published by Stonaker (1954) and Schoonover (1954) indicated the profilometer data evaluated by the use of either a bull key or discriminant function failed to distinguish between clean and carriers of dwarf gene. Schoonover (1958) reported an extensive study of the profilometer in which he tried to associate head form of Hereford heifers with the dwarf factor. He found that certain inbred lines exhibited extreme mid-prominence while others did not. Two lines exhibiting extreme mid-prominence also carried the dwarf gene. Although mid-prominence of the head seems to be related to dwarfism, the problem with the use of the profilometer is that head shape is highly heritable and all animals within a line tend to exhibit similar head form whether they carry the dwarf gene or not. Also, some lines show mid-prominence which have been shown to be clean of the dwarf gene. This method has not shown enough accuracy to be used as a diagnostic test for carriers of "snorter" dwarfism.

Hazel et al. (1956), Emmerson and Hazel (1956), and Bovard et al. (1956) proposed a method based on vertebral abnormalities evident in radiographs of the thoracolumbar spinal region of young calves. Lumbar

vertebrae of "snorter" dwarfs exhibited severe longitudinal compression and irregular protrusion of the body below the usual epiphyseal-diaphyseal union. Radiographs from heterozygous calves exhibited abnormal vertebrae varying from slight to extreme degrees of abnormality. Buchanan et al. (1956) reported similar compression and protrusions of the lumbar vertebrae. Julian et al. (1956) reported a study on 78 "shortheaded" Hereford dwarfs. They indicated that all alterations of the vertebrae were due to a basic growth deficiency of the primary centers of ossification of the vertebrae. Recent reports and summaries by High et al. (1958) and Turman et al. (1957) indicated the x-ray method was effective as a method of detecting dwarfs at birth. However, errors of 10% and 20%, respectively, have been estimated in differentiating between carriers and cleans on the basis of vertebral abnormalities seen in x-rays. The method has not been recommended for routine use by the beef industry.

Another method of detecting carriers was proposed by workers at the Missouri station. Foley et al. (1956) reported on 59 animals in which insulin was injected intravenously and white cell counts were made at time of injection and at one and two hour intervals after injection. Highly significant differences were noted between dwarfs which showed little increase, pedigree clean animals giving a rapid increase, and known carriers which were intermediate in their increase in numbers of white cells one hour post injection. Massey et al. (1958) stated that the "insulin test" had been run on 1,800 animals of various breeds, ages, and sex in different parts of the country and under various climatic conditions. They feel that there is a definite differential response of pedigree clean and carrier animals; however, errors

have been made in predicting genotypes and 10-15% of the animals tested did not give a clear-cut response.

These results were very encouraging and several stations attempted unsuccessfully to confirm these results. Subsequent studies revealed the cells being counted were not all true white blood cells. Although response of the "insulin test" shows some relationship to dwarfism, the characteristic cell types were difficult to distinguish and in general are affected by a large number of variables which make this test an inaccurate method of detecting carriers of the dwarf gene.

Several studies have been concerned with carbohydrate metabolism. Eveleth et al. (1956) indicated dwarfs could not use glucose in the same manner as normal cattle. The blood glucose level content after glucose injection indicated that "snorter" dwarfs were diabetic. Heidenreich et al. (1955) reported that the glucose tolerance curves on nine dwarfs showed no consistent pattern. No apparent differences in the metabolic response were noted between carriers of the dwarf gene and clean cows. Foley et al. (1956) injected insulin intravenously and determined blood sugar levels at regular intervals. The tests showed the blood sugar level dropped much more quickly and to lower levels in dwarfs and failed to return to normal levels as quickly as normals.

Eveleth et al. (1956) described the dwarf heart as almost spheriod in shape while the normal heart is the classical shape. Asplund et al. (1956) reported observations on glutamic acid and creatinine in urine collections. The ratio of creatinine to glutamic ranged from 2.8 to 14.7 for normals and 1.2 to 2.0 for dwarfs. The difference between normals and dwarfs is caused by a greater excretion of glutamic acid by dwarfs. Julian et al. (1956) found the sphenoccipital synchondrosis

of dwarfs closed within the first week of life in contrast to normal closure age of 24-36 months. Fransen and Andrews (1954) reported a higher cerebrospinal fluid pressure for dwarfs than normal beef cattle.

Hematological Values for Cattle

One of the most striking features of hematological values in cattle and other animals is the extreme variation that exists in blood cells under normal conditions. Although much of this variation can be accounted for by the inaccurate procedures utilized in blood work, many other known sources of variation exist. Hematological studies are meaningful only when the importance of these factors is recognized, and an attempt is made to control them as much as possible. Two excellent reviews by Scarborough (1930) and Albritton (1952) are examples of the extreme variation of blood values in cattle.

Red Blood Cells

The normal erythrocyte is shaped like a biconcave disc with a diameter of 5.6 microns (Scarborough, 1930; Dukes, 1955) to 5.9 microns (Albritton, 1952). The actual number of erythrocytes in the blood stream is quite variable. From data based on 200 animals of varying ages and of both sexes, Scarborough (1930), reported a value of 6.62 million red cells per cu. mm. with the normal range from about five to eight million. Records on 2,501 counts from 25 cows and heifers produced a value of 6.33 million with a standard deviation of .889 million (Ferguson et al. 1945). The authors reported a range of 4.1 to 10 million red cells with most of the variation due to differences between animals rather than in weekly counts within animals. Dukes (1955) gives a mean value of 6.3 million per cu. mm. Albritton (1952) reported

a somewhat higher value of 8.05 million red cells per cu. mm. of blood. Among the factors that have been noted as affecting erythrocyte number in healthy cattle are; level of nutrition, breed, age, and sex.

In studying breed differences Long et al. (1952) at the Oklahoma Station failed to find any consistent differences in the erythrocyte counts between Shorthorn, Hereford, and Angus cows in the University herd. All animals fell within a range of four to six million red cells per cu. mm. of blood with an average of 5.35 million cells and a standard deviation of .55 million. Rusoff et al. (1954) reported values of 6.55, 7.49, and 7.84 million red cells per cu. mm., respectively, for mature bulls of the Jersey, Guernsey, and Holstein breeds. These authors found significant differences between animals but no significant differences between breeds. Holman (1955) reviewed studies which showed no consistent differences in African cattle as compared to Ayrshire. He also reported an average of 8.2 million red cells for 32 mature cows of the Indian breeds of cattle. Although no comparison was made to temperate breeds, this value indicates a higher number of erythrocytes for Indian breeds than temperate breeds.

A study of differences between herds of Jersey and Holstein cows at three different stations in Louisiana was reported by Rusoff and Piercy (1946). Significantly different mean values of 5.27, 4.89, 5.72 million red cells per cu. mm. were found, indicating that one herd can vary significantly from other herds within the same area. This indicates that even though herds are maintained within close proximity, there is variation in management, nutrition, disease conditions, and other factors which affect the erythrocyte picture. When attempting to evaluate data on erythrocyte number, there is a

need for detailed information regarding the environmental conditions that are present when the data are collected.

Greig and Boyne (1956) reported work with eight monozygous twin heifer calves, varying in age from seven to nine months on two levels of nutrition. Both members of two sets of twins were put on high and both members of two sets were placed on a low level of nutrition, while the other four sets of twins were divided placing one member of each pair on a high and the other twin on a low level of nutrition. Although values of 7.80 and 8.22 million red cells per cu. mm., respectively, were determined for calves on low and high levels of nutrition, the differences were not significant.

A review by Holman (1955) compared the red cell count of cattle in Africa under similar conditions and ages but at varying levels of elevation. Cows at 4,400 feet had one to two million more red cells per cu. mm. than did cows at 3,500 feet above sea level. He further reported that as 27 cows were moved from 5,400 feet to 9,000 feet the red cell count increased from 8.75 to 9.26 million per cu. mm.

The age of the animal has been found to have an important influence on erythrocyte numbers of cattle. Albritton (1952) in a review of blood values gave a value of 6.8 million red cells for calves at birth. Greatorex (1954) made a comprehensive study of the hematology of 233 calves of mixed breeds in which he reported 7.4 million red cells per cu. mm. at birth, which increased to 8.1 million at 8-12 weeks, and then decreased to near mature values of 6.0 million at 12 months of age. Holman (1956) reported 8.0 million red cells at birth in 12 calves, but no consistent change occurred in red cell number until 12-24 months of age when there was a decrease to six million red blood cells per cu. mm.

There are only a few reports of the influence of sex on red cell values since little data are available on males, and bulls are usually maintained under different environmental conditions which confounds sex with environmental differences. Holman (1955) in his review cited one study which showed no significant difference between males and females between 14 months and three years of age, but two other studies showed higher figures for males than females.

Hematological studies with dwarfs by Cornelius et al. (1956) and Fransen (1955) have found the red cell number to fall within the wide normal range. Fransen (1955) reported 9.7 million red cells for controls and 8.1 million red cells for dwarf. Cornelius et al. (1956) reported a mean value of 9.8 million red blood cells per cu. mm. for dwarfs ranging in age from six days to 14 months.

Hematocrit

The hematocrit, or packed cell volume, is a measure of the percent of blood which is composed of cells, and is, therefore, highly related to the size and number of red cells. Albritton (1952) in a review reported the range to be 37% to 47%. Dukes (1955) gives an average value for cattle of 40%. Rusoff et al. (1954) at the Louisiana Station found values of 46.3%, 42.3%, 39.5%, respectively for Guernsey, Jersey, and Holstein bulls. These values were in the upper range reported by Albritton (1952) and showed significant difference between breeds and seasons with higher values during the summer. Holman (1952) reported a lower hematocrit value of 33.8% in mature Ayrshire cattle. Holman (1955) reported a hematocrit of 33.7% for mature dairy cows. All of these values are within the normal range reported by Albritton (1952). Holman (1956) in a study with calves reported higher values

of 42% at birth which decreased markedly in the first few days after birth to 35% and thereafter remained constant through 24 months of age. Greatorex (1954) reported the corpuscular volume of dairy calves varied between 30% to 50%. A mean value of near 50% was reported at birth with decreasing values from 28 weeks to one year of age.

Fransen (1955), Hafez et al. (1958), and Cornelius et al. (1956) reported the packed cell volume of dwarf cattle to be within the normal range. Fransen (1955) reported 41.3% for controls versus 36.7% for dwarfs, while Cornelius et al. (1956) gave a value of 38.1% for the dwarfs. Hafez et al. (1958) gave no values but reported a lower mean value for hematocrit of dwarfs.

Hemoglobin

The hemoglobin range for normal cattle of all ages varies from 8-14 grams per 100 cc. of blood with a mean of 11.5 grams (Albritton, 1952). At the Oklahoma Station a value of 10.7 grams with a standard deviation of 1.08 grams hemoglobin was found in Shorthorn, Hereford, and Angus cows (Long et al. 1952), with no significant differences between the breeds. Byers et al. (1952) reported a mean value of 11.2 grams of hemoglobin was found in 1,014 mature animals of the Holstein and Jersey breeds. A significant difference was found between Holstein and Jersey breeds, while no significant differences were found between levels of nutrition or sex. Animals with high or low hemoglobin values generally maintained these levels throughout the experiment. Work by Rusoff et al. (1954) reported values of 12.2, 11.6, 11.6 grams of hemoglobin per 100 cc. blood, respectively, for Guernsey, Jersey, and Holstein bulls; these values were not significantly different. Findlay (1950) reported a higher hemoglobin index for tropical breeds than

temperate breeds which he associated with a higher heat tolerance. However, among Indian breeds, which have a high heat tolerance, a study reviewed by Holman (1955) reported a hemoglobin figure of 10.9 grams per 100 cc. of blood.

Numerous studies have indicated differences due to environmental factors and level of nutrition. Rusoff et al. (1954) reported hemoglobin to be higher during the summer. Mullick and Kehar (1952) in India found maximum level of hemoglobin of 12.0 grams during February and lowest in August with a value of 8.4 grams. The variation due to months and animals was highly significant. In general as temperature and moisture decreased there was a decrease in hemoglobin readings. Greig and Boyne (1956) in their study with monozygous twins reported 6.6 Haldane Percent less for those on low level of nutrition than for twins on high level. One hundred percent on the Haldane scale is equal to 14.8 grams of hemoglobin.

Another factor studied has been the effect of age. Greatorex (1954) reported values for hemoglobin ranged from 4.60 to 16.05 grams per 100 ml. of blood for calves at birth, with the majority between 9.0 and 14.5 grams per 100 ml. Their data indicates high levels of hemoglobin at birth which decrease during the first weeks of life and is followed by fluctuations during the remainder of the year with a mean of 9.05 grams per 100 ml. at the age of 52 weeks. Holman (1956) reported 13 grams of hemoglobin at birth which decreased to 11.0 grams at one week and remained relatively constant to 24 months of age.

Hemoglobin readings of 11.7 and 11.3, respectively, were determined for dwarfs by Cornelius et al. (1956) and Fransen (1955) which were in the normal range. Hafez et al. (1958) indicated a lower value was found in dwarf than controls for hemoglobin.

Mean Corpuscular Volume

The mean corpuscular volume is an important consideration of the red cell. It is a calculated value which measures the volume occupied by the average red cell in cubic microns and is computed by the following formula:

$$M.C.V. = \frac{\text{hematocrit reading x 10}}{\text{red blood cells in millions per cu. mm.}}$$

Variation in mean corpuscular volume are important in diagnosing microcytic or macrocytic anemic conditions. Values reported by Albritton (1952) were 53 cubic microns for calves and 50 cubic microns for adult females with a range from 47 to 54 cubic microns. Holman (1955) reported 57.1 cubic microns in mature cows and Holman (1956) reported definite changes in the size of the erythrocyte from calves at birth to maturity. At birth the corpuscular size averaged 44.9 cu. microns, after birth the corpuscle decreases in size until 2 months with an average of 30.8 cu. microns. From this age there was increase in size until 24 months when it reached the size of mature cows. Greatorex (1954) reported no mean values, but a range of 40 to 60 cu. microns were reported at birth.

Mean Corpuscular Hemoglobin

Another useful measurement calculated from erythrocytes is mean corpuscular hemoglobin, which measures the weight of hemoglobin per cell in micro-micrograms (γγ). It is computed by the following formula:

$$M.C.H. = \frac{\text{hemoglobin in grams per 100 cc. x 10}}{\text{red blood cells in millions per cu. mm.}}$$

This also is important in determining whether red cell picture is normal or a pathological anemia is present. Albritton (1952) reported

a normal value of 14.1 for calves at birth and 15.7 for adult females with a range of 13.7 to 18.5 micro-micrograms. Holman (1955) had an average of 19.2 micro-micrograms in mature cows as compared to 13.2 in cows of Indian breeding.

Mean Corpuscular Hemoglobin Concentration

The mean corpuscular hemoglobin concentration describes the red cell picture by giving the ratio of weight of hemoglobin to the volume in which it is contained, and the result is expressed in percent. It is calculated by the following formula:

$$\text{M.C.H.C.} = \frac{\text{hemoglobin, gm. per 100 cc. x 100}}{\text{vol. packed red cells in cc., per 100 cc.}}$$

This measurement has been a valuable tool in man in diagnosing anemia especially of hypochromic and hyperchromic types. Holman (1955) reported a mean of 33.7% and a standard deviation of 2.81% for mature dairy cows. Greatorex (1954) found little variation in mean corpuscular hemoglobin concentration with the majority of calves ranging between 21% and 40%. The only other report available was Holman (1956) in which he reported an average of 31% from birth to 24 months of age.

Fragility of Red Cell

Although little information is available on fragility of red cells in cattle, considerable data is available in man. A disease condition in man known as hereditary spherocytoses or congenital hemolytic jaundice has been one of the main factors which has stimulated interest in the resistance of red cells to hypotonic salt solutions. By placing known quantities of blood in different concentrations of saline, comparisons of osmotic fragility can be obtained. With hemolytic jaundice there is an increase in the number of red cells which are hemolized

(Emerson et al., 1956; Bertles, 1957; Dacie et al., 1938) and is therefore a method that can be used in detecting hemolytic anemias.

The classical explanation for osmotic hemolysis (Stone et al. 1953) is that the interior of the erythrocyte has an osmotic concentration higher than that of the hypotonic medium surrounding it and the cell takes in fluid in an effort to establish osmotic equilibrium. If an excessive amount of fluid is absorbed, the cell ruptures and hemoglobin is released. Although this seems to account for the general procedure, it does not explain the differences that occur between animals.

Ponder (1940) stated "it remains an experimental fact that the red cell, which on some occasions behaves like a perfect osmometer, on other occasions does not. The lack of swelling of the red cell in hypotonic saline may be due to salt loss or presence of bond water." Castle and Daland (1937) stated the difference in the susceptibility of various types of erythrocytes to hemolysis with hypotonic solution of sodium chloride are due largely to differences in form and not to differences in osmotic behavior. By direct observation his results indicated that hemolysis of a given type of erythrocyte is associated with the assumption of a spherical form in a hypotonic solution, the more susceptible the erythrocyte to hypotonic hemolysis, the less hypotonic the solution necessary for erythrocyte to form a spherical shape. Dacie et al. (1938) found that the larger the initial degree of spherocytosis (roundness) the larger the increase in fragility. They also found a positive correlation of 0.86 between the ratio of mean corpuscular thickness divided by mean corpuscular diameter and percent hemolysis. No significant correlations were found between

percent hemolysis with total protein, mean corpuscular volume, or hemoglobin content. Dacie et al. (1938) found the temperature and pH of the saline needed to be controlled in measuring fragility. Jacobs and Parpart (1931) and Parpart et al. (1947) found pH, temperature, and rate of attainment of equilibrium to be important when determining red cell fragility.

Bertles (1957) suggested that the increased fragility with hereditary spherocytosis is due to an abnormal functioning surface membrane of the erythrocyte and altered glycolytic pathways. Johnson et al. (1944) found that high levels of lipids were injurious to erythrocytes. Although all authors indicate factors affecting fragility, the actual mechanism is still unknown.

Lyons (1918) reported initial hemolysis of the blood cells of oxen occurred in .60% NaCl solution and complete hemolysis of red cells in .40% solution. Dukes (1956) reports initial hemolysis at .59% and complete hemolysis in .42% solution. Holman (1955) on data from 81 cows gave a value of .474% NaCl solution for complete hemolysis. A study on calves by Greatorex (1954) reported high resistance of calves to hemolysis at birth, which decreased over the next four months. Complete hemolysis occurred in a .30% NaCl solution at birth, but at five weeks required only a .45% NaCl solution for complete hemolysis. From about 20 weeks of age to one year complete hemolysis occurred in a .50% NaCl solution. Holman (1956) found a similar pattern in fragility, calves at birth showed complete hemolysis in .35% NaCl, in .36% NaCl at one week, in .44% NaCl at two months, in .53% NaCl at four months, and at two years in .47% NaCl which is close to the value reported for mature cattle.

Massey et al. (1958) at the Missouri Station reported tests on dwarfs, carrier animals and pedigree clean animals which were given intravenous injections of insulin 48 hours apart. The first injection was .8 unit of insulin per kilogram of body weight followed by a second dose of .3 unit to test the response of individual animals to a sequence of a higher and lower dosage of the hormone. It was also thought that the two injections would minimize environmental influences and the response of the individual could be more accurately measured. The fragility of the red cells was measured by counting in a hemacytometer the number of red cells in a blood sample diluted 1:200 in a .48% NaCl solution. In the initial samples, the red cells from pedigree clean cows were more resistant to hypotonic solution than were those of known carriers and dwarfs; however, considerable overlap was noted between the three groups. Forty-eight hours after the first injection of insulin, the difference between the three groups in the resistance of the red cells was much greater and less overlap was observed. Massey et al. (1958) also studied the effect of ACTH on red cell fragility on 17 dwarfs, 10 carriers, and 10 pedigree clean cows. A significant difference was observed between pedigree clean and carrier animals in the resistance of red cells to .48% NaCl solution within five hours after injection of ACTH. The carriers were more resistant than cleans before the hormone was administered and became increasingly more resistant within five hours.

White Blood Cells

In the excellent review of Scarborough (1930) a mean of 9,250 leucocytes per cu. mm. with a normal range of 6,000 to 12,000 leucocytes per cu. mm. were determined from 291 animals of all ages. Ferguson

et al. (1945), Rusoff and Piercy (1946), and Holman (1955) reported mean values of 8,912, 9,474, and 7,030 per cu. mm., respectively. Differential counts from dried stained blood smears are used to determine the percent distribution of each type of white cells. Average values of 31.9% neutrophils, 55.4% lymphocytes, 5.2% large mononucleated and transitionals, 7.7% eosinophils and .62% basophils were reported for healthy cattle of all ages by Scarborough (1930). Ferguson et al. (1945) working with 25 females between the ages of 18-24 months reported 41% lymphocytes, 34% neutrophils, 14% eosinophils, and 8% monocytes. Rusoff and Piercy (1946) from 613 mature females showed estimates of 56% lymphocytes, 31% neutrophils, 7% eosinophils, and 6% monocytes. Holman (1955) reported on 81 mature cows a distribution of 52% lymphocytes, 30% neutrophils, 11% eosinophils, and 7% monocytes. The data clearly indicates a lymphocyte dominated picture. All the above authors generally agreed that the white blood picture of healthy normal cattle varies to a great extent, not only between animals but in the same animal. Factors as age, sex, breed, daily and seasonal variations, disease, and pituitary-adrenal system have been indicated as sources of variation.

In studying environmental effects on leucocytes, Braun (1946) studied the effect of pasture on white cells over different seasons with 49 mature females. Leucocyte count increased significantly during the year when ample green forage was available, and decreased when grazing was scarce. In a study with dairy bulls Rusoff et al. (1954) found an increase in leucocytes when the temperature increased over 80 degrees. Greig and Boyne (1956) reported 6,770 leucocytes on low plane as compared to 7,990 leucocytes per cu. mm. on high level of

nutrition, a highly significant difference. Moberg (1955) studied diurnal variation in cows, but found no significant diurnal variation.

Braun (1946) reported a study on age by dividing animals that were under 30 months of age in one group and those over into another group. No difference of any significance was found in leucocytes. Greatorex (1954) reported an average of 8,700 leucocytes per cu. mm. on 233 calves with the following percent of each type, 50.8% lymphocytes, 45.0% neutrophils, 2.8% monocytes, and 1.1% eosinophils. This indicates a neutrophilia condition at birth which by 2-4 months has passed into a lymphocyte dominated picture. Eosinophils increased from 1.1% to 4.9% at four to six months while neutrophils dropped from 45.0% to 21.7%.

Holman (1956) reported an average of 9,000 white cells with little change in number from birth to two years of age. Lymphocytes were 33% of the white cells at birth but increased to 68% by two to four months of age and remained constant. Eosinophils increased from 0.2% at birth to 9% at two years of age. Monocytes were to variable in number to interpret changes.

Little data are available on the effect of breeds and sex on leucocytes. Rusoff (1954) reported highly significant difference between the dairy breeds of Jersey, Guernsey, and Holstein with means of 8,500, 6,444, 7,416 leucocytes per cu. mm., respectively. Moberg (1955) reported a study which found a higher count in females than males, while steers were lower than bulls in white cells.

Studies of hormones and stress have indicated that in general increases in neutrophils and decreases in lymphocytes and eosinophils occur under these conditions.

Merrill and Smith (1954) injected ACTH into dairy cows which caused a rise in leucocyte number which was noted for 18 hours. The percent of eosinophils and lymphocytes decreased markedly after injection, while neutrophils increased over 100%. Johnson et al. (1951) reported that injections of epinephrine in dairy cattle resulted in decreases of eosinophils up to 46% within six to four hours. He explained this change as the result of ACTH release by the pituitary in response to epinephrine. Injections of stilbestrol and oestradiolbenzoate by Moberg (1950) was followed by increase in leucocytes, primarily neutrophils while lymphocytes and eosinophils decreased after treatment.

Studies on dwarfs by Cornelius et al. (1956), Hafez et al. (1958) and Fransen (1955) found leucocyte count to fall within the normal range. Fransen (1955) reported 7,300 leucocytes per cu. mm. and Cornelius et al. (1950) 8,800 leucocytes per cu. mm. while Hafez stated a lower mean value was found for dwarfs than for controls. Fransen (1955) reported the differential white picture to be normal for dwarfs with values of 69.9% lymphocytes, 20.2% neutrophils, 5.5% eosinophils, 3.5% monocytes. Cornelius et al. (1956) reported 55.6% lymphocytes and 33.3% neutrophils in the dwarf. They found these values significantly different from the values obtained in normal animals in their laboratory.

Foley et al. (1955) injected insulin intravenously into 59 animals and measured the changes in numbers of white blood cells. Highly significant differences were noted between genotypes. Dwarfs showed a minor change in leucocytes, while pedigree clean animals gave a rapid increase, and known carriers were intermediate in their increase at one hour after injection. Subsequent investigations revealed that the

cells were not all true white blood cells. Deyoe et al. (1957) reported a significant increase in leucocyte number in known carriers and dwarfs two hours after injection of insulin. No consistent changes were found in leucocyte cell types following insulin injection. Massey et al. (1958) reported work on injection of ACTH on a group of dwarfs, carrier animals, and pedigree clean cows. No clear-cut difference between the groups was found in white cells, although all groups showed increase in number of neutrophils and a decrease in total number lymphocytes within a few hours after injection.

Plasma Proteins

The plasma proteins of mature cattle have generally been divided into the following classes, albumin, alpha-one, alpha-two, beta and gamma globulins, and fibrinogen. Albritton (1952) review reported a value of 8.32 grams of protein per 100 ml. of plasma. Deutsch and Goodloe (1945) using electrophoretic analysis gave the following percent for each fraction of protein; gamma globulin 11.0, fibrinogen 16.3, beta globulin 13.7, alpha-two 8.3, alpha-one 10.7, and albumin 40.6. Although the above values hold true for mature animals, subsequent studies indicate that serum of new born calves contain very little gamma and beta globulin and are characterized by large amounts of alpha globulins (Deutsch, 1954; Jameson et al., 1942). Jameson et al. (1942) found the serum of new-born calves before the ingestion of colostrum contains no gamma globulin and only small amounts of beta globulin. During the nursing period the composition of calf serum changes rapidly, with increases in the composition of both gamma and beta globulin and decrease in albumin and alpha globulin. Smith and Erwin (1959) and Deutsch and Smith (1957) found that direct absorption of protein from

colostrum occurred during the 24 hours after birth and formed a large percent of the gamma globulin fraction. After 24 hours, the permeability of the gut to protein is largely lost.

Cornelius et al. (1956) and Hafez et al. (1958) reported that no consistent differences in plasma protein fractions were found between dwarfs and control animals.

MATERIALS AND METHODS

The data reported in this study were taken during the period from May, 1958, through April, 1959. Initially, data were collected from the purebred cattle in the University herd at Stillwater. Both Angus and Herefords of ages varying from one to eight years were used. The blood collected was utilized in determining the basic procedure for red cell fragility.

As the study progressed, the analytical equipment was moved to Ft. Reno where cattle of known genotypes could be studied. At the Ft. Reno Station hematological data were collected to study the effect of the dwarf gene on the cellular constituents of the blood. Both purebred and grade Hereford and Angus cattle in Projects 873, 650, and 670 were utilized in this study.

The cattle of Project 873, "Evaluation of Methods of Identifying Dwarf Carriers in Beef Cattle," were either dwarf or known carrier Herefords and Angus, or Comprest Herefords. The cattle of Project 650, "The Relation of Nutrition and Age at First Calving to Lifetime Performance of Beef Cattle," were grade Hereford cows considered to be dwarf free since no dwarf calves have been produced in this line. Project 670, "The Improvement of Beef Cattle by the Application of Breeding Methods," consists of one purebred Angus line and two purebred Hereford lines. Ample information is available on these lines to accurately evaluate the mature cows as pedigree clean, possible

dwarf carriers, known dwarf carriers, or comprest. In the younger age groups the possible carrier classification includes cattle with either known carrier parents or parents that were possible carriers. This group includes Hereford, Angus, and crossbred (Hereford X Angus) calves. The known carrier calves all had one dwarf parent and included Angus, Hereford, and crossbred (Hereford X Angus) calves. The pedigree clean group included Hereford and Angus, but no crossbred calves, while the comprest group included only Hereford calves from comprest dams.

The cattle in all projects graze on native grass pasture during the summer months. Winter treatment varies somewhat between projects. The cows of Project 650 are divided into three winter groups to receive either a low, medium, or high level of feeding. The cows in Projects 873 and 670 are wintered under range conditions, and are fed supplemental feed only when in accordance with good range practice. Winter small grain pasture is utilized when available.

The statistical methods used in this study were computed as described by Snedecor (1956). Standard deviations were utilized in measuring the variation rather than standard error since numbers varied between treatments. Standard deviations allow more direct comparison of the variation since it is not divided by the square root of the individuals within the treatment.

All blood was collected from the jugular vein, prevented from coagulation by heparin, and cooled immediately in an ice water bath. The procedures used in the blood analysis follow under section headings.

Red Blood Cell Count

Erythrocyte counts were made according to the procedure of Wintrobe (1956). The heparinized blood was thoroughly mixed by gently

inverting the tube several times before a sample was taken. After mixing, a sample of blood was drawn into a red cell pipette exactly to the 0.5 mark. The .85% saline diluting fluid was drawn into the pipette to the 101 mark resulting in a 1:200 dilution of the blood. The blood cells and diluter were thoroughly mixed by shaking the pipette for two to three minutes in a mechanical shaker. The first four or five drops in the stem of the pipette were discarded before transferring a small portion of the diluted blood to the counting chamber of Spencer "Bright-Line" Hemacytometer with improved double Neubauer ruling. After allowing a few minutes for the cells to settle, all cells in the four corner and middle .04 sq. mm. squares of the central sq. mm. square were counted under the high power lens of a microscope. The number of cells counted was multiplied by 10,000 to obtain the number of red blood cells per cubic millimeter of blood.

White Blood Cell Count

Leucocyte counts were made according to the procedure of Wintrobe (1956). The white cell procedure is very similar to the counting of red cells. After mixing of blood, a sample was drawn into the white blood cell pipette to the 0.5 mark. The .1N HCl diluting fluid was drawn to the 11 mark and the diluted sample was shaken for two to three minutes in a mechanical shaker. Four drops of the diluted blood in the stem of the pipette was discarded and a sample was placed on the Spencer Hemacytometer. All white cells in the four corner sq. mm. squares were counted under low power, and the total count multiplied by 50 to express the count in white blood cells per cubic millimeter of blood.

Differential White Cell Count

The differential white cell count made according to the procedure outlined by Wintrobe (1956) is a determination of the types of leucocytes expressed as a percent of the total leucocytes counted. The coverslip procedure was used in preparing the blood smears for counting. The coverslips were first thoroughly cleaned with alcohol, wiped dry, and a drop of blood placed on one coverslip. A second coverslip was placed over the first and the two slowly pulled apart to obtain a thin, even distribution of blood. The coverslip smears were quickly air dried to prevent an uneven distribution. The cells were stained by flooding with Wright's stain for one to two minutes, adding a buffer for a period of four to five minutes and finally rinsing clean with distilled water. After drying, the coverslips were mounted on microscope slides and counted under oil immersion. The types of leucocytes observed in a sample of 200 cells were recorded and each type expressed as a percent of the total.

Red Cell Fragility

The procedure utilized in determining red cell fragility was a modification of the method of Stewart et al. (1950). The modified procedure was shown by preliminary studies to be a more repeatable procedure. To control as nearly as possible fluctuations resulting from temperature, the blood and saline solutions were cooled in an ice water bath at all times. One-tenth ml. of heparinized blood was added to test tubes containing 10 ml. known concentration of saline, and the blood and saline were thoroughly mixed by inverting the tube four times. After standing for one hour in an ice water bath, the unhemolized cells were removed from the solution by centrifugation for five minutes at 1,500

revolutions per minute. Approximately six ml. of fluid was carefully poured into a reading tube without disturbing the unhemolized cells. The supernatant fluid was read on a Coleman Model 6A Jr. Spectrophotometer set at a wavelength of 540 millimicrons. The remaining unhemolized cells in the centrifuge tube were hemolized by adding saponin and stiring. This solution was mixed with the supernatant fluid in the reading tube to obtain the reading for the completely hemolized sample. The percent hemolysis was calculated as follows:

$$\% \text{ Hemolysis} = \frac{\text{optical density of supernatant fluid}}{\text{optical density after total hemolysis}}$$

Hematocrit

Hematocrit determinations were made by the method described by Wintrobe (1956). A well mixed sample of blood was transferred by means of a hematocrit pipette to a Wintrobe tube. The tube was centrifuged for 30 minutes at 3,000 revolutions per minute. The length of the packed red cell column was determined directly from the reading scale on the tube, and the results expressed as a percent of whole blood.

Hemoglobin

Hemoglobin was determined by the Spencer Hemoglobinometer, a visual filter photometer. The blood was thoroughly mixed, a sample was placed on the open chamber of the Spencer Hemoglobinometer, and completely hemolized by stirring with a saponin treated applicator. The chamber is placed into the Spencer Hemoglobinometer and a reading is made by visually matching the colors of the blood sample with that of a standard. The readings are determined directly in grams of hemoglobin per 100 cc. of blood.

Mean Corpuscular Volume: (Wintrobe, 1956)

The mean volume of the cells in absolute terms may be calculated by this formula,

$$M.C.V. = \frac{\text{vol. packed red cells, ml. per 1000 ml.}}{\text{red cell count, millions per cu. mm.}}$$

This formula was designed so the result may be expressed in cubic microns.

Mean Corpuscular Hemoglobin: (Wintrobe, 1956)

This indicates the weight of hemoglobin in the average corpuscle expressed in micro-micrograms (γγ).

$$M.C.H. = \frac{\text{hemoglobin, grams per 1000 ml.}}{\text{red cell count, millions per cu. mm.}}$$

Mean Corpuscular Hemoglobin Concentration: (Wintrobe, 1956)

This is a measure of the concentration of hemoglobin in the average red corpuscle expressed in percent.

$$M.C.H.C. = \frac{\text{hemoglobin, grams per 100 ml. x 100}}{\text{volume packed red cells, ml. per 100 ml.}}$$

Specific Gravity of Plasma: (Wintrobe, 1956)

The plasma utilized in this study was removed from the hematocrit tube after centrifugation. The plasma was drawn into a pipette and dropped into a series of graded copper sulfate solutions of known specific gravity ranging from 1.021 to 1.031. If the drops of plasma had a higher specific gravity than the copper sulfate solution the drop fell directly to the bottom, while if it had a lower specific gravity than that particular copper sulfate solution the drop rose immediately. When the drop of plasma maintains an equilibrium (neither rises or falls in the first 15-20 seconds) in the copper sulfate solution the specific gravity of the blood is the same as that of the copper sulfate solution.

Electrophoretic Patterns of Plasma

The procedure used was that of Block et al. (1958) utilizing a Durrum-type paper electrophoresis cell with a barbituate buffer of pH 8.6 and of an ionic strength of .075. To prepare the cell about 800 ml. of the buffer was poured directly into the cell, while the remaining 200 ml. of the buffer was poured onto the filter paper strips. Care was taken to see that the strips were in contact with the paper wicks below. The cell was then placed into a cold room for 15 minutes to allow the excess liquid to drain from the strips and the cell to become saturated with water vapor. A sample of .01 ml. of plasma was placed on each strip in the cell. The current was set for 2.5 ma. and 250 volts and run for a period of 18 hours. After completion of the run, the strips are dried in an oven at 120-130° c. for 30 minutes and then stained with bromphenal blue. A Photovolt densitometer with a 615 milli-micron filter was utilized to plot the density of the different plasma fractions. The area of each fraction was determined by use of an integrator and was expressed as a percent of the total plasma protein on the strip.

Electroploretic Patterns of Hemoglobin: (Block et al. 1958)

The hemoglobin was prepared by washing the cells three times in 0.9 NaCl solution to remove the plasma. Distilled water was added to the sample to hemolize the red cells and liberate the hemoglobin, and benzine was added to the sample to dissolve the lipids. Centrifugation of the sample for 20 minutes causes the formation of layers of distilled water and lipids, which are removed by aspiration. The hemoglobin was diluted with .85% saline to a 3% solution and a .01 ml. sample was placed on each strip. The remainder of the procedure is identical with the one used for plasma protein electrophoretic patterns.

RESULTS AND DISCUSSION

Erthrocyte Fragility Studies

A comparison of erythrocyte fragility of normal beef cattle of different ages as determined by percent hemolysis in .52% and .56% saline is given in Table I and Figure I. This is a summary of all non-dwarf cattle used in this study divided into five age groups varying in age from less than one week to mature cows, three to nine years of age. The analysis of variance showed a highly significant difference between age groups ($P<.005$). Observations of the mean values for percent hemolysis in .52% and .56% saline, respectively, show the lowest values, 43.1% and 34.2%, at one week or less of age, increasing to 87.6% and 75.5% at one to two months, and reaching the highest values, 99.1% and 92.6%, at seven to eight months of age. Following the high point at seven to eight months the percent of hemolysis decreased progressively as older age groups were studied. The 18 month age group had means of 97.8% and 84.3% and the mature animals had means of 71.4% and 44.7% hemolysis in .52% and .56% saline solutions, respectively. This study indicates that the erythrocytes of young calves at birth are the most resistant of any age group studied to osmotic fragility. After birth and at the age of one to two months there is a marked increase in erythrocyte fragility which reaches the maximum at seven to eight months. There is a gradual decrease in fragility of erythrocytes with increasing age from the high at seven to eight months to the mature

TABLE I PERCENT ERYTHROCYTE HEMOLYSIS OF DIFFERENT AGES OF NORMAL CATTLE

Group	Percent NaCl .52 %	Percent NaCl .56 %
One week or less	43.1 (60)[b] (16.07)[a]	34.2 (64) (15.45)
One-two months	87.6 (27) (10.43)	75.5 (27) (13.74)
Seven-eight months	99.1[c] (8) (0.25)	92.6[c] (46) (8.02)
Eighteen months	97.8 (8) (1.06)	84.3 (8) (10.41)
Three-nine years	71.4 (25) (20.16)	44.7 (25) (22.18)

[a]standard deviation
[b]number of animals
[c]differences between age groups ($P < .005$)

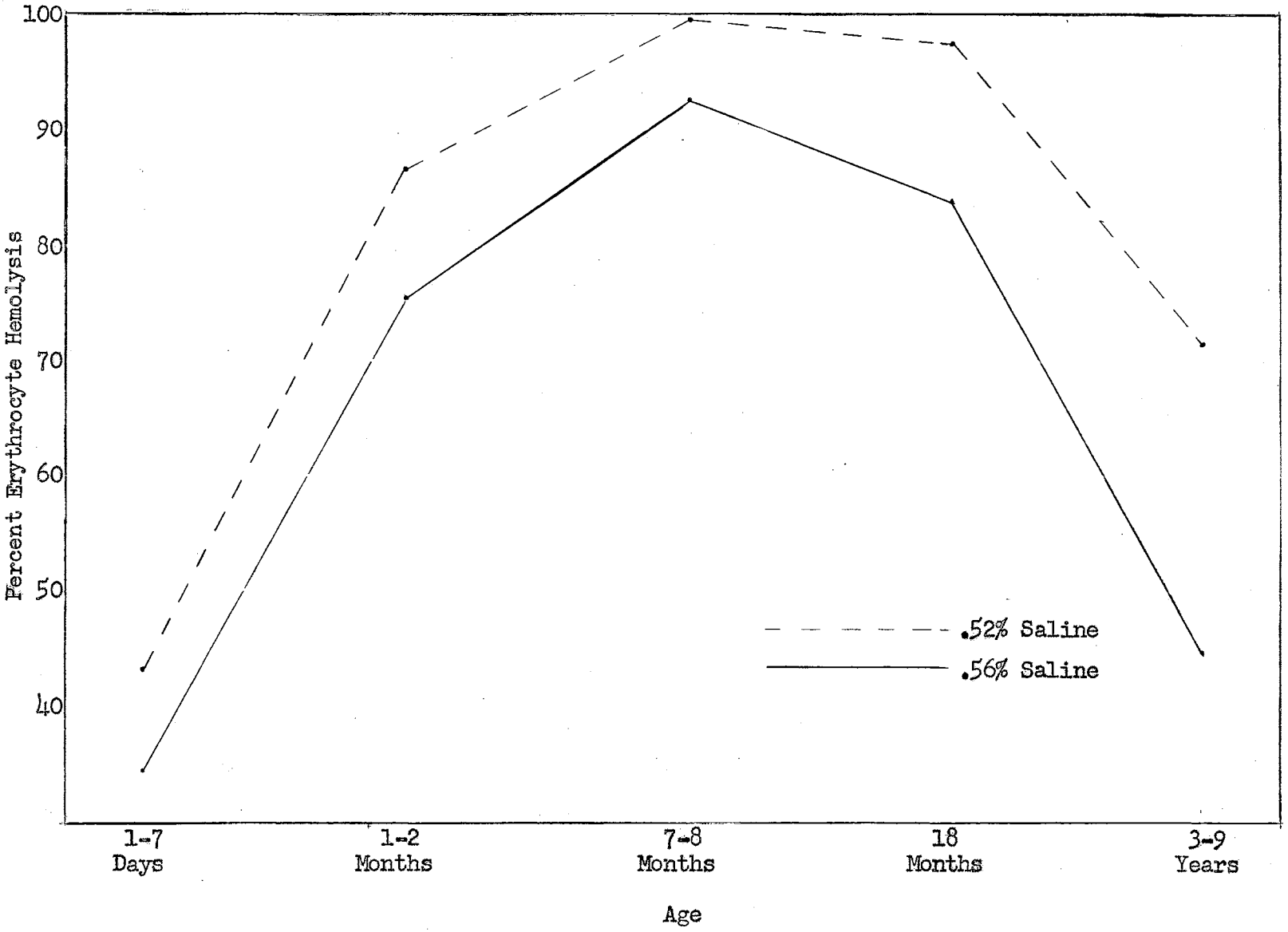

FIGURE 1. PERCENT ERYTHROCYTE HEMOLYSIS OF DIFFERENT AGES OF NORMAL CATTLE

levels at approximately three years of age. The studies of Greatorex (1954) and Holman (1956) agree closely to the results obtained in this study. They found calves at birth to be very resistant to erythrocyte hemolysis, with a decreased resistance of erythrocytes to hemolysis up to four to six months of age. This suggests that age is an important factor to consider and attempt to control in any research dealing with erythrocyte fragility. In this study, the age factor was controlled by making all genotype studies within a limited age group.

Mature Age Group

Table II compares the erythrocyte fragility in four concentrations of saline for pedigree clean, possible carrier, known carrier, and comprest Hereford cows three to nine years of age. These cows included eight comprest and six known carrier cows from Project 873, and four possible carrier and seven pedigree clean cows from Project 670, all of which were non-lactating and pregnant. The data indicates only small differences between the means and large variation within the genotype groups. No significant differences were found between the pedigree clean cows and cows that were known carriers of the snorter dwarf gene. There was, however, a tendency in all of the saline solutions for the pedigree clean cows to be more resistant to hemolysis than known carriers. The mean values for the percent hemolysis in .52% saline are 67.2%, 57.3%, 78.0%, and 77.1%, respectively, for pedigree clean, possible carrier, known carrier, and comprest cows.

Massey et al. (1958) found pedigree clean cows to be more resistant than known carriers, with the differences highly significant. Although the differences were not significant, there was a tendency in the report under study for the erythrocytes of pedigree clean cows to

TABLE II PERCENT ERYTHROCYTE HEMOLYSIS OF PEDIGREE CLEAN, POSSIBLE CARRIER, KNOWN CARRIER, AND COMPREST HEREFORD COWS OF THREE TO NINE YEARS OF AGE

Group	Percent NaCl .48 %	.52 %	.56 %	.60 %
Clean	87.2 (8.83)[a] (7)[b]	67.2 (15.49) (7)	38.1 (15.11) (7)	22.3 (10.56) (7)
Possible Carrier	77.4 (21.65) (4)	57.3 (37.84) (4)	29.4 (11.61) (4)	6.0 (1.99) (2)
Known Carrier	92.0 (8.99) (6)	78.0 (19.74) (6)	52.6 (26.50) (6)	22.6 (13.57) (6)
Comprest	91.2 (9.76) (8)	77.1 (21.60) (8)	52.2 (25.23) (8)	43.4 (14.52) (2)

[a]standard deviation
[b]number of animals

be more resistant than those of known carrier cows, with the comprest cows showing values more similar to known carriers than any other group. The percent hemolysis for the possible carrier group does not fall between the means of the pedigree clean and known carrier as might be expected if both clean and carrier cows were in this classification. Although no ready explanation can be given for this, several possibilities might be proposed. One is that no real difference exists between known carrier and pedigree clean cows; therefore, the mean for the possible carriers, even though the group has both clean and carrier cows, could be either above or below the means for the pedigree clean or known carrier group. A second possibility is that since the possible carrier group is so limited in number, that the chance factor alone might have allowed the mean of the possible carrier group to fall outside the area between the means for carrier and pedigree clean groups. Another consideration is the fact that the possible carrier group consisted of animals of a different line of breeding; therefore, if breeding affects the erythrocyte fragility it could alter the mean of the possible carrier group. This points out the many factors, known and unknown, which might affect the percent hemolysis of erythrocytes and make interpretation of results difficult.

Eighteen Month Age Group

Table III compares the erythrocyte fragility of dwarfs and non-dwarfs 18 months of age as determined by the percent hemolysis of erythrocytes in .52%, .56%, .60%, and .64% saline. The non-dwarf group was composed of eight Angus bulls from Project 873 and two Angus bulls from Project 670. One dwarf Hereford bull and one dwarf Angus bull of comparable ages from Project 873 constituted the dwarf group. No significant

TABLE III PERCENT ERYTHROCYTE HEMOLYSIS OF DWARF AND NON-DWARF BULLS 18 MONTHS OF AGE

Group	No.	Percent NaCl .52 %	.56 %	.60 %	.64 %
Non-Dwarf	8	97.8 (1.06)[a]	84.3 (10.41)	57.6 (17.67)	33.7 (19.28)
Dwarf	2	96.9 (2.97)	95.4 (18.8)	72.8 (18.03)	34.0 (16.97)

[a]standard deviation

TABLE IV PERCENT ERYTHROCYTE HEMOLYSIS OF DWARF AND NON-DWARF CATTLE, SEVEN TO EIGHT MONTHS OF AGE

Group	Percent NaCl .52 %	.56 %	.60 %	.64 %
Non-Dwarf	99.1 (0.26)[a] (8)[b]	94.4 (3.31) (12)	80.7[c] (11.54) (12)	45.9[d] (17.69) (12)
Dwarf	97.2 (1.06) (2)	77.2 (27.60) (4)	49.2 (25.40) (4)	25.3 (12.79) (4)

[a]standard deviation
[b]number of animals
[c]different from dwarf group ($P < .025$)
[d]different from dwarf group ($P < .10$)

differences were found between the dwarf and non-dwarf groups with large standard deviations within groups. The percent hemolysis in .56% saline solution was 84.3% for non-dwarfs and 95.4% for dwarfs. The dwarf group had a higher percent of hemolysis in .56%, .60%, and .64% saline. In .52% saline the percent hemolysis was slightly higher for non-dwarfs. However, it should be pointed out that the percent hemolysis in the .52% saline was above 90%. This study and other research with erythrocyte fragility in humans has indicated that the determination of percent hemolysis is not a critical measure of the fragility of erythrocytes when the percent hemolysis is above 90% or below 10% (Parpart et al., 1947). When the study of erythrocyte fragility is not within the critical area, the differences between animals disappear. Also since not enough information was known regarding the genotype of the eight normal bulls, they were all classified as non-dwarf. However, six animals of this group were possible carriers; therefore, if the dwarf gene decreases resistance to hemolysis as suggested by the older known carrier cows, this would reduce the difference between the means of the dwarf and non-dwarf groups if, as would be expected, some of the bulls were carriers.

Seven to Eight Month Age Group

Comparisons of the percent hemolysis of dwarf and non-dwarf calves seven to eight months of age are reported in Table IV. Twelve calves from known carrier cows of Project 873 constituted the non-dwarf group, while four dwarfs of comparable age composed the dwarf group. Each group consisted of both sexes in equal numbers. In all saline solutions the non-dwarfs showed a higher percent of hemolysis of erythrocytes than dwarfs, with differences of 31.5% in .60% saline ($P < .025$)

and 20.6% in .64% saline ($P<.10$). In .60% saline the percent hemolysis was 80.7% for non-dwarf and 49.2% for the dwarfs, indicating an increased resistance of the erythrocytes of dwarf calves. Comparison of these values with the 18 month group indicates a reverse trend, with the younger dwarf calves showing an increased resistance to osmotic fragility than do non-dwarf cattle. Although mature dwarfs were not available, a similar trend was indicated by the higher rate of hemolysis of carriers as compared to pedigree clean cows.

Three Months Age Group

Since information was not available to group the calves according to their dwarfism genotype, the comparison of twelve calves three months of age was made by classifying them on the basis of their x-rays into a C x-ray group considered to be clean of the dwarf gene, and a B x-ray group considered to be carriers. The results of the erythrocyte fragility of this group are found in Table V. Comparisons of the two groups show the C x-ray to be more resistant than the B x-ray with values of 54.2% for C x-ray and 61.5% for B x-ray in .60% saline. The differences between the groups were small with large standard deviations in each group.

If one assumes that the x-ray method accurately identifies the dwarfism genotype, the results obtained with three months old calves compares favorably with the results of mature cows and 18 month old cattle, but differs with the calves of seven to eight months of age. Since the calves were closer in age to those of seven to eight months of age, it was expected that the calves predicted clean would have more fragile erythrocytes than calves predicted carrier. This difference between predicted and actual values could be due to inaccuracy

TABLE V PERCENT ERYTHROCYTE HEMOLYSIS OF C X-RAY AND B X-RAY CALVES THREE MONTHS OF AGE

Group	No.	Percent NaCl .60 %	.64 %	.68 %
C X-ray	6	54.2 (26.47)[a]	22.0 (13.94)	7.3 (14.50)
B X-ray	6	61.5 (18.92)	33.8 (26.49)	14.5 (14.30)

[a]standard deviation

TABLE VI PERCENT ERYTHROCYTE HEMOLYSIS OF PEDIGREE CLEAN, POSSIBLE CARRIERS, AND KNOWN CARRIERS CALVES ONE TO TWO MONTHS OF AGE

Group	No.	Percent NaCl .60 %	.56 %	.52 %
Pedigree Clean	16	57.8 (15.72)[a]	79.7 (12.14)	91.9[b] (5.19)
Possible Carrier	5	51.1 (19.74)	71.9 (12.19)	86.0 (9.02)
Known Carrier	4	48.5 (10.07	69.9 (3.60)	79.8 (4.43)

[a]standard deviation
[b]different from known carrier group ($P < .01$)

of x-ray methods. Because the genotypes were so indefinite in this study, probably the most important contribution of this group was for comparisons of erythrocyte fragility at different ages.

One to Two Month Age Group

The percent of erythrocyte hemolysis of pedigree clean, possible carrier, and known carrier calves of one to two months are reported in Table VI. The known carrier calves are more resistant than the pedigree clean calves with values in .52% saline of 91.9% and 79.8%, respectively, for pedigree clean and known carrier. This difference between the mean was 12.1% ($P<.01$). The mean values of the possible carrier group fell between the known carriers and pedigree clean groups in all saline solutions for the percent of hemolysis. This would be expected if the possible carrier group included both pedigree clean and known carriers. The increased resistance to osmotic fragility of the known carrier group compares favorably with the dwarfs of seven to eight months of age.

One Week or Less Age Group

The largest data of any age group was the study with calves one week or less of age. Table VII compares the erythrocyte fragility, determined in .60%, .56%, and .52% saline solutions, of pedigree clean, possible carrier, known carrier, and comprest calves of one week or less of age. The pedigree clean group included six Angus and 15 Hereford calves, the possible carrier group included three Angus, 17 Hereford, and nine crossbred (Hereford X Angus) calves, the known carrier group consisted of six Hereford and three crossbred (Hereford X Angus) calves, and the comprest group included five Hereford calves. The known carrier calves were all sired by a dwarf Hereford bull. Of the

TABLE VII PERCENT ERYTHROCYTE HEMOLYSIS OF PEDIGREE CLEAN, POSSIBLE CARRIER, KNOWN CARRIER, AND COMPREST CALVES OF ONE WEEK OR LESS OF AGE

Group	Percent NaCl .60 %	.56 %	.52 %
Pedigree Clean	33.2 (15)[b] (9.92)[a]	44.7 (21) (14.72)	53.0 (19) (15.44)
Possible Carrier	20.7 (13) (9.61)	32.4 (29) (14.07)	42.3 (28) (15.02)
Known Carrier	15.3[c] (4) (5.49)	20.5[c] (9) (6.96)	29.1[c] (9) (9.63)
Comprest	12.2 (3) (3.34)	25.0 (5) (8.12)	32.9 (5) (4.28)

[a]standard deviation
[b]number of animals
[c]different from pedigree clean group ($P < .005$)

29 possible carrier calves, 19 were from known carrier dams, and the remaining 10 were more distantly related to the known carriers in their ancestry.

At the start of this particular age group study it was not known which saline concentrations should be used, therefore, three concentrations, .52%, .56%, and .60%, were tried. It was observed that the hemolysis of some groups in the .60% saline approached the lower limit for most accurate readings. For that reason this concentration was abandoned and only the .52% and .56% concentrations were used during the latter part of the study. There was a correlation of .96 (Table XXII) between values obtained in .52% and .56% saline, indicating values obtained in either concentration were equally accurate in comparing the erythrocyte hemolysis of the different genotype groups.

In all saline solutions the known carrier calves were more resistant to erythrocyte hemolysis than were pedigree clean calves ($P < .005$). The percent hemolysis in .56% saline was 44.7% for pedigree clean, 32.4% for possible carrier, 20.5% for known carrier, and 25.0% for comprest groups. The standard deviations were consistently smaller in the known carrier group than in either the possible carrier or pedigree clean group. The possible carrier group was intermediate between the means of the pedigree clean and known carriers for the percent of erythrocyte hemolysis in all saline solutions. This suggests that the possible carrier group included both carrier and clean calves, assuming that a real difference exists in erythrocyte fragility between pedigree clean and known carriers. The percent hemolysis for comprest calves was similar to the known carrier values. Although this group is limited in number, this indicates that the comprest gene may have

a similar effect to the snorter dwarf gene in increasing the resistance of erythrocytes to osmotic fragility at this age, or that the comprest calves also carry in a high frequency the snorter dwarf gene.

Table VIII compares the erythrocyte fragility determined in known concentrations of saline of male and female calves one week or less of age. This study was made to see if sex was a factor to consider as affecting the percent of hemolysis. The number of males and females was generally equally divided within genotype so that genotype did not confound sex differences. The mean values of 32.1% for males and 35.8% for females in .56% saline is indicative of the small differences between male and females for percent hemolysis in all saline solutions.

Because the differences in percent hemolysis between pedigree clean and known carriers one week or less of age were large ($P < .005$), a more comprehensive evaluation was made to determine if this might be a possible method for detection of carriers. If the average of the means of the known carriers and pedigree clean calves is used as a dividing point, all calves above 32.6% would be predicted clean and those below 32.6% predicted carriers. Examination of the nine known carriers shows one calf with a value over the dividing line, while three calves in the pedigree clean group have a value below 32.6% hemolysis. Of the 29 calves which were classified possible carrier, 17 had values above 32.6% and 12 below.

The means for birth weight of the different genotypes were 69 pounds for pedigree clean calves, 71 pounds for possible carriers, 60 pounds for known carriers, and 67 pounds for comprest calves. The probable reason for the low birth weight of the known carriers was because all of the calves were from 2-year-old cows, while in the

TABLE VIII PERCENT ERYTHROCYTE HEMOLYSIS OF MALE AND FEMALE CALVES OF ONE WEEK OR LESS OF AGE

Group	Percent NaCl .60 %	.56 %	.52 %
Male	27.7 (18)[b] (9.93)[a]	32.1 (34) (13.30)	40.6 (33) (14.70)
Female	24.8 (17) (13.82)	35.8 (30) (17.52)	46.1 (27) (17.42)

[a]standard deviation
[b]number of animals

TABLE IX PERCENT ERYTHROCYTE HEMOLYSIS IN .56% SALINE FOR PEDIGREE CLEAN, POSSIBLE CARRIER, AND KNOWN CARRIER CALVES ONE WEEK OR LESS OF AGE UNCORRECTED AND CORRECTED FOR BIRTH WEIGHT

	No.	Uncorrected .56% NaCl %	Corrected for Birth Weight .56% NaCl %
Pedigree Clean	21	44.7 (14.72)[a]	45.0 (12.67)
Possible Carrier	29	32.4 (14.07)	33.0 (13.75)
Known Carrier	9	20.5 (6.96)	18.3 (7.87)

[a]standard deviation

other groups the calves were out of older cows. This variation in birth weights between genotypes and a correlation of -.18 between erythrocyte fragility and birth weight (Table XXII) indicated that a more valid test of differences between pedigree clean and known carrier calves might be made if erythrocyte fragility values were corrected for birth weights. The regression between birth weight and erythrocyte fragility based on calves of week or less of age in the pedigree clean, known carrier, and possible carrier groups was -.32. Using this regression the erythrocyte fragility of all calves was corrected to the mean birth weight of 69 pounds. Comparisons of corrected and uncorrected erythrocyte hemolysis in .56% saline for pedigree clean, possible carrier, and known carrier calves are shown in Table IX. The mean for pedigree clean calves was changed from 44.7% to 45.0% by correction for birth weight, while the mean of the known carriers decreased from 20.5% to 18.3%. This was an increase of 2.5% hemolysis between the means of the pedigree clean and known carriers after correction for birth weight. The variation within the pedigree clean and possible carrier groups decreased after correction for birth weight, while there was a slight increase from 6.95% to 7.87% for the standard deviations in the known carrier group.

The average of the means of the pedigree clean and known carriers was again used as the dividing point for carriers and clean calves. The calves with the percent of erythrocyte hemolysis in .56% saline above the average of 31.6% were considered to be clean and those below 31.6% were classified carriers. Investigation of the nine known carriers showed all the calves to fall below the average of 31.6%; however, one calf had a value of 31.0% very close to dividing line.

Except for one calf with a value of 17.9%, all other calves in the pedigree clean group had an erythrocyte fragility above 31.6%. One calf had a reading of 32.2% which was close to the dividing point. The 29 calves in the possible carrier group divided into 13 clean calves with a mean of 46.0% and 16 carriers with a mean of 22.4%, these values were very similar to means for the pedigree clean and known carrier groups. By this procedure all known carrier calves were classified as carriers and 20 of the 21 pedigree clean classified as clean. This indicates with this limited data that after correction for birth weight the percent of erythrocyte hemolysis of calves one week or less of age was 100% accurate in predicting all known carriers, while it was 95% accurate in predicting cleans. This compares to an accuracy of 86% for predicting cleans on the average of uncorrected erythrocyte fragility of the pedigree clean and known carrier, and an accuracy of 89% for predicting carriers.

It is impossible to determine the number of carrier and clean offspring which would be expected in the possible carrier group because classification of the sires and dams as either clean or carrier was not possible. If all the bulls used were clean, one would expect 50% of the calves from the 19 known carrier dams to be carriers, and approximately 25% of the calves from dams with more distantly related carriers in their ancestry to be carriers, giving an estimate of 41% of the calves as carriers. Breeding tests of the bulls indicate they are clean; therefore, the estimate of 41% carriers among the offspring is probably most nearly correct. However, if any of the bulls were carriers, it would increase markedly the number of carrier offspring expected. The maximum number of carriers that would be expected

would be 66% of the normal calves if all sires and dams were carriers. The possible carrier group was divided into 16 carrier and 13 clean on the basis of the fragility of erythrocytes corrected for birth weight. Thus the corrected erythrocyte fragility predicted 59% carriers as opposed to the above estimate of 41% expected. Several possibilities can be suggested to explain this difference in the number of carriers observed and number expected. The chance factor itself could be involved because of the limited number of calves in this study. Although the sires are believed to be clean, they have not been tested to a level that eliminates the possibility that one or more may be a carrier. Another possibility is this method is not highly accurate in predicting genotype, in this group of calves predicting too many carrier calves.

To evaluate the corrected erythrocyte fragility from another standpoint, a review of x-rays was made to see how closely the two methods agreed on predicting clean and carrier calves. The nine known carriers were all predicted carrier by both x-ray and corrected erythrocyte fragility. In the pedigree clean group one of the 21 calves was predicted carrier by corrected erythrocyte fragility and nine by x-ray. The one calf predicted carrier in the pedigree clean group by corrected erythrocyte fragility was also predicted carrier by x-ray. Assuming all pedigree clean calves were free of the dwarf gene, the accuracy of the two methods was 57% for x-ray and 95% using fragility. The x-ray method predicted 13 carriers and 15 cleans in the possible carrier group (one calf had no x-ray), while the 28 calves with x-rays were classified into 16 carriers and 12 cleans by corrected erythrocyte fragility. Of the 16 predicted carrier by fragility, nine were also predicted carrier

by x-ray, while of the 12 calves predicted clean by fragility 8 were also predicted clean by x-ray. Of the 26 calves predicted carrier in the known carrier, possible carrier, and pedigree clean groups on the basis of corrected erythrocyte hemolysis, 20 were also predicted carrier on basis of x-ray, an agreement of 73%. Of the 32 calves which were predicted clean on basis of corrected erythrocyte fragility, 20 were predicted clean by the x-ray method, or an agreement of 62.5% in predicting cleans. The overall agreement in predicting cleans and carriers by the two methods would be 67.2%.

This data indicates that correction for birth weight reduced some of the overlap between pedigree clean and known carriers for erythrocyte fragility. It also suggests that this might be a possible method of detecting carriers of the snorter dwarf gene. The agreement, however, between the x-ray method and corrected erythrocyte fragility was only 67.2%.

Effects of Stress and Hormones

Research at the Missouri Station has indicated that the use of hormones as a stressor were valuable in blood studies to separate dwarfism genotypes. This stimulated additional research in this study to determine the effect of fasting and injection of epinephrine on the fragility of erythrocytes. The period of fasting and level of epinephrine used in these studies were determined from preliminary trials with cattle and sheep.

Values of Table X represents the percent hemolysis of erythrocytes of pedigree clean and known carrier Hereford cows three to nine years of age prior to a 72 hour fast, following a 72 hour fast, and after an injection of .25 cc. epinephrine hydrochloride (1:1000) per

TABLE X PERCENT ERYTHROCYTE HEMOLYSIS OF FASTED AND NON-FASTED PEDIGREE CLEAN AND KNOWN CARRIER HEREFORD COWS AND AFTER AN INTRAVENOUS INJECTION OF .25 cc. EPINEPHRINE (1:1000) PER 100 POUNDS FOLLOWING A 72 HOUR FAST

	Non-Fasted		72 Hour Fast		5 Hours after injection of epinephrine	
	Percent NaCl					
Group	.48 %	.54 %	.48 %	.54 %	.48 %	.54 %
Pedigree Clean	67.8 (10.39)[a] (6)	43.0 (25.43) (6)	74.8 (7.18) (4)	37.8 (14.15) (4)	64.1 (9.45) (4)	41.4 (14.01) (4)
Known Carriers	74.1 (12.17) (6)	31.2 (11.82) (6)	56.7 (16.02) (3)	36.0 (21.42) (3)	67.4 (15.04) (3)	40.3 (18.75) (3)

[a]standard deviation

100 pounds of body weight following a 72 hour fast. The pedigree clean group consisted of 12 grade Hereford cows from Project 650 while 11 purebred Hereford cows from Project 873 composed the known carrier group. Prior to fasting all cows were on native grass pasture and were pregnant non lactating cows. These cows were a part of another study dealing with the changes in levels of glucose in the blood. The results in this study were not used in age comparison of erythrocyte fragility because the blood remained in the saline only 10 minutes before the hemolysis was determined as compared to one hour in all of the other studies.

Small differences were found between the pedigree clean and known carrier cows. Prior to fasting, the percent hemolysis of erythrocytes in .48% saline was 67.8% and 74.1%, respectively, for pedigree clean and known carriers. After a 72 hour fast the percent hemolysis was 74.8% and 56.7%, and five hours after a single intravenous injection of epinephrine the percent hemolysis was 64.1% and 67.4%, respectively, for pedigree clean and known carriers in .48% saline. This suggests a picture of higher percent of hemolysis for known carriers prior to fasting, which reverses to a higher percent of hemolysis for pedigree clean after a 72 hour fast and then returns to the initial pre-fasting picture after injection of epinephrine in which the known carriers have a higher percent of hemolysis. The standard deviations were large within all groups. It was not possible to compare percent changes in erythrocyte fragility because pre-fast values were not obtained from the first group of cows, and because different concentrations of saline were used on the same animal between treatments. After the stress of fasting and injection of epinephrine two cows died shortly after the

experiment. Autopsy of the cows revealed extensive edema, but the cause of death could not be definitely established. This part of the study was discontinued because the death of two cows indicated it was a dangerous procedure, and because of extreme variation in the results which were possibly due to the uncontrollable excitability of some cows. The variation in excitability of the cows and the large variation within each group for erythrocyte fragility suggested that the excitability of the cow might affect the percent hemolysis of erythrocytes, possibly through the release of epinephrine.

Another study was conducted with the effect of fasting and epinephrine on erythrocyte fragility of younger animals, to see whether this could be considered as a means of separating dwarf and non-dwarf calves. The fragility of erythrocytes in .60% and .64% saline of dwarf calves and non-dwarf calves seven to eight months of age prior to fasting, after fasting for 48 and 72 hours and 5 hours after injection of epinephrine following a 72 hour fast are found in Table XI. The calves used were the four dwarf calves and eight of the non-dwarf calves in the genotype study with the group of seven to eight months of age. Blood samples were collected from the calves before they were removed from feed and after 48 and 72 hours of fasting. After 72 hours of fasting, the calves were given an intravenous injection of .25 cc. epinephrine hydrochloride (1:1000) per 100 pounds body weight, and a blood sample taken 5 hours post-injection.

The percent hemolysis in .60% saline prior to fasting was 82.6% for non-dwarfs and 49.2% for dwarfs with a difference between the means of 33.4% ($P<.05$). After a 48 hour fast the differences between the means of dwarfs and non-dwarfs remained similar to before

TABLE XI PERCENT ERYTHROCYTE HEMOLYSIS OF NON-DWARF AND DWARF CALVES SEVEN TO EIGHT MONTHS OF AGE PRIOR TO FAST, FASTED FOR 48 AND 72 HOURS AND AFTER AN INTRAVENOUS INJECTION OF .25 cc. OF EPINEPHRINE (1:1000) PER 100 POUNDS FOLLOWING A 72 HOUR FAST

		Prior To Fasting		48 Hour Fast		72 Hour Fast		5 Hours After Injection of Epinephrine	
		Percent NaCl							
Group	No.	.60 %	.64 %	.60 %	.64 %	.60 %	.64 %	.60 %	.64 %
Non-Dwarf	8	82.6 (7.19)[a]	53.4 (14.42)	83.0 (7.47)	54.5 (16.69)	75.1 (15.90)	44.7 (21.86)	69.0 (22.67)	44.1 (22.49)
Dwarf	4	49.2[b] (25.40)	25.3[b] (12.79)	52.7[b] (20.43)	22.6[b] (9.85)	49.6[c] (23.37)	19.9 (10.33)	37.0[c] (12.91)	20.4 (7.94)

[a]standard deviation
[b]different from non-dwarf group ($P < .05$)
[c]different from non-dwarf group ($P < .10$)

fast ($P<.05$), with mean values of 83.0% for non-dwarfs and 52.7% for dwarfs of percent hemolysis. After 72 hours of fast the differences in the mean values of 75.1% and 49.6%, respectively, in .60% saline for non-dwarfs and dwarfs were not as great as before fasting or after 48 hours fast ($P<.10$). Five hours after injection of epinephrine following a 72 hour fast the mean values were 69.0% for non-dwarfs and 37.0% for dwarfs. Again the differences were not as large as they were before the animals were fasted (P .10). This indicates that the use of a stressor agent such as fasting or epinephrine failed to increase the differences in percent hemolysis between dwarfs and non-dwarfs seven to eight months of age. After 72 hours of fast and after injection of epinephrine there was a decrease in the differences. The standard deviations were large for both groups, with especially large increases in standard deviations in the non-dwarf group following 72 hour fast and after injection of epinephrine.

These data were also analyzed by comparing the percent change in erythrocyte fragility from the initial non-fasted samples (Table XII). The tendency was for the non-dwarf to show increases in the percent hemolysis after fasting and after injection of epinephrine, while dwarf groups showed a decrease in the percent hemolysis. Using the percent hemolysis in .64% saline the percent change from non-fasted values was 54.3% for non-dwarfs and -12.2% for dwarfs, the difference was 66.5% ($P<.05$). After a 72 hour fast the percent change of erythrocyte fragility in .64% saline were 21.3% increase in hemolysis for non-dwarfs compared to -25.5% decrease in the hemolysis of dwarfs, or a difference of 46.8% ($P<.10$). The injection of epinephrine after a 72 hour fast eliminated much of the difference

TABLE XII CHANGES IN ERYTHROCYTE HEMOLYSIS OF FASTED DWARF AND NON-DWARF CATTLE SEVEN TO EIGHT MONTHS OF AGE INJECTED WITH .25 cc. EPINEPHRINE PER 100 POUNDS

		Percent Change from Initial Non-Fasted Erythrocyte Hemolysis					
		48 Hour Fast		72 Hour Fast		5 Hours after Injection of Epinephrine	
		Percent NaCl					
Group	No.	.60 %	.64 %	.60 %	.64 %	.60 %	.64 %
Non-Dwarf	8	14.6 (14.81)[a]	54.3 (30.53)	2.6 (16.06)	21.3 (31.20)	-6.4 (24.01)	19.1 (40.14)
Dwarf	4	-3.6 (8.41)	-12.2[b] (17.12)	-11.9 (14.64)	-25.5[c] (19.53)	-28.1 (19.30)	-15.6 (30.79)

[a]standard deviation
[b]different from non-dwarf group ($P < .05$)
[c]different from non-dwarf group ($P < .10$)

observed during fasting with changes from initial non-fasted values of 19.1% and -15.6%, respectively, in hemolysis of erythrocytes of non-dwarfs and dwarfs in .64% saline. The differences decreased between the dwarf and non-dwarf following injection of epinephrine and the standard deviation increased.

The standard deviations were extremely large in both groups. One reason for the large variation in the non-dwarf group was the fact that three individuals within this group showed decreases in the percent change of erythrocyte fragility similar to the dwarf group. Of these three showing a decrease one was a known carrier and the other two both possible carriers. The remaining five, also possible carrier calves, in the non-dwarf group, showed increases in the percent hemolysis from the initial non-fasted values with fasting and hormone treatment. This indicates the need for more information on younger calves regarding their genotype. If this information were available the calves could be more accurately classified and reduce variation within the non-dwarf classification.

Presented in Table XIII is the limited data of six calves one week or less of age injected with 20 U.S.P. units of ACTH per 100 pounds of body weight in an attempt to further divide the differences between genotypes. Since the genotypes of the calves were unknown the calves were classified by the x-ray method. The mean values of percent hemolysis for the B x-ray group in .52% saline were 28.2% prior to injection, 30.1% two hours after injection and 28.3% four hours after injection of ACTH. No significant changes occurred in the percent hemolysis between or within the B x-ray or C x-ray group following ACTH, indicating the procedure on these limited data was

TABLE XIII PERCENT ERYTHROCYTE HEMOLYSIS OF B X-RAY AND C X-RAY CALVES ONE WEEK OR LESS OF AGE INJECTED WITH 25 U.S.P. UNITS OF ACTH PER 100 POUNDS OF BODY WEIGHT

		Non-treated		Two hours after injection of ACTH		Four hours after injection of ACTH	
		Percent NaCl					
Group	No.	.52 %	.56 %	.52 %	.56 %	.52 %	.56 %
B x-ray	4	28.2 (10.28)[a]	17.3 (6.48)	30.1 (10.42)	21.4 (8.40)	28.3 (10.60)	18.1 (7.27)
C x-ray	2	52.4 (13.79)	39.6 (15.70)	52.8 (25.66)	35.8 (22.99)	51.2 (19.94)	32.2 (14.36)

[a]standard deviation

of no value in further dividing the two groups. Contrary to work with these calves, Massey et al. (1958) found a significant increase in the difference between pedigree clean and known carrier cows for erythrocyte fragility following injection of ACTH. A possible reason for the difference in response following ACTH might be the ages of the cattle utilized in the studies. Massey et al. (1958) used mature cows compared to the calves one week or less of age in this study. Also, the level of ACTH injected might have differed, since no dosage levels were reported in the article by Massey et al. (1958).

General Discussion

These data indicate rather clearly that age is a very important factor affecting erythrocyte fragility. The erythrocytes of calves at birth seem to be very resistant to osmotic fragility, however, this is a rapidly passing phase, as calves at one to two months are less resistant, with a further decreased resistance to hypotonic saline of calves at seven to eight months. After the high point at seven to eight months in percent hemolysis of erythrocytes there is a gradual increase in resistance of erythrocytes to osmotic fragility to relatively constant levels after three years of age. These data agree closely with the other studies of the effect of age on erythrocyte fragility (Holman, 1956; Greatorex, 1954).

The study of the relation between genotype and erythrocyte fragility indicates that differences between genotypes is dependent upon the age of the animal. There were non-significant differences between known carrier cows and pedigree clean cows three to nine years of age. The tendency, however, in all saline solutions was for the known carriers to have a higher percent of erythrocyte hemolysis.

Heifers 18 months of age showed a similar trend with the dwarfs having a higher percent of erythrocyte hemolysis than non-dwarfs, but again this difference was not significant. Younger animals, however, showed an opposite trend with the erythrocytes of seven to eight months old dwarfs being more resistant to osmotic hemolysis than were the erythrocytes of non-dwarfs.

The reasons for the change in erythrocyte hemolysis between 18 months old animals and those seven to eight months of age is not clear. This trend was seen in all younger groups with the exception of the three months old group. In the one to two months and one week or less age groups the erythrocytes of known carriers were found to be more resistant than were those of the pedigree clean calves, the differences being highly significant in the youngest age group. In the three months age group, which did not conform to the pattern of the other young age groups, calves predicted to be clean of dwarfism on the basis of their lumbar x-rays were more resistant to erythrocyte hemolysis than were calves predicted to be carriers. However, it should be pointed out that only a limited number of calves were studied, and the differences were not clear cut.

The highly significant differences observed in the fragility of the erythrocytes of known carrier and pedigree clean calves at one week or less of age suggest the need for further study. The accuracy obtained in this study indicates that the fragility of erythrocytes at one week or less of age should be considered as a method of identifying carriers of the snorter dwarf gene. However, extensive research under widely differing environmental conditions must be made before there can be a clear evaluation of this procedure.

Neither fasting or the injection of epinephrine increased the differences in erythrocyte fragility. The injection of ACTH into calves one week or less of age likewise did not increase the differences observed between pedigree clean and known carrier calves. These results indicate that none of the stress techniques, as used in this study, offer any promise as a diagnostic test.

Other Hematological Studies

In addition to the erythrocyte fragility studies several other hematological studies were made on the young calves available in 1959. These studies included hematocrit (packed cell volume), hemoglobin, erythrocyte numbers, mean corpuscular volume (M.C.V.), mean corpuscular hemoglobin (M.C.H.), mean corpuscular hemoglobin concentration (M.C.H.C.), leucocyte differential counts, specific gravity of plasma, and electrophoretic analysis of plasma protein. Not all observations were made on all calves because of time limitations. Studies were made on a total of 59 calves from Projects 873 and 670 that were one week or less of age, divided on the basis of genotype into 21 pedigree clean, 25 possible carriers, 8 known carriers, and 5 comprest calves. There were 25 calves ranging from one month to two months of age divided into 16 pedigree clean, five possible carrier, and four known carrier calves. Studies were also carried on 12 calves that were three months of age, and which were possible carriers since their mothers were known carrier cows from Project 873. Leucocyte differential counts were also reported for nine older animals that received intravenous injections of insulin. This group included four dwarfs varying in ages from one and one-half to two and one-half years of age and five non-dwarfs, three of which were one year of age and two were mature cows.

Hematocrit

Table XIV compares the hematocrit values of pedigree clean, possible carrier, known carrier, and comprest calves of varying ages. At one week or less of age the hematocrit values were higher for pedigree clean than known carriers ($P<.10$), with values of 35.7% for pedigree clean and 32.6% for known carriers. The mean hematocrit value of 33.7% for possible carriers was intermediate to the known carriers and to the pedigree clean groups. The comprest calves had a mean hematocrit value of 34.8% which was higher than the possible carrier calves but slightly less than the pedigree clean calves.

Calves one to two months of age show a trend similar to calves one week or less of age although the differences were smaller. The pedigree clean calves had a slightly higher hematocrit (38.8%) than known carriers (37.8%). The mean values for possible carrier calves (38.2%) was again intermediate to known carriers and pedigree clean for the hematocrit mean values.

The age group consisting of calves three months of age was divided on the basis of x-ray since other information was not available to accurately determine their genotype. The C x-ray group, calves predicted clean, had a mean hematocrit value of 40.4% as compared to the mean value of 38.8% for the B x-ray group which included those calves predicted to be carriers on the basis of their lumbar x-rays. The trend at this age is similar to that seen at younger ages if one assumes the x-ray technique is fairly accurate in identifying clean and carrier calves.

Although no dwarfism genotype studies of hematocrit values have been made on young calves, these results might be compared to studies

on older cattle. Fransen (1955) reported 41.3% for controls versus 36.7% for dwarfs, while Cornelius et al. (1956) gave a value of 38.1% for the dwarfs. As might be expected these hematocrit values were slightly higher for the older cattle than for calves, however, they found the mean values for dwarfs in general to be lower than for non-dwarfs.

Comparison of the means of the three different age groups (Table XIV) shows that calves at birth have a lower hematocrit value than do older calves ($P<.005$). The mean hematocrit value for very young calves of 34.2% increases to 38.5% for the one to two months age group and further increases to a mean of 39.5% in the three months age group. Holman (1956) reported a value of 42% at birth which decreased to 35.0% a few days after birth and remained constant through 24 months of age. Greatorex (1954) also reported a mean value of nearly 50.0% at birth, with decreasing values from 28 weeks to one year of age. The data presented here disagrees with the works of Holman (1956) and Greatorex (1954) in that the hematocrit values increased from birth to three months. Although it is not clear why the mean of calves at one week or less of age in this study was so low, it suggests that some calves were anemic or bordering on an anemic condition. The higher mean values at one to two months and three months indicate the calves were anemic for a short period of time.

These data indicate that only small differences exist between pedigree clean and known carrier calves in hematocrit values. However, there is a tendency in all age groups for the pedigree clean and those predicted clean on x-ray to have slightly higher values than the known carrier and B x-ray calves. This agrees with research

TABLE XIV HEMATOCRIT, HEMOGLOBIN, AND ERYTHROCYTE VALUES FOR PEDIGREE CLEAN, POSSIBLE CARRIER, KNOWN CARRIER, AND COMPREST CALVES AT VARIOUS AGES

Group	Hematocrit %		Hemoglobin gm./100 ml.		Erythrocyte No. million/mm^3	
One Week or Less						
Pedigree Clean	35.7 (3.84)[a]	(21)[b]	10.8 (1.07)	(19)	7.09 (0.862)	(17)
Possible Carriers	33.7 (5.45)	(25)	9.8 (1.68)	(24)	6.50 (1.300)	(13)
Known Carriers	32.6[c] (4.22)	(8)	10.0 (2.01)	(7)	6.87 (1.080)	(7)
Comprest	34.8 (4.32)	(5)	10.8 (1.15)	(4)	6.32 (0.425)	(3)
Group Average	34.2[d] (4.73)	(59)	10.3[e] (1.53)	(54)	6.80[f] (1.062)	(40)
One to Two Months						
Pedigree Clean	38.8 (2.80)	(16)	12.2 (0.91)	(16)	8.21 (1.121)	(16)
Possible Carriers	38.2 (3.49)	(5)	11.9 (1.04)	(5)	8.43 (0.803)	(5)
Known Carriers	37.8 (5.44)	(4)	11.5 (3.16)	(4)	7.75 (0.702)	(4)
Group Average	38.5 (3.28)	(25)	12.0 (1.12)	(25)	8.18 (1.000)	(25)
Three Months						
C X-Ray	40.4 (5.54)	(6)	------		9.16 (1.309)	(6)
B X-Ray	38.8 (4.62)	(6)	------		8.74 (1.545)	(6)
Group Average	39.5 (4.93)	(12)	------		8.95 (1.382)	(12)

[a]standard deviation
[b]number of animals
[c]different from pedigree clean group ($P<.10$)
[d]difference between age groups ($P<.005$)
[e]different from calves of one week or less ($P<.001$)
[f]difference between age groups ($P<.01$)

on older animals where dwarfs have shown slightly lower mean hematocrit values from one week or less of age to three months, which suggests the need for recognition of the age factor and the control, or correction for it, especially in younger animals.

Hemoglobin

Table XIV presents the hemoglobin values of pedigree clean, possible carrier, known carrier, and comprest calves of one week or less of age and one to two months of age. The pedigree clean calves had a value of 10.8 grams of hemoglobin per 100 milliliters of blood compared to the known carrier group with a mean value of 10.0 grams of hemoglobin, a difference in the means of 0.8 of a gram. The mean of the possible carrier calves, 9.8 grams, was nearly the same as the known carriers, while the value for the comprest calves was 10.8 grams, identical with the value for the pedigree dean calves.

The mean hemoglobin levels of calves one to two months of age were 12.2 grams for pedigree clean and 11.5 grams for known carriers, a difference of 0.7 of a gram. The possible carrier group was intermediate to the means of the pedigree clean and known carriers with a mean of 11.9 grams of hemoglobin.

Cornelius et al. (1956) reported hemoglobin values for dwarfs of 11.7 grams which he stated were lower than values obtained from non-dwarf cattle. Fransen (1955) reported a mean hemoglobin level of 11.3 grams for dwarfs which was lower than the mean of 12.5 grams for the controls. Hafez et al. (1958) gave no data but indicated a lower hemoglobin level was observed in dwarfs than in the controls. Although the animals in these reports were of an older age, the same trend was reported for dwarfs as was observed in the known carriers in this study. In general the known carriers and dwarfs

show slightly lower hemoglobin values than do pedigree clean calves and non-dwarf cattle.

The mean hemoglobin level of all calves at one week or less was 10.3 grams compared to 12.0 grams at one to two months, the difference was 1.7 grams of hemoglobin ($P < .001$). Holman (1956) reported 13.0 grams of hemoglobin at birth which decreased to 11.0 grams at one week and remained constant to 24 months of age. Greatorex (1954) found high levels of hemoglobin at birth which decreased during the first few weeks of age. The data presented in this study indicates an increase in hemoglobin values from one week or less of age to one to two months of age, while the work of Greatorex (1954) and Holman (1956) showed a decrease in hemoglobin from birth. Comparison of the hemoglobin value of 11.0 grams at one week of age (Holman, 1956) with the mean value of 10.3 grams in this study, indicates that the mean value in this study is within the normal range. However, some calves had values of six or seven grams of hemoglobin at one week or less of age, which borders on an anemic condition. This low hemoglobin values of a few calves at birth, which increased markedly by one to two months of age, is probably an explanation for the increase in hemoglobin values, within the first few months, that was observed in this study.

The genotype studies suggest small differences between pedigree clean and known carrier calves with a tendency for the pedigree clean to have a higher hemoglobin readings than known carriers. Other studies indicate a similar trend with dwarfs having a lower mean than non-dwarfs. The age study indicates an increase in hemoglobin level of the blood from one week or less of age to one to two months of age.

Erythrocyte Number

The erythrocyte numbers of pedigree clean, possible carrier, known carrier, and comprest calves of various ages are compared in Table XIV. At one week or less of age the pedigree clean calves had a mean value of 7.09 million erythrocytes per cu. mm., compared to 6.87 million erythrocytes for known carriers, or a difference of 0.22 of a million. The possible carriers had a mean of 6.50 million erythrocytes, lower than either the pedigree clean or known carrier group. The comprest calves had the lowest value for erythrocyte number with a mean of 6.32 million per cu. mm. The standard deviations were large within groups, especially in the possible carrier and known carrier groups. At one to two months of age the pedigree clean calves had a value of 8.21 million erythrocytes compared to 7.75 million for the known carriers. The difference was 0.46 million, but not significant because of the large variation within groups. The possible carrier calves at one to two months of age had the highest mean value of 8.43 million erythrocytes per cu. mm. The C x-ray calves three months of age had a mean of 9.16 million erythrocytes per cu. mm., compared to 8.74 million erythrocytes for B x-ray calves. The differences were small with large standard deviations for both the C x-ray and B x-ray groups.

Erythrocyte studies of dwarfs by Fransen (1955) reported 8.1 million erythrocytes for dwarfs as compared to 9.7 million for controls. Cornelius et al. (1956) reported a value of 9.8 million erythrocytes per cu. mm. for dwarfs ranging in age from six days to 14 months. Both authors found the dwarfs to have lower erythrocyte number than non-dwarfs, the differences were small and non-significant.

In this study small differences were also found between known carrier and pedigree clean calves with the pedigree clean calves having higher values.

The comparison of the calves at different ages for erythrocyte number shows a value of 6.80 million erythrocytes per cu. mm. at one week or less of age which increased to 8.18 million at one to two months and further increased to 8.95 million at three months of age. This is a difference of 2.15 million erythrocytes for calves at one week or less of age compared to calves at three months ($P < .01$). Albritton (1952) in a review of blood values gave a value of 6.8 million erythrocytes at birth, while Greatorex (1954) reported 7.4 million erythrocytes at birth which increased to 8.1 million at 8-12 weeks. Holman (1956) reported 8.0 million erythrocytes at birth with no consistent change within the first few weeks after birth. The data presented here agrees with the studies of Greatorex (1954) and Albritton (1952) in which the erythrocyte numbers are lower at birth and increase the first weeks after birth.

The difference in erythrocyte number between pedigree clean and known carrier calves at all ages was small and non-significant. However, the pedigree clean calves had higher erythrocyte number at all ages studied. The age study of erythrocyte number indicated increasing number of erythrocytes after one week or less of age. These differences were significant and suggest that age should be considered as a factor affecting erythrocyte number.

Mean Corpuscular Volume

The values for mean corpuscular volume of various ages are reported in Table XV. The mean value for mean corpuscular volume at

TABLE XV MEAN CORPUSCULAR VOLUME, MEAN CORPUSCULAR HEMOGLOBIN, AND MEAN CORPUSCULAR HEMOGLOBIN CONCENTRATION VALUES FOR PEDIGREE CLEAN, POSSIBLE CARRIER, KNOWN CARRIER, AND COMPREST CALVES AT VARIOUS AGES

Group	Mean Corpuscular Volume Cu. Microns		Mean Corpuscular Hemoglobin Micro-Micrograms		Mean Corpuscular Hemoglobin Concentration %	
One Week or Less						
Pedigree Clean	52.7 (7.83)[a]	(17)[b]	15.7 (2.36)	(15)	30.6 (1.57)	(19)
Possible Carriers	50.7 (4.42)	(13)	15.0 (1.27)	(12)	29.5 (2.18)	(24)
Known Carriers	46.5[c] (3.54)	(7)	13.9 (1.54)	(6)	30.3 (2.81)	(7)
Compest	56.8 (5.41)	(3)	17.3 (2.40)	(2)	30.0 (1.69)	(4)
Group Average	51.3[d] (6.48)	(40)	15.2 (2.00)	(35)	30.0[d] (2.06)	(54)
One to Two Months						
Pedigree Clean	47.9 (6.07)	(16)	15.0 (1.84)	(16)	31.5 (1.10)	(16)
Possible Carriers	45.6 (5.38)	(5)	14.3 (2.27)	(5)	31.3 (1.78)	(5)
Known Carriers	49.0 (8.21)	(4)	14.9 (2.72)	(4)	30.4 (1.02)	(4)
Group Average	47.6 (6.13)	(25)	14.9 (1.99)	(25)	31.3 (1.25)	(25)
Three Months						
C X-Ray	45.8 (6.79)	(6)	------		------	
B X-Ray	44.8 (5.51)	(6)	------		------	
Group Average	45.4 (5.92)	(12)	------		------	

[a]standard deviation
[b]number of animals
[c]different from pedigree clean group ($P<.10$)
[d]difference between age groups ($P<.01$)

one week of age or less was 52.7 cubic microns for pedigree clean and 46.5 cubic microns for known carrier calves, the difference was 6.2 cubic microns ($P<.10$). The mean of the possible carriers was intermediate to the means of known carriers and pedigree clean calves, the value was 50.7 cubic microns. The comprest calves had a mean of 56.8 cubic microns, the highest mean value of any group. The calves at one to two months of age show a higher value for mean corpuscular volume for known carriers than pedigree clean, which is opposite to what is seen in calves at one week or less of age. The mean for pedigree clean calves was 47.9 cubic microns and for known carriers was 49.0, a difference of only 1.1 cubic microns. Calves at three months had values of 45.8 cubic microns for the C x-ray group and 44.8 cubic microns for B x-ray calves, the difference was 1.0 cubic microns, which was non-significant. These data indicate no consistent difference between pedigree clean and known carrier calves. At one week or less of age the pedigree clean calves had a higher value for mean corpuscular volume than known carriers; however, at one to two months of age the known carriers had a slightly higher mean while at three months the C x-ray group, those predicted clean, had a mean which was one cubic micron larger than the B x-ray calves.

The averages of the three different age groups indicated that as the calves increased in age there was a decrease in mean corpuscular volume ($P<.01$). Calves one week or less had a group average of 51.3 cubic microns which decreased to 47.6 cubic microns at one to two months and further decreased to 45.4 cubic microns at three months of age. Holman (1956) reported corpuscular size at birth averaged 44.9 cubic microns, which decreases in size after birth to

30.8 cubic microns at two months of age. The data presented here also indicate a decrease of mean corpuscular volume to two months of age, however, the values reported in this study are higher than those reported by Holman (1956). An explanation for this is the higher erythrocyte number reported by Holman (1956). Since this is a calculated value with the erythrocyte number in the denominator, any increase in erythrocyte number would decrease the mean corpuscular volume.

The genotype studies indicate no consistent or significant difference between pedigree clean and known carriers for mean corpuscular volume. As calves increase in age from birth there is a significant decrease in mean corpuscular volume.

Mean Corpuscular Hemoglobin

The values for mean corpuscular hemoglobin of pedigree clean, possible carrier, known carrier, and comprest calves of one week or less and one to two months of age are found in Table XV. The means were similar for the pedigree clean and known carrier calves with mean values of 15.7 micro-micrograms for pedigree clean and 13.9 micro-micrograms for possible carriers. The possible carriers have a mean value of 15.0 micro-micrograms, intermediate to known carriers and pedigree clean calves. The value for mean corpuscular hemoglobin for comprest calves was 17.3 micro-micrograms the highest mean value for any group.

The means were nearly identical for pedigree clean and known carriers at one to two months of age. The pedigree clean calves had a value of 15.0 micro-micrograms while the known carriers had an almost identical mean of 14.9 micro-micrograms. The possible carriers

had the lowest mean corpuscular hemoglobin at one to two months, 14.3 micro-micrograms. No other research has been conducted on the relation of dwarfism to mean corpuscular hemoglobin except the study here.

The difference between the average of the calves at one to two months and one week or less of age was small and non-significant. The mean corpuscular hemoglobin at one week or less of age was 15.2 micro-micrograms as compared to 14.9 micro-micrograms at one to two months of age. This indicates little change in mean corpuscular hemoglobin in the first few weeks after birth. The only reported value in the literature for calves at birth was 14.1 micro-micrograms (Albritton, 1952). This would indicate the data presented here to be in the normal range, however, the values in this study are slightly higher.

The data here indicate no consistent or significant differences between pedigree clean and known carrier calves in mean corpuscular hemoglobin. No significant trend was found between calves one week or less of age as compared to calves one to two months of age for mean corpuscular hemoglobin.

Mean Corpuscular Hemoglobin Concentration

The mean values of pedigree clean, possible carrier, known carrier, and comprest calves of one week of age or less and one to two months of age for mean corpuscular hemoglobin concentration are reported in Table XV. The means were similar for all genotypes in the youngest age group with values of 30.6% for pedigree clean calves, 29.5% for possible carriers, 30.3% for known carriers, and 30.0% for comprest calves. The mean corpuscular hemoglobin concentration values

at one to two months of age were similar for all groups, the means were 31.5% for pedigree clean calves, 31.3% for possible carriers, and 30.4% for known carriers. Since these values were so similar no significant differences were found between pedigree clean and known carrier calves.

Comparison of the averages for the two age groups shows an increase in mean corpuscular hemoglobin concentration from one week or less of age with a mean of 30.0% to a mean of 31.3% at one to two months of age ($P<.01$). Although the difference was only 1.3%, because of the small standard deviations the increase in mean corpuscular hemoglobin concentration was significant. Greatorex (1954) found little variation in mean corpuscular hemoglobin concentration at birth while Holman (1956) reported an average of 31% from birth to 24 months of age. The values and variation of these data compare favorably with those of Holman (1956) and Greatorex (1954).

The mean corpuscular hemoglobin concentration in these data showed little variation within genotype or age groups. No significant differences were found between pedigree clean and known carriers in this study for mean corpuscular hemoglobin concentration. Comparison of calves one week or less of age with calves one to two months revealed a significant increase in mean corpuscular hemoglobin.

Specific Gravity of Plasma

Specific gravity of plasma was measured in this study as a factor involved in the differences in plasma protein, since protein is the main factor affecting the specific gravity of plasma. Therefore, if differences existed in the specific gravity of plasma between genotypes the amount of protein might be a factor causing the differences

in erythrocyte fragility between pedigree clean and known carrier calves. Table XVI compares the specific gravity of plasma of pedigree clean, possible carrier, known carrier, and comprest calves one week or less of age and one to two months of age. Calves one week or less of age showed similar means for all groups with values of 1.0238 for pedigree clean calves and 1.0240 for known carrier calves. The possible carrier group had the highest mean value of 1.0254, while the comprest calves had a mean of 1.0242, similar to the known carrier calves. At one to two months of age the group means were also similar with values of 1.0233 for pedigree clean calves and 1.0228 for known carrier calves. The fact that the means were similar for known carriers and pedigree clean calves at both ages indicates that no real difference exists between the genotypes for specific gravity of plasma. This is confirmed by the possible carrier group, which at one week or less of age had the highest mean value for specific gravity of any group. The mean for this group should be intermediate if a real difference existed between the known carriers and pedigree clean calves, and if the possible carrier group contained calves of both genotypes, as would be expected.

Comparison of the averages of the two age groups for specific gravity of plasma gives values of 1.0245 for calves one week or less of age and 1.0232 for calves one to two months of age, the difference was .0013 ($P < .025$). This indicates a significant decrease in specific gravity of plasma from one week or less of age to one to two months of age.

The dwarfism genotype studies of specific gravity of plasma indicate non-significant differences between pedigree clean and known

TABLE XVI SPECIFIC GRAVITY OF PLASMA OF PEDIGREE CLEAN, POSSIBLE CARRIER, KNOWN CARRIER, AND COMPREST CALVES OF ONE WEEK OR LESS AND ONE TO TWO MONTHS OF AGE

Group	One week or less of age	One to two months of age
Pedigree Clean	1.0238 (21)[b] (0.0016)[a]	1.0233 (16) (0.0007)
Possible Carrier	1.0254 (21) (0.0029)	--------
Known Carrier	1.0240 (8) (0.0019)	1.0228 (4) (0.0001)
Comprest	1.0242 (5) (0.0027)	--------
Group Average	1.0245 (55) (0.0024)	1.0232[c] (25) (0.0003)

[a]standard deviation
[b]number of animals
[c]different from one week or less of age ($P < .025$)

carrier calves. The difference between calves one week or less of age and those calves at one to two months of age indicated a significant decrease in specific gravity of plasma; therefore, the age factor should be considered in studies with specific gravity of plasma.

Leucocyte Differential Counts

The values reported in Table XVII are the leucocyte differential counts of pedigree clean, possible carrier, known carrier, and comprest calves. These data were collected from 24 calves one week or less of age, divided on the basis of genotype into 13 pedigree clean, 6 possible carriers, 3 known carriers, and 2 comprest calves. No significant differences were found between pedigree clean and known carriers for any of the differential leucocytes types. The means for the pedigree clean calves were 34.2% lymphocytes, 47.9% segmented neutrophils, 11.5% unsegmented neutrophils, 59.4% total neutrophils, 3.0% monocytes, 0.6% eosinophils, and 3.0% undifferential cells compared to known carrier means of 45.3% for lymphocytes, 30% segmented neutrophils, 12.7% nonsegmented neutrophils, 42.7% total neutrophils, 5.3% monocytes, 2.0% eosinophils, and 5.0% undifferentiated cells. Large standard deviations were found within groups for most types of leucocytes. The means of the possible carrier group fell between the means for pedigree clean and known carrier for all types of cells. The values for differential counts of calves in the comprest group were not consistently similar with any of the groups in this study.

The tendency in this study was for the pedigree clean calves to have a higher percent of neutrophils and a lower percent of lymphocytes than the known carriers. It is difficult to make a comparison with other studies because of the difference in ages of cattle studied.

TABLE XVII LEUCOCYTE DIFFERENTIAL COUNTS OF PEDIGREE CLEAN, POSSIBLE CARRIER, KNOWN CARRIER, AND COMPREST CALVES OF ONE WEEK OR LESS OF AGE

Group	No.	Lymphocytes %	Segmented Neutrophils %	Non-Segmented Neutrophils %	Total Neutrophils %	Monocytes %	Eosinophils %	Undifferentiated Cells %
Pedigree Clean	13	34.2 (15.84)[a]	47.9 (16.32)	11.5 (6.27)	59.4 (18.43)	3.0 (3.58)	0.6 (0.60)	3.0 (1.79)
Possible Carriers	6	38.8 (11.20)	46.0 (12.95)	8.0 (2.83)	54.0 (12.87)	4.5 (3.02)	0.7 (0.51)	2.0 (1.67)
Known Carriers	3	45.3 (17.39)	30.0 (9.85)	12.7 (8.33)	42.7 (9.07)	5.3 (4.73)	2.0 (1.73)	5.0 (3.61)
Comprest	2	25.0 (12.73)	55.0 (21.21)	7.5 (0.71)	62.5 (21.92)	6.5 (6.36)	1.5 (0.71)	4.5 (3.54)

[a]standard deviation

The work of Cornelius et al. (1956), with dwarfs ranging in ages from one week to 14 months of age, reported 55.6% for lymphocytes and 33.3% for neutrophils as compared to 68% lymphocytes and 23.7% neutrophils for controls. The difference between normal and dwarf cattle in percent of neutrophils and lymphocytes was highly significant (Cornelius et al., 1956). This shows an opposite trend in lymphocyte and neutrophil percent between this study and the work of Cornelius et al. (1956). A possible explanation is the difference in ages of the cattle studied, since at young ages there is a big change in the leucocyte picture. The neutrophils are the largest percent of leucocytes at birth, and also a few days after birth, but this quickly passes into a lymphocyte dominated picture. This indicates that research dealing with leucocytes should be restricted to a limited age group rather than calves varying considerably in age.

Table XVIII compares the percent of the different types of leucocytes for dwarfs and non-dwarf cattle at time of injection of insulin and one and two hours after an intravenous injection of 36 units zinc insulin per 100 pounds of body weight. This level of injection was the one reported by Foley et al. (1956) and was the one recommended in the "insulin test" for dwarfism. This study was limited by the availability of dwarf test animals. Data are reported on dwarfs varying in age from one and one-half to two and one-half years of age, and a non-dwarf group of five animals, three of which were one year of age and two mature cows.

No significant differences were found between dwarfs and non-dwarfs for the different types of leucocytes. The dwarf group had

TABLE XVIII LEUCOCYTE DIFFERENTIAL COUNTS OF DWARFS AND NON-DWARFS INJECTED WITH INSULIN

Type of Leucocyte	Non-Treated		1 Hour After Injection of Insulin		2 Hours After Injection of Insulin	
	Dwarfs	Non-Dwarfs	Dwarfs	Non-Dwarfs	Dwarfs	Non-Dwarfs
No.	4	5	4	5	4	5
Lymphocytes	63.0 (3.86)[a]	77.4 (12.99)	54.0 (11.37)	69.8 (16.75)	64.0 (10.95)	64.6 (19.73)
Neutrophils	30.0 (6.27)	16.8 (12.11)	34.0 (6.48)	23.0 (13.13)	30.5 (9.26)	30.2 (10.67)
Monocytes	4.8 (3.77)	3.8 (0.84)	2.8 (2.87)	3.6 (2.19)	2.0 (1.58)	1.6 (0.55)
Eosinophils	2.2 (0.96)	2.0 (1.23)	5.5 (3.70)	2.6 (2.08)	2.2 (2.22)	1.4 (0.99)
Non-Segmented Granulocytes	0.2 (0.00)	0.8 (1.30)	3.0 (1.64)	1.4 (2.19)	1.0 (1.53)	1.0 (2.78)

[a]standard deviation

means of 63.0% and 30.0%, respectively, for lymphocytes and neutrophils as compared to means of 77.4% and 16.8% for non-dwarfs before the injection of insulin. The increased percent of lymphocytes and decreased neutrophil percent for non-dwarfs was not significantly different from the dwarf group because of the large standard deviations within groups. The values for monocytes, eosinophils, and non-segmented grandulocytes are very similar between the groups. Comparison of lymphocytes and neutrophils one hour after injection of insulin shows means of 54.0% and 34.0%, respectively, for dwarfs compared to 69.8% and 23.0% for non-dwarfs. One hour after the injection of insulin the differences between the means of non-dwarfs and dwarfs are less than at injection time and the standard deviations also increased. The means for lymphocytes and neutrophils two hours after injection were 64.0% and 30.5% for dwarfs and 64.6% and 30.2% for non-dwarfs which were nearly identical and not significantly different. Monocytes, eosinophils, and non-segmented granulocytes showed no consistent or significant changes or differences between dwarfs and non-dwarfs. This agrees with the work of Fransen (1955) and Deyoe et al. (1957), in which no significant differences were found in the type of leucocyte between dwarfs and non-dwarfs. However, there was a tendency for the dwarfs to have higher values for neutrophils and lower values for lymphocytes than non-dwarfs which agrees with the work of Cornelius et al. (1956), and Fransen (1955). The large variation within the dwarf and non-dwarf groups indicates the need for animals of similar age. The injection of insulin not only failed to increase the difference in types of leucocytes but decreased the difference between dwarf and non-dwarfs.

The limited animals which were studied in these two experiments indicated large variation within genotypes for leucocyte differential counts and non-significant differences between dwarfs and non-dwarfs, and between pedigree clean and known carrier calves. Although age must be considered as an important source of variation, there is need for control of other sources of variation as health of animal, level of nutrition, stress conditions, and refinement in the techniques used in leucocyte counts.

Electrophoretic Analysis of Plasma Proteins

This study indicated that it was not possible to accurately separate each type of plasma protein because of the overlaps within fractions; therefore, values are reported for five distinct fractions designated by numbers. The identification of each fraction was made by comparison with known plasma protein fractions of mature cattle conducted under exactly the same conditions. By this comparison fraction I was composed of albumin, fraction II was alpha-one globulin, fraction III was primarily alpha-two globulin with some beta globulin, fraction IV was primarily beta globulin and fibrinogen with small quantity of gamma globulin, and fraction V was gamma globulin. The area of each fraction was determined by separating the plasma proteins on the strip at the low points between each peak and with an integrator determine the area under each peak. Each fraction is expressed as a percent of the total protein on the strip to eliminate the error due to quantity of plasma placed on the strip.

Table XIX compares the electrophoretic analysis of pedigree clean, possible carrier, known carrier, and compresst calves of one week or less of age. Data are reported on 36 calves divided on the basis of

TABLE XIX PLASMA PROTEIN ANALYSIS OF PEDIGREE CLEAN, POSSIBLE CARRIER, KNOWN CARRIER, AND COMPREST CALVES OF ONE WEEK OR LESS OF AGE

Group	No.	Fraction I %	Fraction II %	Fraction III %	Fraction IV %	Fraction V %
Pedigree Clean	15	28.5 (2.09)[a]	26.8 (3.83)	18.2 (1.87)	19.0 (3.29)	7.7 (3.11)
Possible Carrier	13	26.9 (2.03)	25.0 (3.57)	17.8 (2.16)	21.5 (3.83)	8.8 (4.30)
Known Carrier	6	29.6 (3.55)	29.0 (3.10)	16.7 (0.92)	19.0 (5.09)	5.6 (2.06)
Comprest	2	33.4 (3.11)	22.3 (2.83)	13.0 (0.57)	21.3 (2.12)	4.9 (3.25)

[a]standard deviation

genotype into 15 pedigree clean, 13 possible carrier, six known carrier, and two compress calves. The plasma protein patterns of calves one week or less of age were very consistent between animals as indicated by the small standard deviations. No significant differences were found between pedigree clean and known carriers for any of the plasma protein fractions. The values for fraction II are 26.8% for pedigree clean, 25.0% for possible carriers, 29.0% for known carriers, and 22.3% for compress which indicates the small differences between means. The possible carrier calves showed no consistent pattern in relation with known carriers and pedigree clean calves, being in some cases higher, lower, and intermediate to these groups. Compress calves had the highest value of 33.4% for fraction I and the lowest mean value of 4.9% for fraction V.

Further comparison of plasma proteins made on pedigree clean and known carrier calves one to two months of age are found in Table XX. This study included nine pedigree clean and five known carrier calves. Plasma protein fractions I, II, IV, and V were similar between pedigree clean and known carrier calves while fraction III was different ($P < .10$). The mean values for pedigree clean were 35.5%, 23.1%, 19.8%, 17.4%, and 4.3%, respectively, for fraction I, II, III, IV, and V while the values for known carriers were 35.6%, 23.0%, 17.6%, 18.3%, and 5.5%.

A comparison of plasma protein analysis of calves one week or less of age and calves one to two months of age is presented in Table XXI. Fraction I increased from a mean value of 28.4% for calves one week or less of age to a mean value of 35.4% at one to two months of age ($P < .001$). However, fraction II had decreased from a mean value of 26.3% to 23.2% at one to two months ($P < .01$). Fraction III mean value was 17.8% for

TABLE XX PLASMA PROTEIN ANALYSIS OF PEDIGREE CLEAN AND KNOWN CARRIER CALVES OF ONE TO TWO MONTHS OF AGE

Group	No.	Fraction I %	Fraction II %	Fraction III %	Fraction IV %	Fraction V %
Pedigree Clean	9	35.5 (2.80)[a]	23.1 (1.45)	19.8 (1.74)	17.4 (2.94)	4.3 (1.04)
Known Carrier	5	35.6 (1.36)	23.0 (1.16)	17.6[b] (1.21)	18.3 (2.02)	5.5 (1.50)

[a]standard deviation
[b]different from pedigree clean group ($P < .10$)

TABLE XXI PLASMA PROTEIN ANALYSIS OF CALVES ONE WEEK OR LESS OF AGE AND CALVES ONE TO TWO MONTHS OF AGE

Group	No.	Fraction I %	Fraction II %	Fraction III %	Fraction IV %	Fraction V %
One Week or Less	36	28.4 (2.81)[a]	26.3 (3.89)	17.8 (1.88)	20.0 (3.87)	7.6 (3.39)
One-Two Months	16	35.4[b] (2.31)	23.2[c] (1.73)	19.1[d] (1.83)	17.7[d] (2.71)	4.6[e] (1.29)

[a]standard deviation
[b]different from one week or less of age ($P<.001$)
[c]different from one week or less of age ($P<.01$)
[d]different from one week or less of age ($P<.05$)
[e]different from one week or less of age ($P<.005$)

calves at birth which increased to 19.1% at one to two months ($P < .05$). A mean of 20.0% for fraction IV of calves at birth decreased to 17.7% at one to two months of age ($P < .05$). Fraction V also decreased from a mean value of 7.6% for calves one week or less of age to 4.6% at one to two months ($P < .005$). Although the differences in plasma protein fractions were not large, they were significant because of the small amount of variation within age groups.

An attempt was also made to separate hemoglobin by using the plasma protein procedure since hemoglobin has been separated into fractions in humans (Block et al., 1958). Sixteen calves, eight of which were pedigree clean, four possible carriers, and four known carriers were used in this preliminary study. This aspect of the study was discontinued when no separation of the hemoglobin occurred. It was concluded that a study would be required to find a technique that would separate bovine hemoglobin.

The limited genotype studies of plasma proteins indicate small differences between pedigree clean and known carrier calves for the percent of each fraction of plasma proteins. Only in one instance, fraction III for calves one to two months of age, were the differences approaching significance between pedigree clean and known carrier calves. The calves within groups showed consistent patterns as indicated by the small standard deviations. Studies by Cornelius et al. (1956) and Hafez et al. (1958) support the findings here, as they found no consistent difference in plasma protein patterns between dwarfs and control animals. The age study of plasma protein patterns indicate that the age factor must be considered in analysis of plasma protein. The age factor can be controlled by studying within limited age brackets or by making correction for age differences.

Relationships Between Erythrocyte Fragility, Birth Weight and Other Hematological Measurements

The high relationship between the fragility of erythrocytes of calves one week or less of age and their genotype for dwarfism indicated this measurement showed promise as a possible diagnostic technique. Further studies were made to determine the correlations between erythrocyte fragility and other hematological measurements. It was hoped that if high correlations were found this might indicate the factors affecting the percent of erythrocyte hemolysis, and aid in explaining the mechanisms involved. It is possible that a fuller understanding of the mechanisms responsible for the differences in erythrocyte fragility might suggest another measurement that would be even more accurate in separating the genotypes. The correlations between erythrocyte fragility, birth weight and other hematological measurements are presented in Table XXII.

A positive correlation of .42 was found between erythrocyte fragility and hematocrit values ($P<.01$). This indicates that an increase in the fragility of the erythrocytes was associated with higher hematocrit values. Increases in hematocrit values are the result of either an increase in the volume of each cell, an increase in the total number of cells, or a combination of these two factors.

There was a positive correlation of .65 between erythrocyte number and the hematocrit values in calves one week or less of age. This correlation indicates that variations in hematocrit readings reflects,

TABLE XXII CORRELATIONS BETWEEN ERYTHROCYTE FRAGILITY, BIRTH WEIGHT, AND HEMATOLOGICAL MEASUREMENT OF CALVES ONE WEEK OR LESS OF AGE

	Erythrocyte Fragility in .56% Saline	Birth Weight
Birth Weight	-.18	--
Hematocrit	+.42[a]	+.10
Hemoglobin	+.03	+.14
Erythrocyte Number	-.09	+.23
Mean Corpuscular Volume	+.33[b]	--
Mean Corpuscular Hemoglobin	+.23	--
Mean Corpuscular Hemoglobin Con.	+.11	--
Specific Gravity of Plasma	+.20	--
Erythrocyte Fragility in .52% Saline	+.96[a]	--

[a](P<.01)
[b](P<.05)

to a large degree, differences in erythrocyte numbers. However, there was a small negative correlation of .09 between erythrocyte number and erythrocyte fragility. One would conclude that, although the number of erythrocytes is an important factor in determining the hematocrit values of an individual, it has little influence on erythrocyte fragility.

No direct measurements of erythrocyte volume were made, although the calculated value for mean corpuscular volume is an estimate of cell volume. There was a positive correlation of .33 between mean corpuscular volume and erythrocyte fragility ($P<.05$), which indicates that as the erythrocytes increase in volume there was a decrease in their ability to withstand hemolysis in saline solutions. This suggests that, in this study, the volume of the erythrocyte was a factor determining the resistance of the cell to hemolysis in hypotonic saline solutions. However, other unknown factors are involved since the coefficient of determination indicates that only 11% of the variation in erythrocyte fragility can be accounted for by mean corpuscular volume.

Several reports have suggested that the volume, and size of the erythrocytes are possible factors affecting the percent of erythrocyte hemolysis. Castle and Daland (1937) found a high correlation between the degree of hemolysis of human erythrocytes in hypotonic saline and the volume, diameter, and surface area of the erythrocyte. A study by Dacie *et al.* (1938), also with human erythrocytes, found that the ratio of mean corpuscular thickness to mean corpuscular diameter had a positive correlation of .86 with the percent erythrocyte hemolysis. Emerson *et al.* (1956) found that increased erythrocyte fragility

reflected the fact that the surface membrane of the erythrocyte is reduced in size relative to volume, with the result that its capacity to swell before reaching the rupture point is reduced.

The correlation between erythrocyte fragility and hemoglobin was only a positive .03. This small non-significant correlation indicates that the level of hemoglobin had little effect on the fragility readings. This suggests that in the determination of the percent hemolysis, the division of the optical density of the supernatant fluid by the optical density when all cells are hemolized and hemoglobin is in solution, has corrected for the differences in hemoglobin levels between animals. Although the level of hemoglobin had little correlation with erythrocyte fragility, it is possible that differences in the amount of hemoglobin per cell could affect the fragility readings. Two measures of the amount of hemoglobin per cell are the calculated values of mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration. The correlation between mean corpuscular hemoglobin and erythrocyte fragility was .23 and between mean corpuscular hemoglobin concentration and erythrocyte fragility .11. This indicates that the amount of hemoglobin per cell or the percent of hemoglobin in relation to hematocrit shows a higher association with the fragility of erythrocytes than the level of hemoglobin in the blood. This suggests that the amount of hemoglobin per cell may influence the overall picture of erythrocyte fragility. However, it is possible that these calculated values include not only hemoglobin, but also other factors, such as size of the cell, which influence the fragility.

The specific gravity of plasma has a non-significant positive correlation of .20 with erythrocyte fragility. Since specific gravity

of plasma is primarily determined by the amount of protein, this suggests that level of plasma protein shows a small relationship with the percent of erythrocyte hemolysis; therefore, as the amount of plasma protein increased, there was also a slight increase in the percent of erythrocyte hemolysis.

The highly significant positive correlation of .96 between the percent hemolysis measured in .56% saline and .52% saline indicates that erythrocyte fragility measured in either solution is equally as accurate. This suggests that in future studies, either solution could be as accurately used for testing erythrocyte fragility of calves one week or less of age.

Correlations were determined between some hematological measurements and birth weight to see if these measurements were related to weight. As shown in Table XXII, the correlations of birth weight to hematological measurements are as follows: .10 with hematocrit, .14 with hemoglobin, .23 with erythrocyte number, and -.18 with erythrocyte fragility.

The -.18 correlation between erythrocyte fragility and birth weight was discussed with the erythrocyte fragility studies and will not be further discussed here. Although these correlations are small and indicate in general little relationship with weight, observation of individual records indicates that calves light in weight also have low hematocrit, hemoglobin, and erythrocyte number. This indicates a higher relationship between weight and these hematological measurements in lighter calves. However, in larger calves there seems to be little relationship between weight and hemotocrit, hemoglobin values, and erythrocyte number. Although the correlations reported

here are relatively small, it is advisable to consider correcting hematological values for birth weight. This is especially true if valid comparisons are to be made between calves that vary widely in birth weights.

SUMMARY AND CONCLUSIONS

A study was conducted on 183 beef cattle to determine: (1) whether there was any relationship between hematological measurements and snorter dwarfism in beef cattle, and if a high relationship existed to investigate it further; (2) to study other factors which affect hematological measurements; and (3) whether one or more of the hematological measurements could be used as a method of identifying carrier animals.

Studies were conducted to compare the values of erythrocyte fragility, hemoglobin, hematocrit, erythrocyte number, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, specific gravity of plasma, electrophoretic analysis of plasma protein, and differential counts of known carrier, possible carrier, pedigree clean, and comprest calves. Age studies were also conducted with calves comparing values of one week or less, one to two months, and three months of age. Additional dwarfism and age studies were conducted on erythrocyte fragility of cows three to nine years of age, bulls 18 months of age, and calves seven to eight months of age. The effect of fasting and injection of epinephrine and ACTH on erythrocyte fragility was also studied. A limited study was conducted on leucocyte differential counts prior to injection, and one and two hours after injection of insulin.

The data indicated that:

1. There was a highly significant difference between different

age groups in erythrocyte fragility as measured in hypotonic saline solutions. Erythrocytes of calves one week of age or less were the most resistant of all age groups studied, while mature animals ranked second. The least resistant group was the calves seven to eight months of age.

2. There was a highly significant difference in erythrocyte fragility between genotypes at one week or less of age with known carrier calves being more resistant than pedigree clean calves. Differences in values for percent hemolysis in .56% saline, corrected for birth weight, was a highly accurate method of separating clean and carrier calves in this study. There was a 100% accuracy in predicting carriers and 95% accuracy in predicting cleans.
3. There was also a significant difference in erythrocyte fragility between genotypes at older ages. Known carrier calves at one to two months of age, and dwarfs at seven to eight months of age were more resistant to erythrocyte hemolysis than were pedigree clean and non-dwarf calves. The difference in erythrocyte fragility was not as large as for calves one week or less of age.
4. There was a tendency for C x-ray calves at three months of age, non-dwarfs at 18 months of age, and mature pedigree clean cows to be more resistant to erythrocyte hemolysis than B x-ray calves, dwarfs, and known carrier cows; however, the differences were not significant.

5. Subjecting cattle to the stress of fasting or injection of epinephrine or ACTH failed to increase differences in erythrocyte fragility between dwarfism genotypes.

6. There were increases in hematocrit, hemoglobin, erythrocyte number, and mean corpuscular hemoglobin concentration values with increasing age; a decrease in mean corpuscular volume and both increases and decreases in the different plasma protein fractions with increasing age. All of these age changes were highly significant. A significant decrease in specific gravity of plasma occurred with increasing age.

7. There were no significant differences between dwarfism genotypes in calves for hemoglobin, hematocrit, erythrocyte number, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, specific gravity of plasma, plasma protein fractions, and leucocyte differential counts.

8. There was no significant difference in leucocyte differential counts prior to injection of insulin or after injection in older cattle; however, there was a tendency for dwarfs to have a higher percent of neutrophils and lower percent of lymphocytes than non-dwarfs.

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A P P E N D I X

TABLE XXIII DESCRIPTION OF ANIMALS IN STUDY

Animal No.	Sex (M-F)	Breed	Genotype	Birth Weight (lbs.)
OK 9-40	F	Hereford	pedigree clean	69
OK 9-38	F	Hereford	pedigree clean	79
OK 9-33	F	Hereford	pedigree clean	71
OK 9-31	F	Hereford	pedigree clean	60
269	M	Angus	pedigree clean	73
259	F	Angus	pedigree clean	64
339	M	Angus	pedigree clean	64
309	M	Angus	pedigree clean	60
329	M	Angus	pedigree clean	60
OK 9-04	F	Hereford	pedigree clean	53
049	F	Angus	pedigree clean	54
OK 9-10	M	Hereford	pedigree clean	72
OK 9-05	F	Hereford	pedigree clean	68
OK 9-06	F	Hereford	pedigree clean	74
OK 9-08	M	Hereford	pedigree clean	60
OK 9-44	M	Hereford	pedigree clean	80
OK 9-48	M	Hereford	pedigree clean	90
OK 9-57	F	Hereford	pedigree clean	62
OK 9-51	M	Hereford	pedigree clean	83
OK 9-58	M	Hereford	pedigree clean	63
OK 9-55	F	Hereford	pedigree clean	86
T902	F	Hereford	known carrier	56
T904	F	crossbred(HXS)	known carrier	74
OK 9-29	M	Hereford	known carrier	63
OK 9-28	M	Hereford	known carrier	54
OK 9-46	M	Hereford	known carrier	64
OK 9-47	M	Hereford	known carrier	60
OK 9-43	F	Hereford	known carrier	66
OK 9-53	M	Hereford	known carrier	58
T949	M	crossbred(HXA)	known carrier	62
T951	F	crossbred(HXA)	known carrier	46
OK 9-11	M	Hereford	known carrier	60
T905	M	Hereford	possible carrier	74
T903	M	crossbred(HXA)	possible carrier	68
T907	F	Hereford	possible carrier	77
OK 9-34	M	Hereford	possible carrier	81
OK 9-24	F	Hereford	possible carrier	63
T933	F	crossbred(HXA)	possible carrier	62
OK 9-54	M	Hereford	possible carrier	81
289	M	Angus	possible carrier	68
T938	F	crossbred(HXA)	possible carrier	70
T937	F	crossbred(HXA)	possible carrier	59
T942	F	crossbred(HXA)	possible carrier	54
T935	M	crossbred(HXA)	possible carrier	75
T940	M	crossbred(HXA)	possible carrier	61
T936	F	crossbred(HXA)	possible carrier	70

TABLE XXIII (Continued)

Animal No.	Sex (M-F)	Breed	Genotype	Birth Weight (lbs.)
T939	F	Hereford	possible carrier	69
T941	M	Hereford	possible carrier	80
OK 9-60	F	Hereford	possible carrier	62
OK 9-64	M	Hereford	possible carrier	75
T944	F	Hereford	possible carrier	64
T945	M	Hereford	possible carrier	81
T943	M	crossbred(HXA)	possible carrier	68
529	F	Angus	possible carrier	73
T950	M	Hereford	possible carrier	94
T948	M	Hereford	possible carrier	72
OK 9-71	F	Hereford	possible carrier	64
549	F	Angus	possible carrier	76
OK 9-65	F	Hereford	possible carrier	65
OK 9-67	M	Hereford	possible carrier	86
T922	M	Hereford	possible carrier	66
OK 9-07	M	Hereford	comprest	59
OK 9-09	M	Hereford	comprest	60
OK 9-49	M	Hereford	comprest	72
OK 9-59	F	Hereford	comprest	76
OK 9-02	F	Hereford	comprest	68
T891	M	Hereford	possible carrier	
T888	M	Hereford	possible carrier	
T886	F	Hereford	possible carrier	
T887	M	crossbred(HXA)	possible carrier	
T890	F	crossbred(HXA)	possible carrier	
T880	M	crossbred(HXA)	possible carrier	
T872	M	Hereford	possible carrier	
T876	F	Hereford	possible carrier	
T889	M	Hereford	possible carrier	
T873	F	crossbred(HXA)	possible carrier	
T871	F	crossbred(HXA)	possible carrier	
T882	M	crossbred(HXA)	possible carrier	
T802	M	Angus	possible carrier	
T808	M	Angus	possible carrier	
T804	M	Angus	possible carrier	
T824	M	Angus	possible carrier	
T812	F	Angus	possible carrier	
T801	F	Angus	possible carrier	
T800	F	Angus	known carrier	
T817	F	Angus	possible carrier	
T805	F	Angus	possible carrier	
T848	M	Angus	possible carrier	
T857	F	Angus	possible carrier	
T838	F	Angus	possible carrier	
T895	F	Angus	dwarf	
T896	F	Angus	dwarf	

TABLE XXIII (Continued)

Animal No.	Sex (M-F)	Breed	Genotype	Birth Weight (lbs.)
T897	F	Angus	dwarf	
T869	M	Hereford	dwarf	
T813	F	Hereford	possible carrier	
T829	F	Hereford	possible carrier	
T840	F	Hereford	possible carrier	
T809	M	Angus	possible carrier	
T854	M	Hereford	possible carrier	
T851	M	Angus	possible carrier	
T860	F	Hereford	possible carrier	
T827	M	Angus	possible carrier	
T832	F	Hereford	possible carrier	
T806	F	Hereford	possible carrier	
T853	M	Angus	possible carrier	
T837	F	Hereford	possible carrier	
T823	F	Hereford	possible carrier	
T859	F	Angus	possible carrier	
T834	F	Angus	possible carrier	
261	F	grade Angus	probably clean	
474	F	grade Angus	probably clean	
453	F	grade Angus	probably clean	
361	F	grade Angus	probably clean	
377	F	grade Angus	probably clean	
362	F	grade Angus	probably clean	
359	F	grade Angus	probably clean	
336	F	grade Angus	probably clean	
248	F	grade Angus	probably clean	
344	F	grade Angus	probably clean	
372	F	grade Angus	probably clean	
329	F	grade Angus	probably clean	
358	M	grade Angus	probably clean	
234	M	grade Angus	probably clean	
337	M	grade Angus	probably clean	
452	M	grade Angus	probably clean	
124	M	grade Angus	probably clean	
162	M	grade Angus	probably clean	
OK 7-26	M	Hereford	pedigree clean	
OK 7-96	M	Hereford	pedigree clean	
T770	M	Angus	possible carrier	
T709	M	Angus	possible carrier	
T710	M	Angus	possible carrier	
237	M	Angus	pedigree clean	
147	M	Angus	pedigree clean	
T767	M	Angus	possible carrier	
T742	M	Angus	possible carrier	
T763	M	Angus	possible carrier	
OK 7-90	M	Hereford	pedigree clean	

TABLE XXIII (Continued)

Animal No.	Sex (M-F)	Breed	Genotype	Birth Weight (lbs.)
T661	M	Hereford	dwarf	
T690	M	Hereford	dwarf	
T90	F	Hereford	dwarf	
D-100	F	Angus	dwarf	
T047	M	Angus	dwarf	
B-124	F	Hereford	pedigree clean	
B-73	F	Hereford	pedigree clean	
326	F	Hereford	comprest	
264	F	Hereford	comprest	
252	F	Hereford	comprest	
3-30	F	Hereford	comprest	
273	F	Hereford	comprest	
230	F	Hereford	comprest	
184	F	Hereford	comprest	
3	F	Hereford	comprest	
591	F	Hereford	pedigree clean	
2-36	F	Hereford	pedigree clean	
5-17	F	Hereford	pedigree clean	
2-08	F	Hereford	pedigree clean	
5-14	F	Hereford	pedigree clean	
592	F	Hereford	pedigree clean	
593	F	Hereford	pedigree clean	
19	F	grade Hereford	pedigree clean	
12	F	grade Hereford	pedigree clean	
56	F	grade Hereford	pedigree clean	
11	F	grade Hereford	pedigree clean	
25	F	grade Hereford	pedigree clean	
31	F	grade Hereford	pedigree clean	
90	F	Hereford	possible carrier	
86	F	Hereford	possible carrier	
80	F	Hereford	possible carrier	
049	F	Hereford	possible carrier	
T46	F	Hereford	known carrier	
T86	F	Hereford	known carrier	
T49	F	Hereford	known carrier	
T127	F	Hereford	known carrier	
T68	F	Hereford	known carrier	
T64	F	Hereford	known carrier	
T25	F	Hereford	known carrier	
ST44	F	Hereford	known carrier	
T2	F	Hereford	known carrier	
T24	F	Hereford	known carrier	
T4	F	Hereford	known carrier	
T3	F	Hereford	known carrier	

TABLE XXIV HEMATOLOGICAL DATA OF CALVES ONE WEEK OR LESS OF AGE

Animal No.	% Hemolysis in Saline Concentrations of .60%	.56%	.52%	Hematocrit	Hemoglobin	MCV	MCH	MCHC	RBC No.	Sp. Gr. Plasma
	%	%	%	%	gm./100 ml.	cu. m.	m.-m. gm.	%	M/mm^3	
(Pedigree Clean)										
OK 9-40	32.9	42.9	61.6	40.5	11.0	52.6	14.3	27.2	7.70	1.0260
OK 9-38	31.4	40.2	49.4	35.5	11.5	47.9	15.5	32.4	7.41	1.0223
OK 9-33	26.5	36.0	49.5	41.0	12.5	49.8	15.2	30.5	8.24	1.0230
OK 9-31	52.9	80.1	87.3	30.5	9.8	43.5	13.9	32.0	7.01	1.0243
269	25.5	39.8	50.6	35.5	10.2	53.0	15.2	28.7	6.70	1.0245
259	48.3	54.9	70.0	37.0	10.5	65.0	18.7	28.4	5.61	1.0232
339	35.9	45.6	53.5	41.0	12.0	56.9	16.7	29.3	7.20	1.0240
309	25.9	35.1	46.4	35.5	11.2	67.3	21.3	31.5	5.25	1.0230
329	46.8	53.5	64.9	41.8	12.8	63.1	19.3	30.5	6.62	1.0236
OK 9-04	20.3	49.5	--	39.5	--	57.3	--	--	6.89	1.0220
049	30.5	63.6	--	39.0	--	61.3	--	--	6.36	1.0260
OK 9-10	27.8	55.2	59.7	31.0	9.8	--	--	31.5	--	1.0221
OK 9-05	36.2	62.5	67.5	31.0	10.2	--	--	32.9	--	1.0255
OK 9-06	37.9	57.3	67.1	31.5	9.9	--	--	31.4	--	1.0240
OK 9-08	20.0	33.3	35.8	30.0	9.3	--	--	31.0	--	1.0202
OK 9-44	--	40.2	48.8	36.5	11.0	47.6	14.3	30.1	7.67	1.0233
OK 9-48	--	31.0	38.8	35.5	11.0	42.0	13.0	32.3	8.45	1.0250
OK 9-57	--	36.0	51.8	34.2	10.6	45.0	14.0	31.0	7.59	1.0250
OK 9-51	--	29.1	38.2	31.0	8.8	47.8	13.6	28.4	6.48	1.0268
OK 9-58	--	39.6	50.0	38.0	12.0	48.4	15.3	31.6	7.85	1.0230
OK 9-55	--	12.5	16.4	35.0	11.0	46.6	14.6	31.4	7.51	1.0223
(Compprest)										
OK 9-07	13.6	27.2	30.6	34.0	10.3	--	--	30.1	--	1.0220
OK 9-09	14.6	36.2	39.0	32.0	10.1	--	--	31.6	--	1.0280
OK 9-49	--	19.2	32.6	41.0	12.5	62.3	19.0	30.5	6.58	1.0224
OK 9-59	--	15.2	29.4	37.0	10.2	56.5	15.6	27.6	6.55	1.0225
OK 9-02	8.4	27.0	--	30.0	--	51.5	--	--	5.83	1.0260

TABLE XXIV (Continued)

Animal No.	% Hemolysis in Saline Concentrations of .60%	.56%	.52%	Hematocrit	Hemoglobin	MCV	MCH	MCHC	RBC No.	Sp. Gr. Plasma
	%	%	%	%	gm./100 ml.	cu. m.	m.-m. gm.	%	M/mm³	
(Possible Carriers)										
T905	27.4	31.9	36.1	29.5	9.0	--	--	30.5	--	1.0270
T903	24.1	44.4	--	30.5	--	51.3	--	--	5.95	1.0290
T907	18.7	26.2	31.8	36.2	11.0	--	--	30.4	--	1.0245
OK 9-34	12.3	17.8	28.6	35.5	11.5	44.7	14.5	32.4	7.95	1.0243
OK 9-24	9.3	19.3	26.5	45.0	13.6	47.4	14.3	30.2	9.50	1.0220
T933	--	53.3	68.6	24.8	7.8	49.0	15.4	31.5	5.06	1.0250
OK 9-54	--	27.6	39.3	29.0	8.5	48.8	14.3	29.3	5.94	1.0225
289	42.3	55.0	62.6	37.5	10.2	57.7	15.8	27.3	6.50	1.0230
T938	--	58.3	64.2	32.5	10.0	--	--	30.8	--	1.0285
T937	--	37.4	42.4	26.5	8.0	--	--	30.2	--	1.0298
T942	--	29.6	45.5	31.0	9.5	--	--	30.6	--	1.0315
T935	--	48.0	56.9	27.0	7.5	--	--	27.8	--	1.0280
T940	--	59.2	69.2	40.5	12.0	--	--	29.6	--	1.0270
T936	--	43.9	52.4	39.0	12.0	--	--	30.8	--	1.0250
T939	--	19.2	25.4	34.0	9.4	--	--	27.6	--	---
T941	--	17.4	25.8	41.5	12.0	--	--	28.9	--	---
OK 9-60	--	29.1	33.3	29.5	7.8	--	--	26.4	--	---
OK 9-64	--	23.0	29.1	41.0	11.0	--	--	26.8	--	---
T944	13.0	21.3	35.4	32.0	10.0	49.8	15.6	31.2	6.43	1.0226
T945	26.7	37.2	49.4	39.0	11.5	47.2	13.9	29.5	8.26	1.0235
T943	27.9	35.0	58.6	35.0	7.8	58.1	13.0	22.3	6.02	1.0225
529	16.9	22.4	36.4	29.0	9.0	49.6	15.4	31.0	5.85	1.0240
T950	--	7.8	13.2	--	--	--	--	--	--	---
T948	--	28.5	42.6	--	--	--	--	--	--	---
OK 9-71	--	21.1	33.9	--	--	--	--	--	--	---
549	--	50.7	62.1	--	--	--	--	--	--	---
OK 9-65	10.5	16.9	36.4	28.0	8.8	55.8	17.4	31.2	5.02	1.0226
OK 9-67	27.3	36.8	51.8	32.0	9.8	54.4	16.7	30.6	5.88	1.0228
T922	12.2	21.8	27.5	28.0	8.4	45.9	13.8	30.0	6.10	1.0282

TABLE XXIV (Continued)

Animal No.	% Hemolysis in Saline Concentrations of .60%	.56%	.52%	Hematocrit	Hemoglobin	MCV	MCH	MCHC	RBC No.	Sp. Gr. Plasma
	%	%	%	%	gm./100 ml.	cu. m.	m.-m. gm.	%	M/mm³	
(Known Carriers)										
T902	18.1	--	--	32.0	--	48.7	--	--	6.56	1.0250
T904	9.0	23.6	29.2	38.0	12.0	--	--	31.8	--	1.0285
OK 9-29	21.3	32.9	45.9	30.0	8.8	52.3	15.2	29.2	5.74	1.0232
OK 9-28	12.7	21.7	28.2	30.2	9.7	43.9	14.1	32.1	6.57	1.0228
OK 9-46	--	12.6	17.8	36.5	10.8	44.6	13.2	29.6	8.18	1.0237
OK 9-47	--	9.8	14.6	25.0	6.2	46.8	11.6	24.8	5.34	1.0223
OK 9-43	--	26.1	36.2	35.0	11.2	41.5	13.3	31.3	8.44	1.0227
OK 9-53	--	17.6	24.0	34.5	11.5	47.6	15.9	33.3	7.24	1.0240
T949	--	21.8	30.0	--	--	--	--	--	--	---
T951	--	18.4	35.7	--	--	--	--	--	--	---

TABLE XXV HEMATOLOGICAL DATA OF CALVES ONE-TWO MONTHS OF AGE

Animal No.	% Hemolysis in Saline Concentrations of .60%	.56%	.52%	Hematocrit	Hemoglobin	MCV	MCH	MCHC	RBC No.	Sp. Gr. Plasma
	%	%	%	%	gm./100 ml.	cu. m.	m.-m. gm.	%	M/mm³	
(Pedigree Clean)										
OK 9-05	45.2	72.3	87.0	36.0	11.2	40.9	12.8	31.2	8.81	1.0234
OK 9-10	38.4	55.2	87.0	40.0	12.5	39.9	12.5	31.2	10.02	1.0245
OK 9-40	74.9	87.3	95.5	38.0	11.4	41.8	12.5	30.0	9.09	1.0236
OK 9-04	61.1	77.0	93.0	37.5	11.5	52.9	16.2	30.7	7.09	1.0228
269	44.0	73.0	91.3	46.0	14.0	46.5	14.1	30.4	9.90	1.0242
329	51.2	78.4	88.8	35.0	10.7	56.0	17.1	30.6	6.25	1.0236
259	73.8	92.4	97.9	39.0	12.3	49.3	15.5	31.5	7.91	1.0235
049	78.6	92.4	96.6	41.0	13.0	56.0	17.8	31.7	7.32	1.0250
OK 9-06	49.5	82.7	95.3	39.5	12.5	42.5	13.5	31.6	9.29	1.0233
339	69.6	89.7	95.4	38.0	12.0	43.4	13.7	31.6	8.76	1.0227
OK 9-33	49.4	78.9	92.2	36.5	12.4	43.1	14.6	34.0	8.47	1.0228
OK 9-38	62.5	90.1	94.8	37.5	12.7	43.0	14.5	33.9	8.73	1.0226
OK 9-31	80.9	93.0	97.3	39.0	12.3	48.9	15.4	31.5	7.98	1.0228
OK 9-08	32.3	59.2	81.2	41.5	13.1	51.3	16.2	31.6	8.09	1.0230
OK 9-55	41.4	64.7	82.4	35.0	10.7	52.3	16.0	30.6	6.69	1.0228
OK 9-44	71.7	88.7	94.8	41.0	12.9	58.6	18.4	31.5	7.00	1.0226
(Known Carriers)										
OK 9-28	62.8	74.2	85.4	32.0	9.5	39.6	11.7	29.7	8.09	1.0227
OK 9-53	47.1	69.8	76.5	36.0	10.6	53.1	15.6	29.4	6.78	1.0228
OK 9-43	44.6	70.3	81.3	38.0	11.9	45.2	14.2	31.3	8.40	1.0228
OK 9-11	39.4	65.4	76.0	45.0	14.1	58.1	18.2	31.3	7.74	1.0230
(Possible Carriers)										
OK 9-34	76.1	89.7	97.3	40.0	11.9	45.5	13.5	29.8	8.80	1.0228
289	61.6	77.4	93.6	43.0	12.9	49.1	14.7	30.0	8.75	1.0238
OK 9-60	55.3	67.9	81.4	34.0	10.7	42.5	13.4	31.5	8.00	1.0224
OK 9-64	33.3	57.5	75.9	36.0	11.1	38.6	11.9	30.8	9.33	1.0223
OK 9-54	29.0	67.0	81.9	38.0	13.0	52.3	17.9	34.2	7.27	1.0225

TABLE XXVI HEMATOLOGICAL DATA OF CALVES THREE MONTHS OF AGE

Animal No.	% Hemolysis in Saline Concentrations of .60%	.64%	.68%	RBC	Hemato-crit	MCV
	%	%	%	M/mm3	%	cu. m.
T891	46.7	17.0	4.5	9.69	40.0	41.3
T888	41.2	18.4	4.9	7.27	34.0	46.8
T886	33.9	11.2	4.5	8.48	48.0	56.6
T877	29.3	8.5	2.5	8.72	43.0	49.3
T890	81.0	45.3	18.9	11.10	34.0	36.5
T880	93.3	31.6	8.6	9.70	43.0	44.3
T872	62.0	28.9	9.0	7.22	39.0	54.0
T876	67.3	28.2	8.9	10.18	46.0	45.2
T889	46.0	22.0	7.0	8.29	34.5	41.6
T873	93.9	87.0	43.8	7.10	34.0	47.9
T871	40.0	15.6	10.9	10.90	42.0	38.5
T882	59.6	21.4	7.4	8.78	37.0	42.1

TABLE XXVII ELECTROPHORETIC ANALYSIS OF PLASMA PROTEIN DATA OF CALVES ONE WEEK OR LESS OF AGE

Animal No.	Fraction I	Fraction II	Fraction III	Fraction IV	Fraction V
	%	%	%	%	%
(Pedigree Clean)					
OK 9-55	29.2	31.1	16.5	16.0	7.2
OK 9-51	27.4	22.0	19.6	19.9	11.0
OK 9-57	32.7	31.9	18.3	14.4	2.7
OK 9-31	29.3	25.5	14.9	25.0	5.3
OK 9-33	28.4	27.3	18.0	22.4	3.8
OK 9-38	30.6	23.2	20.1	20.1	10.7
259	31.0	23.2	20.0	18.4	7.4
OK 9-40	26.3	22.8	15.3	25.1	10.5
269	26.6	23.9	19.8	22.1	7.6
OK 9-48	23.7	28.5	18.3	16.9	12.6
OK 9-44	28.3	24.9	17.9	17.2	11.6
OK 9-58	28.1	33.0	15.5	18.5	5.0
329	29.9	33.6	18.2	14.8	3.4
309	28.9	27.7	20.8	15.9	6.6
339	27.3	23.9	20.3	18.7	9.8
(Compress)					
OK 9-59	31.2	20.3	18.4	22.8	7.2
OK 9-49	35.6	24.3	17.6	19.8	2.6
(Known Carriers)					
OK 9-53	31.6	27.0	15.5	17.0	8.8
OK 9-43	15.5	24.3	17.4	27.8	5.1
OK 9-47	35.5	33.0	15.6	12.4	3.5
OK 9-46	29.3	28.9	17.1	17.3	7.5
OK 9-28	27.1	31.4	17.0	20.2	4.3
OK 9-29	28.6	29.5	17.6	19.6	4.6
(Possible Carriers)					
T940	27.1	22.3	17.5	23.9	9.2
T933	26.8	25.8	17.9	22.9	6.6
T939	25.7	21.4	15.7	26.8	10.4
OK 9-54	25.7	24.5	18.9	27.9	3.0
T935	28.5	21.9	19.2	14.8	15.6
T936	27.0	25.1	16.8	18.1	13.1
T941	28.1	26.3	16.9	18.3	10.4
OK 9-64	27.5	24.4	15.5	20.9	11.7
OK 9-60	31.3	28.3	19.5	17.3	3.9
OK 9-24	26.1	34.6	18.4	19.5	1.4
OK 9-34	26.9	26.1	15.6	22.5	8.9
289	26.9	21.3	23.3	21.7	6.8
T922	22.2	23.6	16.1	24.6	13.5

TABLE XXVIII ELECTROPHORETIC ANALYSIS OF PLASMA PROTEIN DATA OF CALVES ONE TO TWO MONTHS OF AGE

Animal No.	Fraction I	Fraction II	Fraction III	Fraction IV	Fraction V
	%	%	%	%	%
(Pedigree Clean)					
OK 9-04	36.9	20.9	23.3	15.3	3.5
259	39.8	25.2	18.9	12.3	3.9
269	36.1	21.4	19.3	18.0	5.2
OK 9-06	38.2	21.8	19.4	17.0	3.6
339	37.0	23.9	21.4	14.3	3.4
OK 9-10	33.7	23.6	20.4	18.8	3.6
OK 9-05	33.9	23.8	18.0	20.0	4.3
OK 9-40	31.8	22.7	17.7	21.3	6.6
049	31.9	24.3	20.1	19.5	4.3
(Known Carriers)					
OK 9-11	34.4	23.9	19.1	18.2	4.5
OK 9-46	36.3	23.2	17.0	17.9	5.7
OK 9-28	37.3	21.0	16.0	21.8	3.9
OK 9-43	35.8	23.7	17.6	17.1	5.8
OK 9-53	34.0	23.1	18.4	16.7	7.8

TABLE XXIX LEUCOCYTE DIFFERENTIAL COUNTS OF CALVES ONE WEEK OR LESS OF AGE

Animal No.	Lymphocytes	Segmented Neutrophils	Non-Segmented Neutrophils	Total Neutrophils	Monocytes	Eosinophils	Non-Differentiated Cells
	%	%	%	%	%	%	%
(Pedigree Clean)							
OK 9-04	43	24	23	47	4	2.0	6.0
049	27	43	10	53	14	0.5	5.5
OK 9-10	12	65	16	81	3	1.0	2.0
OK 9-05	34	55	5	60	0	1.0	4.0
OK 9-08	21	65	6	71	4	0.0	3.0
OK 9-40	19	59	17	76	4	0.0	1.0
OK 9-38	17	69	12	81	1	1.0	1.0
339	19	55	19	74	2	0.0	5.0
OK 9-33	62	31	3	34	2	0.0	2.0
OK 9-31	62	31	4	35	2	0.0	1.0
269	24	58	14	72	1	0.0	3.0
329	37	46	13	59	2	2.0	2.0
309	67	22	17	29	0	0.0	4.0
(Possible Carriers)							
T922	32	61	5	66	1	0.0	1.0
T903	44	34	12	46	7	1.0	3.0
T905	55	32	7	39	4	1.0	1.0
T907	35	42	7	49	9	1.0	5.0
OK 9-34	23	62	11	73	2	1.0	1.0
289	44	45	6	51	4	0.0	1.0
(Known Carriers)							
T902	39	22	22	44	7	1.0	9.0
T904	32	41	10	51	9	4.0	4.0
OK 9-28	65	27	6	33	0	1.0	2.0
(Comprest)							
OK 9-02	34	40	7	47	11	1.0	7.0
OK 9-09	16	70	8	78	2	2.0	2.0

TABLE XXX LEUCOCYTE DIFFERENTIAL COUNTS OF DWARFS AND NON-DWARF CATTLE BEFORE AND AFTER INJECTION OF INSULIN

Animal No.	Lymphocytes	Neutrophils	Monocytes	Eosinophils	Unsegmented Granulocytes
	%	%	%	%	%
(Non-dwarf, before injection of insulin)					
OK 7-26	56	36	4	1	3
OK 7-96	80	14	4	2	0
OK 7-90	86	9	3	2	0
B-73	76	20	5	4	1
B-124	89	5	3	1	0
(Non-dwarf, one hour after injection of insulin)					
OK 7-26	43	45	3	5	4
OK 7-96	78	18	4	0	2
OK 7-90	87	11	1	1	0
B-73	66	24	7	3	0
B-124	75	17	3	4	1
(Non-dwarf, two hours after injection of insulin)					
OK 7-26	36	57	1	0	5
OK 7-96	57	38	1	1	3
OK 7-90	78	17	2	2	1
B-73	65	31	2	1	1
B-124	87	8	2	3	0
(Dwarf, prior to injection of insulin)					
T90	66	23	8	3	0
D-100	59	38	1	3	0
T690	61	31	8	1	0
T661	67	28	2	2	1
(Dwarf, one hour after injection of insulin)					
T90	41	41	7	6	5
D-100	49	37	1	10	3
T690	67	26	2	5	1
T661	59	32	1	1	2
(Dwarf, two hours after injection of insulin)					
T90	56	37	3	3	1
D-100	58	36	0	5	1
T690	80	17	4	0	0
T661	62	32	1	1	3

TABLE XXXI ERYTHROCYTE FRAGILITY DATA OF MATURE COWS

Animal No.	Percent Hemolysis in Saline Concentrations of .48%	.52%	.56%	.60%
	%	%	%	%
(Pedigree Clean)				
591	82.1	53.7	26.2	11.6
2-36	90.6	86.0	55.8	36.0
5-17	86.0	59.0	25.1	10.3
2-08	94.0	70.0	43.4	18.1
5-14	97.9	90.3	59.8	36.2
592	77.3	60.0	32.8	24.4
593	77.0	51.6	23.3	19.6
(Known Carriers)				
T25	92.8	83.2	51.9	17.2
ST44	99.5	95.3	83.3	38.3
T2	97.4	85.4	58.6	23.8
T24	89.0	60.6	23.5	8.6
T4	97.8	96.1	77.8	38.9
T3	75.4	47.4	20.2	9.1
(Possible Carriers)				
90	94.5	79.2	39.8	7.4
86	73.6	37.8	31.5	4.6
80	93.1	76.2	33.4	--
049	48.2	36.1	12.8	--
(Comprest)				
3	97.8	93.2	84.4	--
326	86.2	76.6	39.9	46.6
264	97.4	93.8	60.7	40.2
252	69.2	31.2	12.7	--
3-30	93.2	66.0	29.0	--
273	97.0	97.4	84.1	--
230	97.4	85.3	59.5	--
184	91.1	73.0	47.0	--

TABLE XXXII ERYTHROCYTE FRAGILITY DATA OF DWARF AND NON-DWARF BEEF CATTLE

Animal No.	Percent Hemolysis in Saline Concentrations of .52%	.56%	.60%	.64%
	%	%	%	%
(Non-dwarf 18 months of age)				
T770	96.2	68.7	33.4	10.0
T709	98.2	88.5	64.4	38.5
T710	97.9	95.9	82.9	49.6
237	98.9	88.4	69.2	50.4
147	98.5	96.4	59.8	44.0
T767	98.9	87.4	67.0	53.6
T742	96.3	67.6	31.9	8.5
T763	97.8	91.6	52.6	15.0
(Dwarf 18 months of age)				
T757	99.0	95.4	85.6	46.0
T789	94.8	68.8	60.1	22.0
(Non-dwarf 7 to 8 months of age)				
T802	99.3	97.4	92.6	24.4
T808	99.2	86.6	51.4	13.8
T804	99.3	92.3	87.6	46.7
T824	99.3	95.1	75.6	39.1
T812	99.0	94.9	76.6	65.2
T801	99.0	94.8	84.6	58.2
T800	98.7	96.0	78.2	39.8
T805	--	97.9	93.0	69.8
T848	--	91.0	71.7	30.6
T857	--	97.4	90.6	59.2
T838	--	92.8	80.6	40.2
T817	98.7	97.2	85.4	64.0
(Dwarf 7 to 8 months of age)				
T895	97.9	89.3	35.1	34.8
T896	96.4	91.4	71.7	22.6
T897	--	92.4	69.5	35.5
T869	--	35.9	20.6	8.3

TABLE XXXIII ERYTHROCYTE FRAGILITY DATA OF CALVES SEVEN TO EIGHT MONTHS OF AGE USED IN AGE COMPARISONS

	Percent Hemolysis in Saline Concentrations of				
Animal No.	.56%	Animal No.	.56%	Animal No.	.56%
	%		%		%
T813	96.2	T837	66.2	336	97.0
T829	88.9	T823	63.3	248	81.5
T814	96.4	T859	92.3	344	96.8
T840	99.0	T834	97.3	372	92.7
T809	93.3	261	70.4	329	96.5
T854	92.3	474	95.4	358	97.1
T851	96.5	453	93.4	234	98.1
T860	98.1	361	94.2	337	98.3
T827	97.8	377	87.6	452	93.9
T832	91.9	362	95.0	124	99.0
T806	81.8	359	93.4	162	96.9
T853	98.1	--	--	--	--

TABLE XXXIV ERYTHROCYTE FRAGILITY DATA OF CALVES SEVEN TO EIGHT MONTHS OF AGE AFTER FASTING AND INJECTION OF EPINEPHRINE

					5 hours after epinephrine	
	48 hour fast		72 hour fast			
Animal	Percent Hemolysis in Saline Concentrations of					
No.	.60%	.64%	.60%	.64%	.60%	.64%
	%	%	%	%	%	%
(Non-dwarfs)						
T857	91.9	78.0	92.6	75.0	93.3	74.0
T805	95.3	82.2	94.3	76.1	91.1	78.2
T848	75.3	41.2	85.0	56.0	85.8	46.0
T801	84.7	52.4	73.3	35.2	85.8	50.6
T812	74.6	36.3	66.3	32.9	58.1	29.0
T800	78.0	45.9	46.0	17.2	33.5	19.4
T817	81.6	50.7	66.8	28.6	52.5	22.2
T838	82.4	49.1	76.2	36.4	51.6	33.1
(Dwarfs)						
T896	60.8	24.2	72.2	23.8	35.4	27.1
T895	65.6	28.6	57.9	30.2	41.7	22.4
T897	62.2	29.3	51.1	19.9	50.9	23.2
T869	22.2	8.2	17.1	5.8	20.2	8.9

TABLE XXXV ERYTHROCYTE FRAGILITY DATA OF CALVES ONE WEEK OR LESS OF AGE BEFORE AND AFTER INJECTION OF ACTH

Animal No.	Prior to injection of ACTH		1 hour after injection of ACTH		2 hours after injection of ACTH	
	Percent Hemolysis in Saline Concentrations of					
	.52%	.56%	.52%	.56%	.52%	.56%
	%	%	%	%	%	%
(B x-ray calves)						
T949	30.0	21.8	35.6	27.8	29.8	22.8
T951	35.7	18.4	35.4	25.9	35.9	20.6
T950	13.2	7.8	14.5	9.2	12.9	7.3
OK 9-71	33.9	21.1	35.0	22.7	34.6	21.8
(C x-ray calves)						
549	62.1	50.7	70.9	52.0	65.3	42.4
T948	42.6	28.5	34.6	19.5	37.1	22.1

TABLE XXXVI ERYTHROCYTE FRAGILITY DATA FROM MATURE COWS; FASTED, NON-FASTED, AND AFTER INJECTION OF EPINEPHRINE

Animal No.	Non-fasted		72 hour fast		5 hours after epinephrine	
	Percent Hemolysis in Saline Concentrations of					
	.48%	.54%	.48%	.54%	.48%	.54%
	%	%	%	%	%	%
(Pedigree Clean)						
19	60.6	21.2	65.7	19.8	53.4	21.4
12	75.5	57.2	77.4	50.9	61.1	48.1
56	63.9	20.1	73.2	33.5	76.0	43.0
11	75.0	35.4	82.7	47.1	65.8	53.3
25	79.1	39.7	--	--	--	--
31	52.5	84.7	--	--	--	--
(Known Carriers)						
T46	79.9	31.0	69.4	59.2	84.5	60.2
T86	54.5	14.9	38.7	17.0	56.2	23.0
T49	81.7	36.4	62.0	31.7	61.5	37.6
T127	72.2	22.0	--	--	--	--
T68	67.6	33.9	--	--	--	--
T64	88.9	48.9	--	--	--	--

VITA

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Doctor of Philosophy

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