# HEMATOLOGICAL INVESTIGATION OF DWARF AND NORMAL BEEF CATTLE

by

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# INTRODUCTION

In the last 10 to 15 years the frequency of dwarf calves produced in purebred beef herds has increased. This has prompted studies of the mode of inheritance of different types of dwarfism. The specific kind of dwarfism with high incidence has been described as "snorter" dwarfism. The symptoms most characteristic of the snorter dwarf are short neck, body and legs, protruding tongue, undershot jaw and lack of coordination while walking. A tendency to bloat easily causes many of the dwarf calves to die prior to weaning, although dwarfs of both sexes have lived to reproduce. The term "snorter" was suggested by the heavy breathing caused by respiratory difficulty.

This type of dwarfism is due to a single autosomal recessive gene. No apparent phenotypic differences exist between the "carrier" or heterozygous animal and the "clean" or homozygous normal animal. Since the frequency of the dwarf gene has increased in the past few years, it seems possible that the breeders have unwittingly preferred animals with the heterozygous genotype.

The dwarf calves themselves have little economic value. Animals which are known to be carriers of the dwarf gene have less value for breeding than animals of the clean (dwarf-free) genotype. Therefore, a method of identifying the carrier ani-

mals more conveniently and quickly than a lengthy progeny test would be helpful. These carrier animals could then be eliminated from the herd before reaching breeding age.

If the allele of the dwarf gene is incompletely dominant for any of the effects of the dwarf gene, either anatomically or physiologically, then classification of carrier (heterozygous) animals might be made. The abnormalities in the phenotype of the dwarf animal must have some physiological basis and these physiological peculiarities might possibly be detectable to some degree in the heterozygous animal. Several studies of the liver, heart, urine, blood, etc. in relation to dwarfism have been made. The differences between the carrier and clean animals are the important concern in trying to reduce the frequency of the dwarf gene since the dwarfs can be easily detected and eliminated.

The blood reflects many of the functions of the body and hence might indicate some physiological abnormality. A more complete hematological study of animals carrying the dwarf gene might give some insight to the problem. The purpose of this work was to make such a study of some of the hematological techniques used in trying to classify carriers of the dwarf gene. The hematological techniques of greatest interest in diagnosing dwarf carriers were the white cell count and

fragility of erythrocytes. Red cell count, hemoglobin content of the blood, and differential white cell counts were studied in conjunction with the other tests, not only as methods of diagnosis but in an effort to gain more insight to the fragility of red cells. The effects of age and sex of the animals and atmospheric temperature on the above blood characteristics were also studied.

# REVIEW OF LITERATURE

The "snorter" type of dwarfism in beef cattle was first reported by Johnson et al. (1950). Even before this time the increased frequency of dwarfism had become a matter of some concern to breeders. These workers felt that the animals normal in phenotype which produced the dwarfs were of an intermediate type but concluded that a single autosomal recessive gene was responsible for the abnormality. Later Lush and Hazel (1952) showed that a ratio of 3 normal appearing animals to one dwarf was obtained from matings of carrier bulls with carrier cows.

Roubicek et al. (1955), Cooper (1957) and Swiger (1958) discussed the appearance of dwarfs and their mode of inheritance in other species of animals as well as cattle.

Hazel (1955) suggested a progeny test as the only accurate method of detecting carriers of the snorter dwarf gene. However, the progeny test is usually unduly expensive and time-consuming. The carrier bulls could be eliminated, as quickly as found, but to prove a female clean with a reasonably high degree of probability would require most of her productive life. By delaying the use of a bull in the breeding herd for one season, a satisfactory test of the bull could be made by using dwarf cows, carrier cows or even daughters of known carrier bulls. Because the progeny test is not always practi-

cal, several methods have been proposed in an attempt to identify the carrier animals by their own anatomy or physiology.

Gregory et al. (1951) described the head of the snorter dwarf as the brachycephalic type with prominences in the midforehead. Later Gregory et al. (1953) stated that by studying the profile of the frontal bones of horned Hereford bulls 15 months of age or older the heterozygous and the homozygous normals could be differentiated with a high degree of accuracy. However, Schoonover and Stratton (1954) applied the same method to Hereford cows and were unable to separate carriers and non-carriers. Other workers, Stonaker (1954) and Stonaker and Hughes (1956), also were unable to make reliable predictions of the genotypes of animals by using the profile method. Cooper (1957) studied head measurements and found that both dwarf and normal animals had a forehead prominence and a dish face. However, these characteristics tended to be noticeable at early ages and in the normal animals, usually disappeared at about three months of age. Cooper also found small differences between animals classified by radiographs as carriers or clean, but these differences were small and the groups overlapped considerably.

Emmerson and Hazel (1956) described a method of classifying dwarf, carrier and normal Hereford calves with radiographs of the lumbar region. They reported that dwarf calves usually exhibited a longitudinal compression and an increase in depth

of the last four or five thoracic vertabrae and the lumbar vertebrae. The radiographs of dwarfs also showed marked undulations of the ventral profiles of these vertebrae. Normal animals which had vertebral abnormalities, intermediate between dwarfs and calves free from the dwarf gene, were thought to be carriers. Hazel et al. (1956) reported that of 26 animals known to be carriers by progeny test or pedigree analysis only two had been rated normal by radiographic classification. The other 24 animals had been classified as exhibiting various classes of abnormalcy. In a summary of 736 radiographs on pedigree-clean (i.e. presumed free from dwarf genes) calves, most of which were taken in breeders herds, Hazel (1958) reported that 74 percent had no vertebral abnormality, 15 percent exhibited mild vertebral abnormalities, eight percent had intermediate abnormalities and three percent showed extreme vertebral abnormalities.

Turman (1958) reported that of 44 radiographs of calves sired by known carrier bulls and out of pedigree-clean cows, 54.5 percent showed no abnormal vertebrae, 15.9 percent showed mild abnormalities, 22.7 percent had intermediate abnormalities, 6.8 percent had extreme abnormalities but were not as extreme as radiographs from dwarf calves. In these matings, one expects 50 percent of the calves to be clean and 50 percent to be carriers. Turman also gave data on 15 known carrier animals. Three of these animals had normal radiographs comparable to those of dwarf-free animals, one had mild abnormalities,

five had intermediate abnormalities and six showed extreme abnormalities. In a herd designated as "probably clean", Turman (1957) reported that of 235 calves produced, 77 percent had normal radiographs, 16.6 percent had mild abnormalities and 6.4 percent had intermediate abnormalities. Koch and Arthaud (1958) reported that of cattle believed to be free of dwarfism, 77 percent had normal lumbar vertebrae. However, the incidence of lumbar abnormalities varied between lines of clean cattle. These workers felt that other genes affected the appearance of the lumbar vertebrae.

Hazel (1957) summarized a large number of radiographs and estimated that 90 percent of the carriers and 80 percent of the pedigree-clean animals were classified correctly. The latter figure was based on approximately 3000 animals while the data on the carriers concerned 186 animals. These data suggest that these classifications were conservative in that more clean animals were classified as carriers than carriers were classified as cleans.

Bovard et al. (1956) studied the length of the metacarpus and the combined length of the six lumbar vertebrae as a means of classifying carriers and cleans. Small average differences in these measurements were noted between groups of carriers and groups of clean animals. Several body measurements were taken by Bovard (1954) on dwarf and normal calves. Using

radiographic classification as a criterion, Bovard computed discriminant functions from several measurements. Applying these functions to another group of 21 calves, he predicted radiographic type correctly for 14 and incorrectly for four. Three were in the overlap area. The actual genotypes of these animals were not known, so the accuracy of these functions cannot be assessed for predicting dwarf carriers.

Another attempt to classify animals carrying the snorter dwarf gene is through hematological studies. Massey et al. (1956) found that dwarfs are less tolerant to insulin injections than normal animals, since blood sugar declines more rapidly and to lower levels in dwarfs than in normals. Massey et al. (1958) stated that both dwarfs and carriers were more sensitive to insulin than pedigree-clean individuals. These workers suggested that the dwarfs, and the carriers to a lesser extent, may be deficient in one or more of the pituitary hormones or that these hormones are present but unable to function properly in the regulation of carbohydrate metabolism. These workers also noted a dosage effect. A dose of 0.8 unit of insulin per kilogram body weight gave a differential response in the three genotypes, but a dose of 0.3 unit failed to produce definite differences.

Clark (1957) reported significant differences between cleans and carriers and between cleans and dwarfs in blood

sugar level following the administration of adrenalin. Clark found among the three genotypes significant differences in mean response to adrenalin injections. The clean animals tended to show a larger increase in blood sugar from the time of injection of adrenalin to one hour after injection than either dwarfs or carriers. He concluded that, even though average differences in response to adrenalin did exist among genotypes, the usefulness of this test was doubtful since much variation and overlap between individuals of different genotypes is present.

Turman (1958) also indicated that clean animals showed a quicker and larger response in blood sugar level to injections of adrenalin after a 72-hour fast, than did the carrier animals. His data, although obtained from small numbers, suggested a difference in magnitude of response between ages within a given genotype. The mature animals seemed slower in their response than the yearling heifers.

Buchanan (1957) studied glucose tolerance and found that snorter dwarfs and known carriers of the dwarf gene required more time to return to normal glycemic levels following intraveneous injections of glucose than did pedigree-clean animals. Buchanan also studied glycogen storage levels in the liver and reported that after starvation for 60-72 hours, the pedigree-clean cattle showed a glycogen level in the liver of almost

twice that found in snorter dwarfs and carriers. Following prolonged high carbohydrate feeding, the carrier cattle fell into two distinct groups in liver glycogen values. The low group of carriers averaged less than 2000 mg. per gram of tissue, while the high group of carriers averaged more than 6000 mg. per gram of liver. Following the same treatment, the pedigree-clean cattle were intermediate between these two groups with a liver glycogen level of 4140 mg. per gram. Buchanan further stated that when similar tests were made on cattle receiving a good maintenance ration, the differences in liver glycogen levels between genotypes failed to exist. He postulated that these findings "point to the carbohydrate mechanism as being definitely involved in the production of the dwarfism syndrome" but, even though significant differences between genotypes do exist, the accuracy with which animals can be classified is subject to question.

The fact that the glucose tolerance tests indicate that the dwarf cattle cannot utilize glucose in the same manner as normal cattle and that these dwarfs are perhaps diabetic was reported by Eveleth et al. (1956). In humans the offspring of a diabetic mother may show an abnormality in the shape of the heart. Eveleth reported that this abnormality also has been found in many cases of snorter dwarfism in beef cattle. Dinkel (1958) reported that the heart abnormalities can be readily

demonstrated radiographically. The radiographs of dwarfs and carriers indicate the heart to be spheroid in shape, while the pedigree clean animals have a normal shaped heart.

The differences in response in insulin injections between dwarf and normal animals, as pointed out by Massey et al. (1956), suggested the possibility that either the adrenals or the pituitary gland do not function properly in the dwarf. These workers devised a test to measure the differential response by making a white cell count before and at various times following the insulin injection. The dwarf animals showed little response after the administration of insulin, while the carriers responded with white cells increasing for a period up to two hours. The non-carriers, however, tended to decline in white cell count in the third sample of blood which was taken two hours after the insulin injection.

Lasley (1957) reported on the same technique. He stated that the cell count obtained by Massey and his associates differed from standard white cell counts because small cells or objects other than standard white cells were present in the blood samples and were counted. The change in the number of these smaller objects gives this test its diagnostic value. Lasley further reported that differential white cell counts have some value in helping confirm results of total cell counts but that the differential counts alone cannot be used for dis-

tinguishing genotypes because variation and overlap exist in these groups.

Swiger (1957) found little difference between snorter dwarfs, carriers of the snorter gene and pedigree-clean animals in their responses to adrenalin as measured by total white cell count. Swiger's technique utilized adrenalin instead of insulin and there was some question as to whether he was counting the same small cells reported by the Missouri workers. He pointed out that, even though the carrier cows were a fair representation of the Hereford breed, the clean animals were all from one inbred line. This fact could have been important since animals from this line are extremely excitable. A large increase in neutrophils was found in the clean animals following injections of adrenalin. This increase caused the clean animals to increase more than the carriers in white cells during a period from one to two hours following the injections.

The various genotypes in both the Hereford and Angus breeds were classified in an insulin test workshop at Ft. Reno, Oklahoma (1957). These data came from E. J. Turman, Oklahoma State University, by private communication. When classification was made on a white cell count without the aid of a differential count on Hereford animals, ten of 18 were classified correctly, four were classified incorrectly, and four were called indeterminant without aid of a differential white cell

count. The test was even less successful on the Angus animals.

Massey et al. (1958) reported a differential response of clean and carrier animals as measured by the insulin test, but 10 to 15 percent of the animals tested did not give a clear-cut response. Several factors affect the accuracy of the test, the most important of which is the human error in making the cell counts. Exciting the animals before the test seems to affect the results of the test. No animal should be tested unless it has been quiet for several hours. Changes in temperature and intense heat also interfere with the accuracy of the test.

Measuring the response of blood sugar and white cell count to the hormone ACTH also was tried by Massey (1958) but he found no differential response between the three genotypes in either blood glucose or white cell count.

Work done on nine known carrier calves and thirteen presumed clean calves by Koch and Arthaud (1958) showed highly significant average differences in total phosphorus level between these two groups but considerable individual overlap. The clean calves had a higher total phosphorus level than the carriers, and the two groups differed significantly in percent of beta globulin. The dwarf free calves had higher percentage of beta globulin than the carrier animals. These two groups did not differ significantly in the other blood serum components.

Bennett (1958) described the use of paper chromatography in the study of dwarfism in beef cattle. He found more glutamic acid in the urine of snorter dwarfs than in the urine of animals normal in phenotype. Chromatograms of the urine of dwarf, carrier and clean animals indicated that the carriers differed from the homozygous normal animals in the absence of a particular spot known as the "14" spot. This spot was absent in the carriers, with only a few exceptions. Later the presence of this spot in the clean animals was found to be quantitative rather than qualitative because concentration of the urine made the spot detectable in both genotypes.

Johnson (1958) studied erythrocyte fragility after the administration of ACTH and insulin. This study indicated significant differences between dwarf, clean and carrier animals in red cell fragility. Hazel (1958) tried to separate the genotypes by differences in fragility of erythrocytes without aid of administering hormones. Of 31 carrier animals eight were reported to have high fragility and 23 reportedly had low fragility. In a test of 37 clean animals, 35 were high and two were low. Turman (1958) measured erythrocyte fragility in twelve clean and eleven carrier cows before and four hours after an injection of epinephrine. The carrier cows increased in fragility of the red cells following epinephrine, while the clean cows decreased in fragility of red cells. There was

some overlap as one carrier cow decreased and three clean cows increased.

Since "fragility of erythrocytes" is rather vague, some explanation of the meaning of the term is warranted. Dukes (1955) defined hemolysis as the discharging of the hemoglobin from the erythrocytes into the plasma. Lowering the osmotic pressure of the medium surrounding the cells causes hemolysis by water passing into the corpuscle through its semipermeable membrane. This action causes the red cell to swell until the cell membrane bursts. Exposing the blood to hypotonic salt solutions will produce this action.

Evidence that hormones of the gonads, thyroid, adrenal cortex and anterior pituitary are involved in the control of hemopoiesis was discussed by Wintrobe (1956). He stated that hormones are of little value in stimulating erythropoiesis even though reticulocytosis does occur and nucleated red cells may appear in the circulation following administration of the hormones. Cruz et al. (1941) gave evidence that the young red corpuscles are less resistant to hemolysis than are the older cells.

Dacie and Vaughan (1938) reported that several factors are apt to affect the degree of hemolysis. Intrinsic factors such as red cell dimension and chemical composition of the cell and cell membrane are important. Any extrinsic factor

which modifies the ionic status and hence the water content of the erythrocyte will alter its resistance to hemolysis. These workers listed the following factors as being important in this respect: (1) oxygen and carbon dioxide content of the blood, (2) temperature, (3) the pH of the saline solution, and (4) the presence and degree of anemia.

Wintrobe (1956) pointed out that many methods of measuring the degree of resistance to fragility of the red cell exist. Most of the techniques measure the resistance of the cells to hemolysis in decreasing strengths of hypotonic saline solutions. Hunter (1940) described a colorimetric method for estimating the degree of hemolysis. Later Ham (1950) developed a method whereby varying strengths of saline solutions were used and the degree of hemolysis in each solution was determined by an Evelyn colorimeter. In cases of congenital hemolytic jaundice, Singer (1940) used a technic measuring the resistance of the erythrocytes to solutions of lysolecithin. Guest and Wing (1939) developed a method for determining erythrocyte fragility using Van Allen hematocrit tubes. A known quantity of blood was placed in each of several tubes containing different salt solutions. The tubes were then centrifuged and the quantity of unhemolyzed red cells was measured. Enumeration of red cells remaining after dilution of blood in red cell pipettes was described by Whitby and Hynes (1935).

Dukes (1955) stated that initial hemolysis in bovine blood will begin in 0.59 percent NaCl solution; complete hemolysis is obtained in 0.42 percent NaCl solution. When the percent hemolysis is plotted against the corresponding salt solution, a sigmoid or cumulative curve results. Bolton (1949) presented such a curve. He also plotted a curve of the actual amount of hemolysis caused by saline solutions differing in strength. He described this curve as not following the normal distribution.

Wintrobe (1956) reported that increased osmotic fragility occurs in cases of acquired hemolytic anemia and is usually found in hereditary spherocytosis. Increased level of bilirubin in the blood plasma as well as an increased number of reticulocytes and nucleated red cells are evidence of such erythrocyte destruction.

Wintrobe suggested that normocytic anemia, defined as no decrease in mean volume or hemoglobin content of the red corpuscles even with severe anemia, may be caused by (1) hemorrhage (2) increased destruction of blood, and (3) decreased blood formation. In the case of humans he indicated that if blood loss is prolonged over a 24 hour period, an individual can lose 50 percent of the blood volume without fatality. However, if the loss is rapid, a 33 percent decrease may cause death. After hemorrhage the first noticeable effect in the blood is

an increase in platelets followed by polymorphonuclear leukocytosis, which may reach a maximum in two to five hours. An increase in white cell count up to 10 to 20 thousand is not unusual after hemorrhage. After 24 to 48 hours an increase in reticulocytes may be found and a maximum of five to fifteen percent increase is noted four to seven days after heavy blood loss. The red cell count, the volume of packed red cells, and the hemoglobin content decrease for several days after an initial increase following hemorrhage.

Various chemical agents may cause increased destruction of red cells in the blood. Cruz et al. (1941) described the use of acetylphenylhydrazine for producing experimental anemia in dogs and rabbits. Lawrason et al. (1949) studied the production of experimental anemia with phenylhydrazine in swine. Chanutin and Word (1955) found severe anemia in rats one week after administering acetylphenylhydrazine and 500 r of x-irradiation. When 500 r of x-irradiation alone was administered, a moderate degree of anemia was noted several weeks after the treatment.

Normal hematological values for the ox are given by Dukes (1955) as follows:

| | |
|---|---|
| Red cell count | 6.3 millions per cu. mm. |
| Leukocyte count | 5-12 thousand per cu. mm. |
| Lymphocytes | 2.7-6.9 thousand per cu. mm. |
| Neutrophils | 1.2-4.8 thousant per cu. mm. |

| | |
|---|---|
| Basophils | 0-100 per cu. mm. |
| Eosinophiles | .18 to 1.8 thousand per cu. mm. |
| Monocytes | .15 to 1.8 thousand per cu. mm. |
| Hematocrit | 40 percent packed red cells |
| Hemoglobin | 12.03 gm. per 100 ml. |

These values vary widely even in the normal healthy animal. They may be influenced by age, sex, muscular activity, psychic states, season, barometric pressure, life habits of the species and presence of disease. Rusoff and Piercy (1946) observed wide variations in blood values in dairy cattle. Hemoglobin values range from 8.91 to 18.60 mg. percent. Significant differences were found between herds (locations) as well as between months. Significant herd differences were found for erythrocyte count, leucocyte count, percentages of lymphocyte, neutrophils and monocytes. Wintrobe (1956) pointed out that in humans, males have a higher average red cell count, higher hemoglobin value and a higher hematocrit value than females. The red cell count in humans decreases with age, as do the hematocrit and hemoglobin values. Numbers of leucocytes decrease with an increase in age.

Several early workers, Student (1907), Berkson (1938), and Berkson et al. (1939) showed that the distribution of erythrocytes in the hemocytometer followed the Poisson distribution.

More recently however, Turner and Eadie (1957) discussed the possibility that the Poisson distribution does not describe fully the distribution of such relatively large objects as erythrocytes when in considerable density. These workers were concerned primarily with precision of the counts. In this respect, the binomial model appeared to be an improvement over the Poisson. The workers concluded that the Poisson distribution would apply to red cell counts better with more extreme dilutions of the cells than is usual in present techniques. This latter conclusion seems reasonable since the Poisson is a limiting form of the binomial distribution.

# SOURCE OF DATA AND TECHNIQUE

## Description of Animals

In these studies 118 Herefords were used. The genotype for the dwarf locus was known for each, barring the possibility of pedigree errors and mutation. The group included animals of three ages and were all normal in phenotype but were either homozygous or heterozygous with respect to the dwarf locus. The cows and yearlings of both sexes were raised at the Iowa State College beef experimental farm at Ankeny, as were part of the calves. The remainder of the calves were raised on an outlying beef experimental farm at Albia, Iowa.

The carriers either had a dwarf parent or, in the case of the cows, had produced a dwarf calf. The animals considered clean were either from lines presumed free of the dwarf gene, because no dwarfs had been produced in several generations, or their parents had passed a progeny test sufficient to make it highly probable that they were not carriers.

## Experiments

Four experiments were conducted to compare the blood characteristics of carrier and clean animals. The first experiment was on the effects of age and temperature in the two genotypes. This experiment was of a general nature to see which ages and which blood characteristics would be the most useful in a de-

tailed study. Since considerable variation exists in an animal bled at different times, a second experiment was set up on male and female calves of both genotypes to study in more detail the effect of age and the repeatability of observations taken six weeks apart. Another aspect of the problem was the difference in response of the two genotypes to certain hormones. Experiment three was an effort to clarify the usefulness of adrenalin as a stress agent in the study of dwarfism. The effect of adrenalin on animals of different genotypes had been studied previously by other workers and discrepancies existed in their results. To explore further the physiological response of animals of different genotypes to stress, hemorrhagic anemia was studied in experiment four.

Experiment 1

In this experiment 21 carrier and 13 clean cows, 16 carrier and six clean calves, and six carrier and six clean yearling steers were studied. Each had one blood sample taken, with no additional treatment of any kind being used on the animal. A carrier and a clean animal were bled consecutively so as to minimize the confounding of genotype with environmental sources of variation such as weather, feeding and handling.

A group of animals was bled in the first part of the morning and another group was bled soon after lunch so that ample

time was allowed for analysis of the samples. The atmospheric temperatures at these times were obtained from the Weather Bureau so that the effect of temperature on the animals could be studied.

The blood characteristics analyzed in this experiment were red cell count, white cell count, white cell differentials, percent hemoglobin, percent packed red cells and fragility of the erythrocytes. The differential white cell count was accomplished by counting 200 cells of the lymphocyte, neutrophil, monocyte, basophil and eosinophil types. The numbers obtained are in percent of total white cell count. For example, the actual number of lymphocytes was determined by dividing the number counted on the slide by two (putting on basis of 100 percent) and multiplying this result by the total white cell count. The percent packed red cells (hematocrit) is the portion of whole blood that the erythrocytes constitute. The other portion of the whole blood, then, is serum.

Experiment 2

The results of Experiment 1 indicated wide differences between ages (cows, yearlings, calves) in several of the blood characteristics. To examine more closely the age effect, calves of varying ages and of both sexes were bled without treatment. The calves were bled twice, six weeks apart, so the change which takes place could be estimated. Repeatability of

these two determinations was studied on 51 calves. Fifty-eight calves including 20 carrier males, 12 clean males, eight carrier females and 18 clean females were bled the first time. However, seven calves (two carrier males, four clean males, and one clean female) were not available for the second bleeding.

The information from Experiment 1 on red cell count and differential white cell counts did not appear to be helpful in classifying carrier and clean animals. These counts require considerable time to make so they were not continued in Experiment 2. The characteristics studied in Experiment 2 included percent hemoglobin, hematocrit, erythrocyte fragility and total white cell count.

Experiment 3

The effect of adrenalin as an aid in discriminating between carrier and clean animals was studied on 24 cows and 24 calves, 12 of each genotype age-subgroup. Preliminary work suggested that the maximum response to an injection of adrenalin occurred about 20 minutes after the injection. Also at 20 minutes the maximum difference existed between carrier and clean animals. After taking the first sample at "zero" time, the bleeding needle was left in the jugular vein after the blood was collected and adrenalin was injected immediately. Blood

samples were taken at 20 minutes and again at two hours after the injection to permit studying the physiological response to adrenalin. The dosage of adrenalin used was .4 cc. of 1:1000 dilution adrenalin chloride per 100 pounds body weight. This amount was diluted with eight cc. of 0.85 percent physiological saline solution before injection.

Erythrocyte fragility, percent hemoglobin, hematocrit and total white cell count were measured on all animals. The differential white cell count was examined on cows only.

Experiment 4

The recovery rate and production of erythrocytes in the different genotypes were studied by inducing hemorrhagic anemia in four carrier and four clean cows. A six inch bleeding cannula was inserted in the jugular vein and four cc. of blood per pound of body weight were removed. This operation required from 10 to 30 minutes for each animal. Blood samples were taken by the standard method 1, 2, 3, 4, 5, 9, 13 and 20 days after the original bleeding. Erythrocyte fragility, percent hemoglobin, hematocrit, erythrocyte count and total white cell numbers were studied.

The data from these eight animals were analyzed and, since no trends which seemed to discriminate between carrier and clean cows were found, no other animals were hemorrhaged. The cows used were of different ages but all were in about the same

stage of pregnancy.

## Hematological Techniques

The bleeding technique and the handling of the blood until analysis was similar for all experiments. The blood was drawn from the jugular vein into a 5 cc. or 10 cc. collection tube containing a powdered mixture of 3 parts ammonium oxalate and 2 parts potassium oxalate. The tube was then kept at approximately 37° centigrade until the analyses were made. A small temporary laboratory with necessary equipment to perform the desired blood analyses was set up at the Ankeny station. Thus, the blood could be kept in a thermostatically controlled oven and all of the analyses made within two hours after the samples were taken.

With the exception of the method for determining osmotic fragility of erythrocytes, conventional hematological laboratory procedures were used in all experiments.

### Standard techniques

The number of erythrocytes was determined by the method described by Bergman et al. (1948). Hayem's solution, the diluting solution used, was freshly prepared as needed. The percentage of packed red cells in the blood was determined with the Wintrobe hematocrit as described by Wintrobe (1956). The blood was drawn from the collection tube into a 10 cc. syringe

and then slowly expelled into the hematocrit. The syringe was selected for use because it was easier to wash and handle than the capillary pipette described by Wintrobe. The tubes were centrifuged for 30 minutes at 3350 R.P.M. to obtain complete packing.

The amount of total blood hemoglobin was determined by the acid hematin method described in the Klett-Sumerson clinical manual. Two hundredths of a milliliter of blood was mixed thoroughly with five milliliters of one-tenth normal hydrochloric acid and allowed to stand for 30 minutes. The samples were shaken gently and read in the Klett colorimeter with a green (54) filter against a distilled water blank. The hemoglobin factor used in converting the colorimeter readings to gram percent hemoglobin was obtained by using several samples of known hemoglobin concentration in the colorimeter, obtaining a calibration factor for each, and averaging these factors. All of the calibration factors were quite similar.

The techniques employed in determining the packed red cell volume and the hemoglobin content of the blood had not been used extensively by the author, so considerable time was spent in practising these techniques. Two determinations on each of 68 samples were made and the repeatability of techniques was calculated. Repeatabilities of 0.88 and 0.95 were obtained for hemoglobin determination and for percent packed red cells, respectively. Since two determinations on each

sample required considerable time and repeatabilities were relatively high, only one determination was made on each subsequent sample.

Total white cell counts were made as described by Bergman et al. (1948). The diluent used was five percent acetic acid. No attempt was made to count particles or objects as described by Swiger (1957), and Massey et al. (1956).

Smears of fresh whole blood were made and stained with a modification of Field's stain as described by Bergman. Two hundred cells were counted on each slide, differentiating among lymphocytes, neutrophils, eosinophiles, basophils and monocytes.

Fragility techniques

The measurement of erythrocyte fragility has been based on amount of hemolysis by varying the strength of sodium chloride solutions. Since these techniques involve several different solutions, they are time-consuming and require making a visual estimate of first appearance of hemolysis. In this work a more objective measurement of degree of hemolysis was needed. The method described by Hunter (1940) seemed adequate for this purpose and was studied.

In using this technique, eight cc. of sodium chloride solution were introduced into each of two colorimeter tubes

using a ten cc. pipette graduated in tenths of cc's. Into each of these tubes .4 cc. of whole blood was pipetted. One cc. of blood was drawn into a one cc. pipette graduated in tenths and .4 cc. was released into each of two tubes, with .2 cc. still remaining in the pipette. Two kinds of errors are possible with this replication. One is that unknowingly the technician may allow slightly more or slightly less than the standard amount to be released into the first tube. If this error were consistently in the same direction for all tubes, the correlation between determinations might be high, even though the estimation of each determination was biased. Secondly, if individual pipettes were not identical in their capacity, the variance between samples on different animals would be increased. These sources of error were checked by using individual pipettes for each aliquot of blood and both were undetectable.

After the blood was introduced into the saline solution, the tubes were inverted five times to insure thorough mixing and then were placed in a rack for 30 minutes to allow full hemolysis. The tubes were then centrifuged for ten minutes at 1700 R.P.M. The supernatant fluid was decanted and read in the Klett colorimeter against a distilled water blank. The samples having the higher degree of hemolysis gave the higher colorimeter reading. The colorimeter reading was the re-

corded observation for erythrocyte fragility.

Early work on this technique by Hazel (1958) indicated that a .56 percent saline solution gave readings in a range of 0 to 400 on the colorimeter, this being the desirable range described in the Klett-Sumerson manual. Later trials by the author indicated that a .58 percent saline solution gave values more nearly in the desired range. Values in Table 1 on four steers show the effects of strength of solution on the fragility of red blood cells.

Table 1. Hemolysis values in three different strengths of saline solution on two carriers and two clean yearling steers as determined by colorimeter reading

| Steer no. | Replicate | Carrier | | Clean | |
|---|---|---|---|---|---|
| | | 484 | 490 | 586 | 593 |
| 8 cc. of .56 percent saline with .2 cc. of blood | 1 | 266 | 710 | 382 | 674 |
| | 2 | 284 | 705 | 392 | 675 |
| 8 cc. of .58 percent saline with .4 cc. of blood | 1 | 132 | 634 | 187 | 519 |
| | 2 | 133 | 642 | 193 | 519 |
| 8 cc. of .60 percent saline with .4 cc. of blood | 1 | 80 | 402 | 118 | 329 |
| | 2 | 82 | 417 | 115 | 327 |

That animals of different age groups differed markedly in hemolysis was noted in a preliminary experiment, the results of which are presented in Table 2. The effect of age appeared linear, since cows showed least hemolysis, calves

Table 2. The effect of age on fragility of erythrocytes as determined by colorimeter reading

| Age | Animal number | Genotype | .58% saline | | Average of group | .60% saline | | Average of group |
|---|---|---|---|---|---|---|---|---|
| | | | Sample 1 | Sample 2 | | Sample 1 | Sample 2 | |
| Calves | 40 | Carrier | 421 | 427 | | 276 | 283 | |
| | 56 | Carrier | 351 | 342 | | 213 | 213 | |
| | 66 | Clean | 860 | 850 | 577 | 790 | 790 | 461 |
| | 33 | Clean | 682 | 682 | | 560 | 562 | |
| Year-lings | 484 | Carrier | 135 | 136 | | 69 | 65 | |
| | 475 | Carrier | 247 | 243 | | 153 | 148 | |
| | 586 | Clean | 228 | 228 | 212 | 111 | 109 | 121 |
| | 576 | Clean | 239 | 240 | | 152 | 164 | |
| Cows | 180 | Carrier | 53 | 49 | | 33 | 38 | |
| | 637 | Carrier | 37 | 34 | 140 | 23 | 29 | 93 |
| | 250 | Clean | 357 | 347 | | 230 | 228 | |
| | 263 | Clean | 118 | 122 | | 83 | 80 | |

showed the most and yearlings were intermediate between the two extremes. From this and other preliminary work, it was decided to use a .58 percent saline solution for cows and yearlings and a .60 percent saline solution for experiments concerning calves only.

The effect of temperature of the blood at the time the technique was performed was studied. Agreement between replicated samples was highest when the blood and the sodium chloride solution were kept at a constant temperature of 37° centigrade.

After considerable time had been spent in perfecting this technique, repeatabilities were calculated. The estimate of repeatability was 0.999 with 12 samples and two determinations on each sample. This estimate seemed so high that the experiment was repeated. Nearly the same estimate was obtained, this time being 0.998 with 22 samples involved. Owing to this high repeatability only one determination per animal was made in later work. Since the amount of equipment needed for a given set of animals was reduced by half, more animals could be sampled at any given time.

# ANALYSIS OF DATA

The main interest in the analysis is to learn how to discriminate between carrier and clean animals, that is, being able to tell a carrier animal from a clean by some diagnostic test. This type of analysis requires little statistics except in trying to better describe the results. Statistical analyses were used and tests of significance were made on the data in the first three experiments but, because the numbers were so few, statistical analyses were not made in Experiment 4. Graphs describe the results of Experiment 4 adequately.

The analyses will be presented separately by experiments, i.e. Experiment 1, Experiment 2, etc. Combining results from all experiments for a single blood characteristic so as to make it most useful in discriminating genotypes will be done in the discussion.

## Experiment 1 - Effect of Maturity and Genotype on Several Characteristics of Blood

### Erythrocyte fragility

The data for each blood characteristic were examined to see if they followed the normal distribution since in most analyses this is an underlying assumption. This was checked by plotting the distributions for the characteristics on normal probability paper. Except for erythrocyte fragility, all distributions appeared normal.

The distributions for erythrocyte fragility for the three age groups studied are shown in Figure 1. The measure of fragility is the colorimeter value. The distribution of the calves seemed rectangular, while the distribution of the cows resembled the Poisson. The steers appeared more normally distributed. Where all animals are grouped together, as represented by the solid black line, the distribution resembles the Poisson. The calves fall into two distinct groups, one of which overlaps the cows and yearlings, while the other is considerably higher. In Figure 1 the line for total at times coincides with the line for an age group, such as the group of calves on the right side of the figure. They are drawn just far enough apart that the two lines can be followed separately.

If a distribution is Poisson, a square root transformation of the data will make the distribution more normal. The distribution by this transformation is shown in Figure 2. The grouping is more compact, but the distribution still resembles a Poisson. The distribution of the untransformed data when plotted on normal probability paper is shown by the solid black line in Figure 3. Clearly the raw data do not follow a normal distribution, since normal data plotted on this paper would form a straight line. However, the transformed data represented by the broken line in Figure 3 do not appear wholly normal either.

Figure 1. Distribution of erythrocyte fragility among animals of different ages. The values for fragility are colorimeter readings.

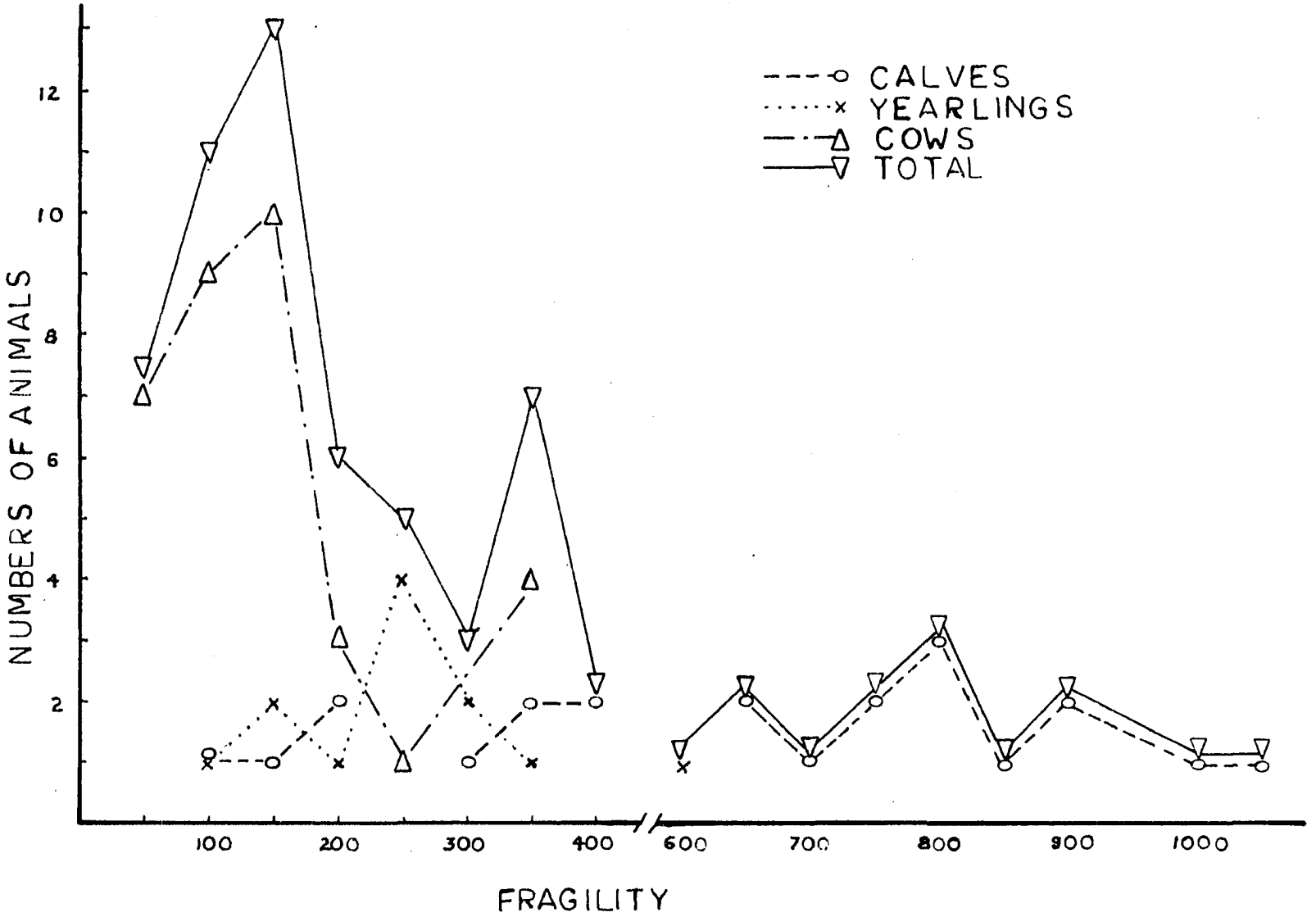

CALVES
YEARLINGS
COWS
TOTAL
NUMBERS OF ANIMALS
2
4
6
8
10
12
100
200
300
400
600
700
800
900
1000
FRAGILITY

Figure 2. Distribution of the square roots of fragility values.

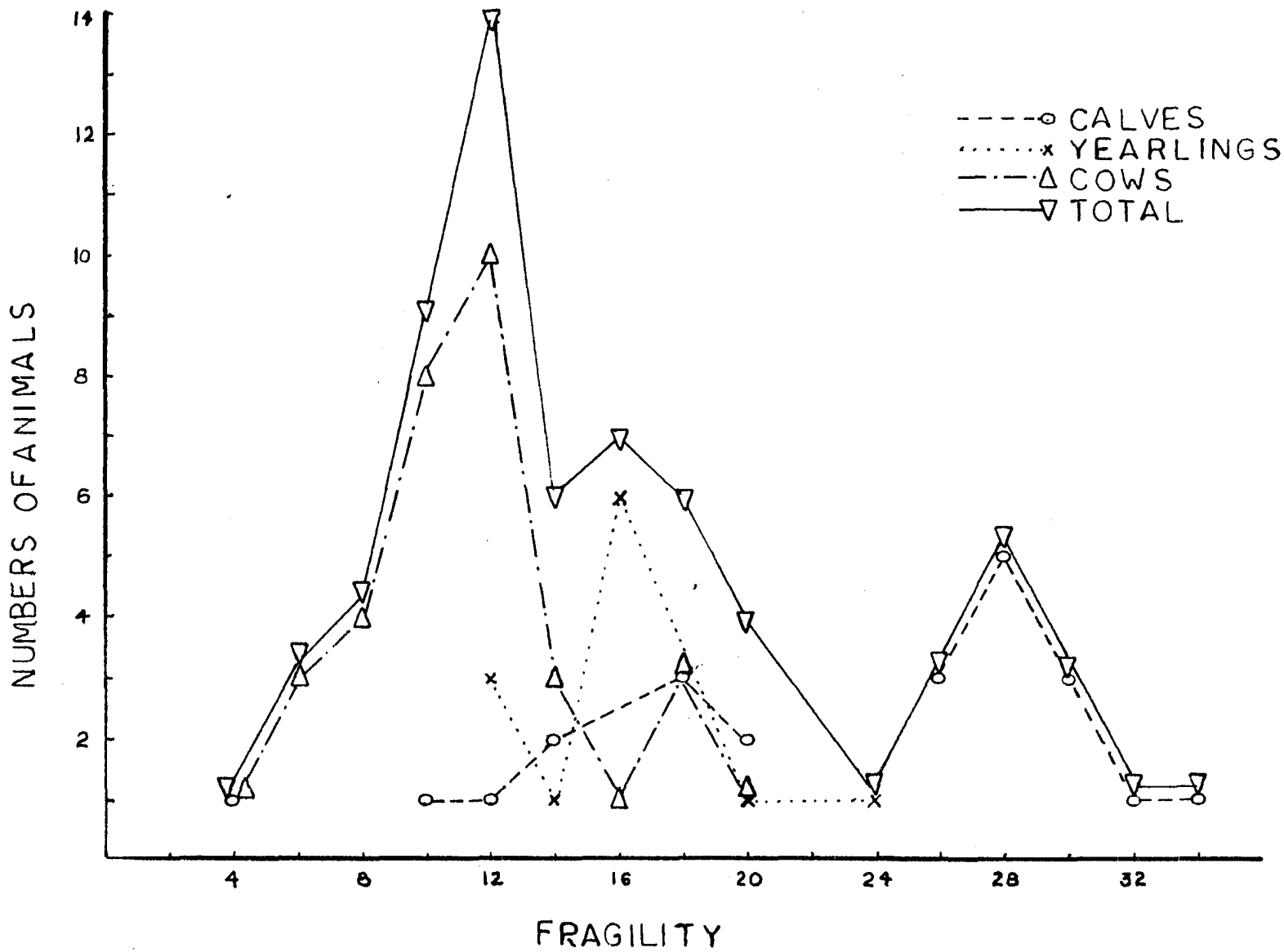
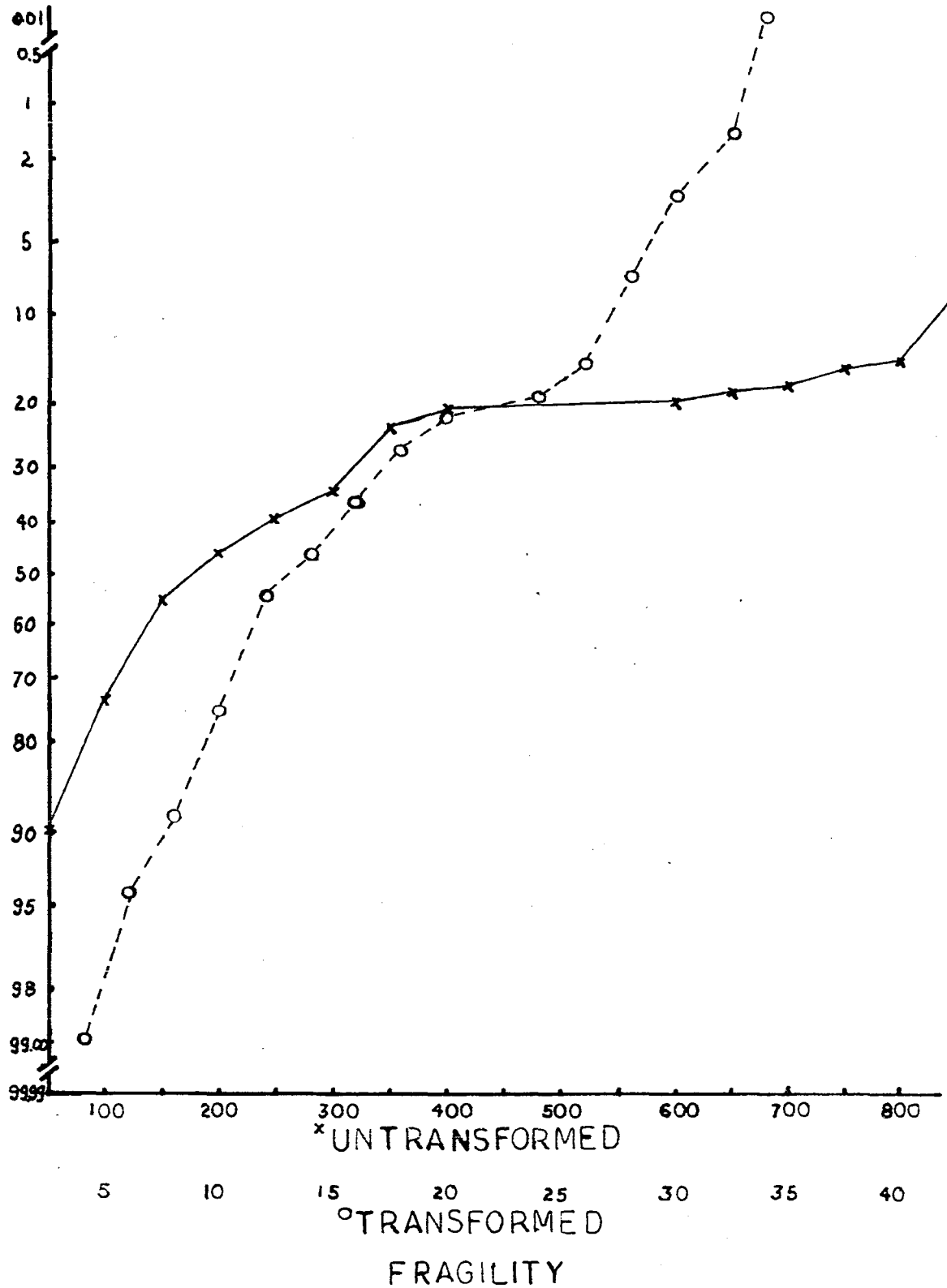

NUMBERS OF ANIMALS
14
12
10
8
6
4
2
4
8
12
16
20
24
28
32
FRAGILITY
CALVES
YEARLINGS
COWS
TOTAL

Figure 3. Frequency distribution of untransformed and transformed erythrocyte fragility values plotted on normal probability paper.


.001
0.5
1
2
5
10
20
30
40
50
60
70
80
90
95
98
99.8
99.9
100
200
300
400
500
600
700
800
×UNTRANSFORMED
5
10
15
20
25
30
35
40
°TRANSFORMED
FRAGILITY

In examining further the nature of the distribution of erythrocyte fragility, the means and variances of the age-genotype subgroups were calculated and are given in Table 3. Figure 4 indicates that in the raw data some proportionality exists between the mean and the variance. Figure 5 shows the corresponding relation for the square root transformation. The points are more on a horizontal line as they would be if mean and variance were independent except for point 6, the carrier calves. However, it is obvious that no transformation would bring point 6 into line with the other sub-groups.

Table 3. Means and variances of erythrocyte fragility, by age-genotype subgroups

| Age group | Genotype | No. of animals | Untransformed data | | Transformed data | |
|---|---|---|---|---|---|---|
| | | | Mean | Variance | Mean | Variance |
| Calves | Carrier | 16 | 578 | 113,775 | 22.9 | 57.2 |
| | Clean | 6 | 668 | 34,511 | 25.6 | 16.0 |
| Yearlings | Carrier | 6 | 206 | 4,338 | 14.2 | 5.6 |
| | Clean | 6 | 313 | 26,217 | 17.2 | 18.5 |
| Cows | Carrier | 21 | 130 | 9,356 | 10.7 | 16.1 |
| | Clean | 13 | 165 | 7,319 | 12.5 | 9.3 |

The variances given in Table 3 and the distributions of the different age groups as shown in Figures 1 and 2, suggest a lack of homogeneity of the variances in these data on erythrocyte fragility. The calves were distributed over a wide

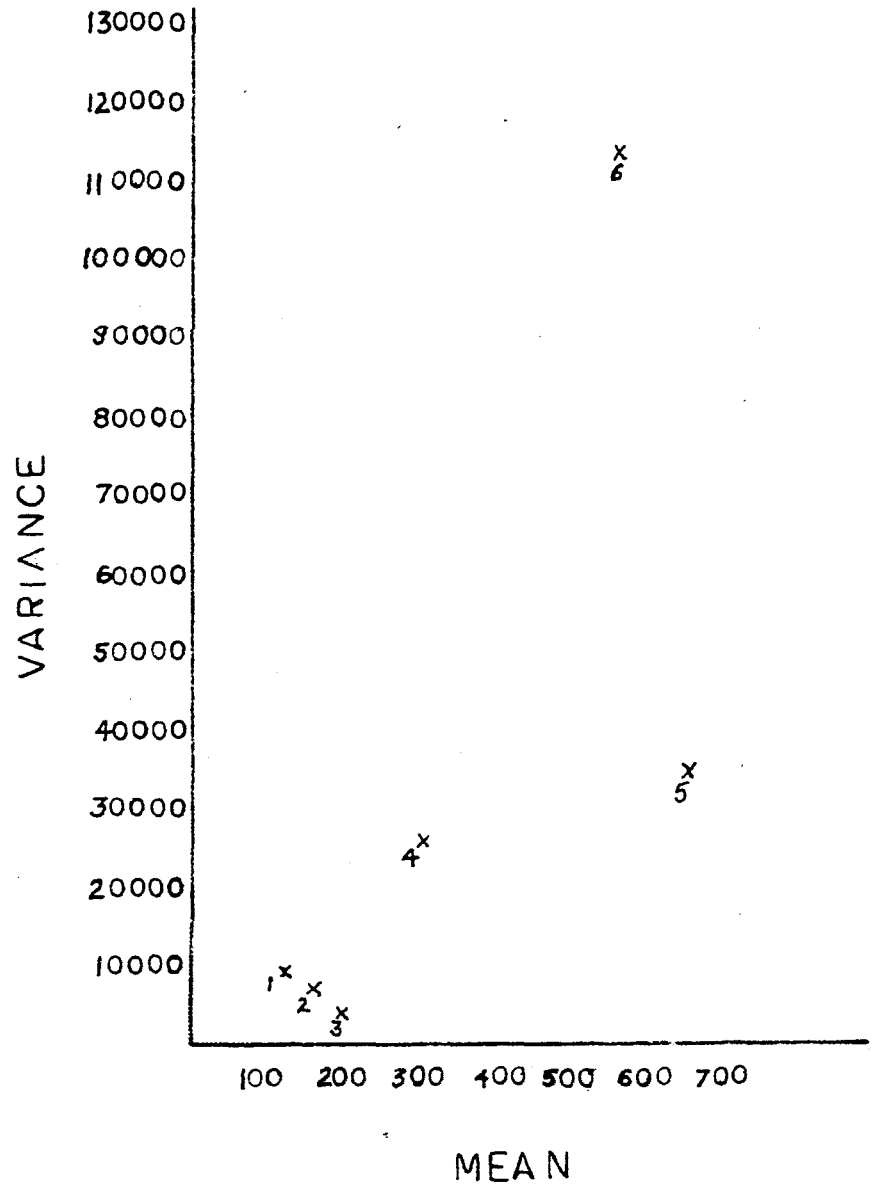



Figure 4. Means and variances for the untransformed fragility values.

1. carrier cows
2. clean cows
3. carrier steers
4. clean steers
5. clean calves
6. carrier calves

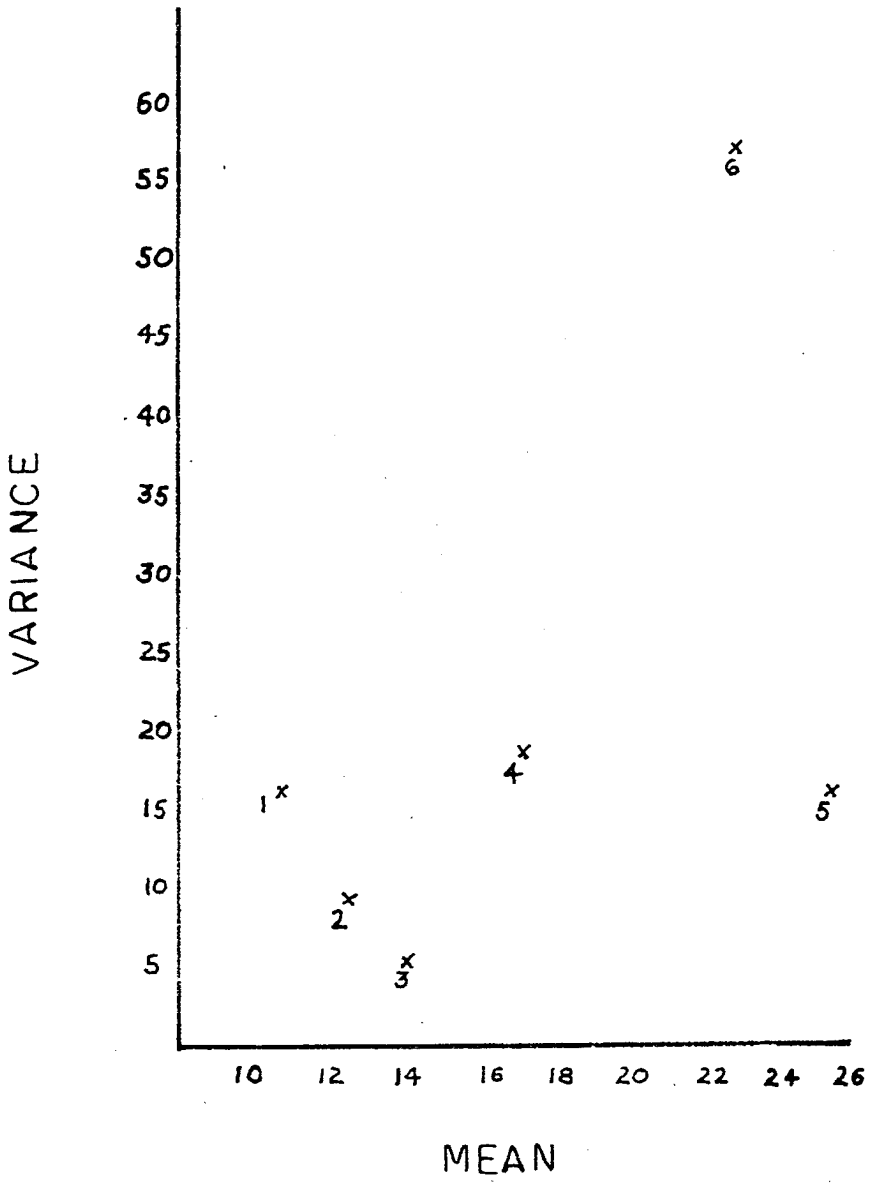



Figure 5. Means and variances for the transformed fragility values.

1. carrier cows
2. clean cows
3. carrier steers
4. clean steers
5. clean calves
6. carrier calves

range of fragilities, while the cows and yearlings occupied a much smaller segment of the entire range. Bartlett's test for homogeneity of variances gave Chi square values of 37.13 in the untransformed material and 11.11 in the transformed figures. These tests indicate that the variances were not homogeneous either in the untransformed or in the transformed data. No further efforts to find a transformation that would correct for non-normality and unequal variances were made since the number of animals in the various groups seemed too small to warrant a more detailed search for such a transformation.

The method of fitting constants was used in statistically analyzing the effects of age and genotype for each characteristic in Experiment 1 since the subclasses had unequal numbers. The analyses followed the method for a two-way classification described by Kempthorne (1952). This type of analysis is made on the premise that the main effects are uncorrelated. The correlations between the effects of age and genotype were checked for each characteristic by using the constants for each age and each genotype and the frequency of animals in each sub-group. The largest correlation, numerically, between the effects of age and genotype was for erythrocyte fragility which was -0.11. None of the correlations were significant.

The interactions between age and genotype were tested for significance as shown in Table 4. If an interaction was non-

Table 4. Analysis of variance (mean squares) for interaction of age and genotype of blood characteristics in Experiment 1

| Source of variation | Age x genotype | Residual |
|---|---|---|
| d.f. | 2 | 62 |
| Erythrocyte fragility | 7612 | 37,207 |
| Square root transformation of fragility | 4.31 | 7.78 |
| Percent hemoglobin | 9.93** | .65 |
| Percent hemoglobin adjusted for temperature | 7.75** | .78 |
| Hematocrit | 42.89 | 11.18 |
| Hematocrit adjusted for temperature | 55.99** | 9.90 |
| White cell count | 3007 | 1580 |
| Red cell count | 3.34* | .78 |
| Lymphocytes | 2092 | 705 |
| Neutrophils | 2102** | 489 |
| Mean corpuscular hemoglobin | .83 | 2.62 |
| Mean corpuscular volume | 15 | 41 |
| Mean corpuscular concentration | 14.08** | 1.59 |

*Significant at P = .05

**Significant at P = .01

significant, a test for significance of the main effects was made and is shown in Table 5. If the interaction was significant, a test for the separate significance of age and genotype was not made, but the difference between genotypes was analyzed within each age-group.

As shown in Table 6, erythrocyte fragility appeared to be of little value for discriminating between carrier and clean animals. There was no definite separation between genotypes in any of the age groups. The animals with a clean genotype averaged higher than the carrier animals in each of the three age groups, but none of the differences were significant as shown in Table 5. A significant difference between ages was found but it must be remembered that these data did not appear to follow the normal distribution nor were variances of the age groups homogeneous. The use of the square root transformation offered no better means of separating the carrier and clean animals than the untransformed values due to overlap between genotypes. This is shown in Table 7. However, the averages for the genotypes differed significantly in the transformed data.

Erythrocyte count, hematocrit and percent hemoglobin

These characteristics of the blood were more nearly normally distributed when plotted on normal probability paper than was erythrocyte fragility. The variances for the age

Table 5. Analysis of variance (mean squares) of blood characteristics in which there was no interaction between age and genotype

| Source of variation | d.f. | Erythrocyte fragility | Square root transformation of fragility | White cell count | Lymphocytes | Mean corpuscular hemoglobin | Mean corpuscular volume |
|---|---|---|---|---|---|---|---|
| Ages | 2 | 1,458,281** | 1,034.89** | 36,295** | 56,539** | 119.43** | 2374** |
| Genotypes | 1 | 64,145 | 80.85** | 315 | 904 | 30.88** | 189 |
| Residual | 64 | 36,283 | 7.60 | 1,625 | 749 | 2.56 | 40 |

**Significant at P = .01

Table 6. Distribution of fragility values by ages and genotypes

| | Calves | | Yearlings | | Cows | |
|---|---|---|---|---|---|---|
| Fragility | Carrier | Clean | Carrier | Clean | Carrier | Clean |
| 1050 | 1 | | | | | |
| 1000 | 1 | | | | | |
| 950 | 2 | | | | | |
| 900 | | | | | | |
| 850 | | 1 | | | | |
| 800 | 3 | | | | | |
| 750 | | 2 | | | | |
| 700 | | 1 | | | | |
| 650 | 1 | 1 | | | | |
| 600 | | | | 1 | | |
| 550 | | | | | | |
| 500 | | | | | | |
| 450 | | | | | | |
| 400 | 2 | | | | | |
| 350 | 2 | | | 1 | 2 | 2 |
| 300 | | 1 | 1 | 1 | | |
| 250 | | | 2 | 2 | 1 | |
| 200 | 2 | | 1 | | 2 | 1 |
| 150 | 1 | | 1 | 1 | 4 | 6 |
| 100 | 1 | | 1 | | 5 | 4 |
| 50 | | | | | 7 | |
| Mean | 577.7 | 667.9 | 206.0 | 312.6 | 129.9 | 164.8 |
| Number of animals | 16 | 6 | 6 | 6 | 21 | 13 |

Table 7. Distribution of the square root of the fragility values, by ages and genotype

| Square root of fragility colorimeter reading | Calves | | Yearlings | | Cows | |
|---|---|---|---|---|---|---|
| | Carrier | Clean | Carrier | Clean | Carrier | Clean |
| 34 | 1 | | | | | |
| 32 | 1 | | | | | |
| 30 | 2 | 1 | | | | |
| 28 | 3 | 2 | | | | |
| 26 | 1 | 2 | | | | |
| 24 | | | | 1 | | |
| 22 | | | | | | |
| 20 | 2 | | | 1 | 1 | |
| 18 | 2 | 1 | | | 1 | 2 |
| 16 | | | 3 | 3 | 1 | |
| 14 | 2 | | 1 | | 2 | 1 |
| 12 | 1 | | 2 | 1 | 4 | 6 |
| 10 | 1 | | | | 5 | 3 |
| 8 | | | | | 3 | 1 |
| 6 | | | | | 3 | |
| 4 | | | | | 1 | |
| Mean | 22.91 | 25.58 | 14.19 | 17.24 | 10.70 | 12.50 |
| No. of animals | 16 | 6 | 6 | 6 | 21 | 13 |

Table 8. Distribution of red cell counts by ages and genotypes

| Millions of red cells | Calves | | Yearlings | | Cows | |
|---|---|---|---|---|---|---|
| | Carrier | Clean | Carrier | Clean | Carrier | Clean |
| 12.5 | 1 | | | | | |
| 12.0 | | | | | | |
| 11.5 | 4 | 1 | | | | |
| 11.0 | 4 | | | | | |
| 10.5 | 4 | 2 | | | | |
| 10.0 | 2 | 2 | | | | |
| 9.5 | | | | | | |
| 9.0 | 1 | 1 | | 2 | | 2 |
| 8.5 | | | 1 | 3 | 1 | |
| 8.0 | | | | 1 | 1 | 6 |
| 7.5 | | | 1 | | 5 | 1 |
| 7.0 | | | 3 | | 5 | 2 |
| 6.5 | | | | | 3 | 1 |
| 6.0 | | | | | 3 | |
| 5.5 | | | 1 | | 2 | |
| 5.0 | | | | | | 1 |
| 4.5 | | | | | 1 | |
| Mean | 10.80 | 10.38 | 7.11 | 8.54 | 6.79 | 7.51 |
| No. of animals | 16 | 6 | 6 | 6 | 21 | 13 |

groups were homogeneous. Therefore the tests of significance are more dependable for these characteristics. Erythrocyte count showed the least promise for discriminating genotypes. The overlap of the genotypes for erythrocyte count is shown in Table 8. A significant interaction between age and genotype existed, so the difference between genotypes was analyzed within each age group. No significant difference was found

between genotypes for erythrocyte count in any of the age groups but, since the numbers were small, the fact that the differences between genotypes were not significant does not mean that they were not real.

The distributions for hematocrit (Table 9) and for percent hemoglobin (Table 10) show significant interactions between age and genotypes. The tests for interaction are shown in Table 4. When examined within age groups, significant differences between genotypes were found. Separation of the genotypes for both these characteristics apparently can be made in calves and yearlings with only slight errors. This is a bit perplexing since reversal of genotypes occurred in the calves and yearlings. The overlap between carrier and clean cows was sufficient to prevent any clear cut separation or significant differences in either blood characteristic in this age group.

Genotypes of calves and yearlings were predicted by looking at the data and separating the two genotypes at whatever level would result in the fewest mistakes. The separation was made at different points in calves and yearlings, as represented by the dotted lines in Tables 9 and 10. Any tests of significance on the correlations between predicted and actual genotypes are biased since the point of separation was not established prior to the test. However, these correla-

Table 9. Distribution for hematocrit by ages and genotype

| Hematocrit | Calves | | Yearlings | | Cows | |
|---|---|---|---|---|---|---|
| | Carrier | Clean | Carrier | Clean | Carrier | Clean |
| 47 | | | | | | 1 |
| 46 | 1 | | | | 2 | |
| 45 | 2 | | | | | |
| 44 | 1 | 1 | | | 2 | 2 |
| 43 | 2 | | | | 1 | 3 |
| 42 | 4 | | | | 2 | 1 |
| 41 | 2 | | | 2 | | |
| 40 | | | | 1 | 2 | |
| 39 | 1 | 2 | 1 | 3 | 3 | |
| 38 | 3 | 1 | | | | |
| 37 | | | 1 | | 2 | 1 |
| 36 | | 1 | 1 | | | 1 |
| 35 | | 1 | 1 | | 3 | 1 |
| 34 | | | 1 | | | 1 |
| 33 | | | | | | |
| 32 | | | | | | |
| 31 | | | | | | |
| 30 | | | | | | |
| Mean | 41.8 | 38.4 | 36.4 | 39.6 | 39.2 | 40.1 |
| No. animals | 16 | 6 | 6 | 6 | 21 | 13 |

Table 10. Distribution for percent hemoglobin by ages and genotype

| Percent hemoglobin | Calves | | Yearlings | | Cows | |
|---|---|---|---|---|---|---|
| | Carrier | Clean | Carrier | Clean | Carrier | Clean |
| 10.4 | 2 | | | | | |
| 10.0 | 3 | | | | 1 | |
| 9.6 | 2 | | | 1 | 1 | |
| 9.2 | 1 | | | 1 | 2 | 3 |
| 8.8 | 1 | | 1 | 4 | 3 | 2 |
| 8.4 | 2 | 1 | 1 | | 3 | 1 |
| 8.0 | 4 | | 3 | | 6 | 2 |
| 7.6 | 1 | | 1 | | 2 | 3 |
| 7.2 | | 2 | | | 2 | 1 |
| 6.8 | | 2 | | | | 1 |
| 6.4 | | 1 | | | 1 | |
| Mean | 9.1 | 7.1 | 8.2 | 9.0 | 8.3 | 8.2 |
| No. animals | 16 | 6 | 6 | 6 | 21 | 13 |

tions are useful in suggesting which blood characteristic affords more accurate prediction. They are shown in Table 11. The numbers of animals are small and little reliance on these high correlations is justified.

Table 11. Correlation between actual and predicted genotypes based on hemoglobin and hematocrit in calves and yearlings

| Predicted genotype | Actual genotypes | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Percent hemoglobin | | | | Hematocrit | | | |
| | Calves | | Yearlings | | Calves | | Yearlings | |
| | NN | Nn | NN | Nn | NN | Nn | NN | Nn |
| Clean | 5 | 0 | 6 | 1 | 5 | 4 | 6 | 1 |
| Carrier | 1 | 16 | 0 | 5 | 1 | 12 | 0 | 5 |
| Correlation | .88 | | .84 | | .53 | | .84 | |

To explore further the age-genotype interaction for percent hemoglobin and erythrocyte count, logarithm and square root transformations were used. Changing the scale of measurement by a transformation often helps to increase homogeneity of variance and decrease non-additivity of effects, in this case the effects of age and genotype. These transformed data were analyzed both for percent hemoglobin and for erythrocyte count, with the results shown in Table 12. The F value of the age-genotype interaction was larger for the transformed data in both cases than in the untransformed data.

These transformations, then, helped little to diminish or clarify the interactions between age and genotype.

Table 12. Analysis of variance for the age-genotype interaction when transformations of percent hemoglobin and erythrocyte count were used

| | d.f. | Log of percent hemoglobin | Square root of percent hemoglobin | Log of Erythrocyte count | Square root of erythrocyte count |
|---|---|---|---|---|---|
| Age x genotype | 2 | .0470* | .5049* | .0195* | .2055* |
| Residual | 62 | .0017 | .0195 | .0028 | .0263 |

*Significant at .01 level

Mean corpuscular concentration, mean corpuscular hemoglobin, and mean corpuscular volume

These are useful ratios calculated from the red cell count, hematocrit and the hemoglobin content. The M.C.C., mean corpuscular concentration, is the ratio of the amount of hemoglobin to the percent packed red cells. This indicates the concentration of hemoglobin for a given volume of red cells. These values are shown in Table 13. This criterion of classification appeared to give good results in classifying the genotypes of calves but failed to separate the genotypes in the other age groups. The interaction between age and genotype for M.C.C. was significant, as shown in Table 4.

Table 13. Distribution of mean corpuscular concentration by ages and genotype

| Percent mean corpuscular concentration | Calves | | Yearlings | | Cows | |
|---|---|---|---|---|---|---|
| | Carrier | Clean | Carrier | Clean | Carrier | Clean |
| 24.4 | 1 | | | | | |
| 24.0 | | | | | | |
| 23.6 | | | | 1 | 1 | |
| 23.2 | 2 | | 2 | 1 | 1 | |
| 22.8 | 1 | | 1 | | 1 | |
| 22.4 | 2 | | 1 | 3 | 2 | |
| 22.0 | 1 | | | 1 | | 1 |
| 21.6 | 2 | | 1 | | 5 | 2 |
| 21.2 | 3 | | | | 1 | 1 |
| 20.8 | | | 1 | | 3 | 2 |
| 20.4 | | | | | 1 | 5 |
| 20.0 | | | | | 3 | 1 |
| 19.6 | 3 | | | | 1 | |
| 19.2 | 1 | | | | | |
| 18.8 | | 2 | | | 2 | |
| 18.4 | | 2 | | | | |
| 18.0 | | 1 | | | | |
| 17.6 | | 1 | | | | |
| 17.2 | | | | | | 1 |
| Mean | 21.6 | 18.4 | 22.4 | 22.7 | 21.2 | 20.5 |
| No. of animals | 16 | 6 | 6 | 6 | 21 | 13 |

The mean corpuscular hemoglobin, M.C.H., is the ratio of percent hemoglobin to number of erythrocytes. This is an estimate of the amount of hemoglobin per red cell for a given sample of blood. These values for the different ages and genotypes are given in Table 14. M.C.H. has only slight value for discriminating between carrier and clean genotypes but the

means of the clean animals are consistently and significantly lower than the means of the carrier animals for this characteristic.

Table 14. Distribution of mean corpuscular hemoglobin by ages and genotypes

| Mean corpuscular hemoglobin | Calves | | Yearlings | | Cows | |
|---|---|---|---|---|---|---|
| | Carrier | Clean | Carrier | Clean | Carrier | Clean |
| 17.0 | | | | | | 1 |
| 16.5 | | | 1 | | | |
| 16.0 | | | | | 1 | |
| 15.5 | | | | | | |
| 15.0 | | | | | 1 | |
| 14.5 | | | | | | |
| 14.0 | | | 1 | | | |
| 13.5 | | | | | 2 | 1 |
| 13.0 | | | | | 2 | |
| 12.5 | | | 1 | | 2 | |
| 12.0 | | | 1 | | 7 | 1 |
| 11.5 | | | | | 1 | 2 |
| 11.0 | | | | 2 | 4 | 1 |
| 10.5 | | | 1 | 2 | 1 | 1 |
| 10.0 | 1 | | | 2 | | 3 |
| 9.5 | 3 | | 1 | | | 1 |
| 9.0 | 2 | | | | | 1 |
| 8.5 | 4 | | | | | 1 |
| 8.0 | 1 | | | | | 1 |
| 7.5 | 3 | 1 | | | | |
| 7.0 | 1 | 2 | | | | |
| 6.5 | 1 | 2 | | | | |
| 6.0 | | 1 | | | | |
| Mean | 8.4 | 6.8 | 12.5 | 10.5 | 12.3 | 11.2 |
| No. of animals | 16 | 6 | 6 | 6 | 21 | 13 |

The mean corpuscular volume, M.C.V., is the ratio of the percent packed red cells to the red cell count. This characteristic also was of little help in classifying carrier and clean genotypes. The distribution of the animals for this characteristic is presented in Table 15. The average of the clean animals is significantly lower than that of the carrier animals. No significant interaction existed between age and genotype.

Table 15. Distribution for mean corpuscular volume by ages and genotype

| Mean corpuscular volume | Calves | | Yearlings | | Cows | |
|---|---|---|---|---|---|---|
| | Carrier | Clean | Carrier | Clean | Carrier | Clean |
| 85 | | | | | | 1 |
| 80 | | | | | 1 | |
| 75 | | | | | | |
| 70 | | | | | | |
| 65 | | | 1 | | 4 | |
| 60 | | | | | 5 | 2 |
| 55 | | | 1 | | 8 | 4 |
| 50 | | | 3 | 1 | 3 | 3 |
| 45 | | | | 5 | | 2 |
| 40 | 10 | 3 | 1 | | | 1 |
| 35 | 6 | 3 | | | | |
| Mean | 38.8 | 37.2 | 52.1 | 46.4 | 48.3 | 54.6 |
| No. of animals | 16 | 6 | 6 | 6 | 21 | 13 |

Total white cell count and differential white cell count

Swiger (1957) and Massey et al. (1956) studied these characteristics as a means of discriminating between genotypes. They worked on the white cell response to a hormonal injection. However, in this work, Experiment 1, each white cell count was on a single blood sample with no treatment of the animal. The total white cell counts for the different ages and sexes were distributed as shown in Table 16. Classifying the animals into two genotypes by white cell count was impossible because the genotypes overlapped so much but ages differed significantly.

Table 16. Distribution for white cell count by ages and genotypes

| Thousands of white cells | Calves | | Yearlings | | Cows | |
|---|---|---|---|---|---|---|
| | Carrier | Clean | Carrier | Clean | Carrier | Clean |
| 17 | 2 | | | | | |
| 16 | | | | | | |
| 15 | | | | | | |
| 14 | | 1 | | 1 | | |
| 13 | 2 | | | | | |
| 12 | 5 | 2 | 3 | | | 1 |
| 11 | 4 | 1 | 1 | 1 | 2 | 3 |
| 10 | 2 | | 1 | 2 | 1 | 2 |
| 9 | 1 | 2 | | 1 | 5 | 1 |
| 8 | | | 1 | 1 | 2 | 3 |
| 7 | | | | | 5 | 1 |
| 6 | | | | | 6 | 2 |
| Mean | 12,112 | 11,158 | 10,925 | 10,358 | 7,824 | 8,988 |
| No. of animals | 16 | 6 | 6 | 6 | 21 | 13 |

The kinds of cells included in the total white cell count are the lymphocyte, neutrophil, eosinophil, basophil and monocyte. Differential white cell counts were made and the actual number of each kind were determined. The overlap between genotypes was large for lymphocytes and no significant difference between genotypes was found. The distributions of lymphocytes are shown in Table 17. Ages differed significantly in lymphocyte number with the calves having the highest count, cows the lowest and yearlings intermediate. The overlap between genotypes was also large for numbers of neutrophils as shown in Table 18. The interaction between age and genotype for neutrophil count was significant. The carrier and clean calves differed significantly, when the differences between genotypes were analyzed separately for each age. The genotypes for the other age groups did not differ significantly. The eosinophils, basophils and monocytes are relatively scarce in normal counts. None of these three kinds of cells showed trends for age or genotype.

Effect of temperature

The bleeding of animals in Experiment 1 extended over a period of six weeks. The atmospheric temperature varied considerably over this period. To see whether temperature and these characteristics varied together, regression and correlation coefficients were calculated and are shown in Table 19.

Table 17. Distribution for number of lymphocytes by ages and genotypes

| Thousands of lymphocytes | Calves | | Yearlings | | Cows | |
|---|---|---|---|---|---|---|
| | Carrier | Clean | Carrier | Clean | Carrier | Clean |
| 11.5 | 1 | | | | | |
| 11.0 | | 1 | | | | |
| 10.5 | | | | | | |
| 10.0 | | 1 | | | | |
| 9.5 | 1 | 1 | | | | |
| 9.0 | 2 | | 1 | | | |
| 8.5 | 2 | | 1 | | | |
| 8.0 | 2 | 1 | | | | |
| 7.5 | 4 | | 2 | 1 | | |
| 7.0 | 1 | | 1 | 1 | | |
| 6.5 | 1 | 1 | | 2 | | 1 |
| 6.0 | 2 | 1 | 1 | | 1 | 1 |
| 5.5 | 2 | | | 1 | | |
| 5.0 | | | | 1 | 2 | 3 |
| 4.5 | | | | | | 2 |
| 4.0 | | | | | 3 | 2 |
| 3.5 | | | | | 3 | |
| 3.0 | | | | | 5 | 2 |
| 2.5 | | | | | 5 | 2 |
| 2.0 | | | | | 2 | |
| Mean | 7,904 | 8,477 | 7,714 | 6,446 | 3,362 | 4,295 |
| No. of animals | 16 | 6 | 6 | 6 | 21 | 13 |

Table 18. Distribution for number of neutrophils by ages and genotypes

| Thousands of neutrophils | Calves | | Yearlings | | Cows | |
|---|---|---|---|---|---|---|
| | Carrier | Clean | Carrier | Clean | Carrier | Clean |
| 5.75 | 1 | | | 1 | | |
| 5.50 | 1 | | | | | |
| 5.25 | 1 | | | | | |
| 5.00 | | | | | 1 | 1 |
| 4.75 | 1 | | | | 1 | |

Table 18. (Continued)

| Thousands of neutrophils | Calves | | Yearlings | | Cows | |
|---|---|---|---|---|---|---|
| | Carrier | Clean | Carrier | Clean | Carrier | Clean |
| 4.50 | | | | | 1 | |
| 4.25 | 1 | | | | | |
| 4.00 | 2 | | | | | 2 |
| 3.75 | 2 | | | | 1 | 1 |
| 3.50 | 2 | | | | 3 | 1 |
| 3.25 | | | 1 | | 2 | |
| 3.00 | 1 | | 3 | 2 | | 4 |
| 2.75 | | 1 | | | 2 | 3 |
| 2.50 | 1 | | | | 2 | |
| 2.25 | | 1 | | 1 | 1 | 1 |
| 2.00 | 2 | 1 | 1 | 2 | 5 | |
| 1.75 | 1 | 2 | | | 2 | |
| 1.50 | | 1 | | | | |
| 1.25 | | | 1 | | | |
| Mean | 3,835 | 1,982 | 2,571 | 2,987 | 2,932 | 3,283 |
| No. of animals | 16 | 6 | 6 | 6 | 21 | 13 |

Table 19. The correlation coefficients and the regressions of blood characteristics on atmospheric temperature at time of bleeding

| Statistic | d.f. | Fragility | Percent hemoglobin | Hematocrit | Erythrocyte count | White cell count | Lymphocyte count | Neutrophil count |
|---|---|---|---|---|---|---|---|---|
| b | 61 | .08 | -.02* | -.10** | .00 | -.09 | -.11 | -.24 |
| r | 61 | .01 | -.28* | -.34** | .00 | -.03 | -.05 | -.13 |

*Significant at P = .05

**Significant at P = .01

Temperature was the independent variable in all of the regressions.

The regressions of percent hemoglobin and hematocrit on temperature were both significant, while the regressions of the other blood characteristics on temperature were not. These regressions indicate that as the temperature rose one degree, the hemoglobin decreased by 0.02 grams per ml. blood and the hematocrit decreased by 0.1 percent. The hematocrit may be interpreted another way by saying that the serum portion of the whole blood increased 0.1 percent as the temperature increased one degree.

Since there was a significant regression of percent hemoglobin and hematocrit on atmospheric temperature, the raw data for these two characteristics were corrected for temperature. This correction made little difference in the attempt to distinguish between carrier and clean animals and, as shown in Table 4, the interaction between age and genotype remained significant.

## Experiment 2 - Effect of Sex and Age on Blood Characteristics of Carrier and Clean Calves

The calves were bled twice, at intervals of six weeks, in order to study the repeatability of blood values on the same animal. The two bleedings were analyzed separately since

some of the calves in the first group did not appear in the second. The fragility, percent hemoglobin, percent packed red cells, mean corpuscular concentration, and total white cell count were studied. All laboratory procedures applied in Experiment 2 were the same as in Experiment 1 except for erythrocyte fragility. Since readings above 400 on the colorimeter tend to be inaccurate, .60 percent saline solution was used for the fragility tests instead of 0.58 percent in order that fragility readings would be in a more desirable range.

Unequal numbers also existed in the sub-classes in this experiment so constants were fitted as in Experiment 1. Sex and genotype were the main effects considered and no significant correlations were found between these for any of the blood characteristics.

No significant interaction was found between sex and genotype for any of the characteristics studied in the first bleeding of Experiment 2. The error term used in testing the sex-genotype interaction was pooled with this interaction to form the "residual" error term used in testing the main effects, age and genotype. The mean squares for the main effects are shown in Table 20.

Erythrocyte fragility was the only characteristic of the blood in which significant differences between means of

Table 20. Mean squares for the first bleeding of calves in Experiment 2

| Source of variation | d.f. | Fragility of erythrocytes | Percent hemoglobin | Percent packed red cells | White cell count | Mean corp. concent. | White cell count adjusted for age of calf |
|---|---|---|---|---|---|---|---|
| Sex | 1 | 124,404 | 14.29** | 22.26 | 1,727 | 51.12** | 5234 |
| Genotype | 1 | 356,383** | 2.61 | 22.84 | 1,461 | 2.77 | 1879 |
| Residual | 55 | 41,221 | 1.18 | 14.35 | 2,354 | 3.00 | 2130 |

**Significant at P = .01

carrier and clean animals were found. The clean calves averaged higher in fragility than the carrier calves, but the overlap between genotypes prohibited discriminating between carriers and cleans. No significant difference was found between sexes in erythrocyte fragility in the first bleeding but significant differences did exist between sexes for percent hemoglobin and for mean corpuscular concentration. The means of the various characteristics are given in Table 21 for each sex and genotype sub-group.

In the second bleeding of Experiment 2, a significant interaction between sex and genotype was found for erythrocyte fragility. No significant difference between genotypes was found in erythrocyte fragility when the sexes were analyzed separately. No significant interaction between sex and geno-

type was found for the other characteristics studied, percent hemoglobin, hematocrit, white cell count and mean corpuscular hemoglobin. Sex and genotype were separately tested for significance by the same method as in the first bleeding. The mean squaresfor these main effects are shown in Table 22. No significant difference was found between genotypes for any of the characteristics studied in the second bleeding. The sexes differed significantly for percent hemoglobin and for white cell count but not for mean corpuscular hemoglobin.

Differences between the first and second bleeding were calculated for each animal. The interactions between sex and genotype for the difference between bleedings were non-significant for all characteristics studied. The main effects were then tested by the pooled residual term as shown in Table 23. The difference between bleedings was tested to see if this difference (bleeding 1 - bleeding 2) was significantly different from zero. The difference between sexes changed significantly from one bleeding to the next for all characteristics except erythrocyte fragility, and white cell count. The significance of the mean difference between first and second bleeding was not tested for these characteristics because of the real interaction between sex and bleeding period. In all characteristics of the blood studied in Experiment 2 there appeared to be no interaction between genotype and bleeding period. Since there was no interaction be-

Table 21. Means of sex and genotype sub-groups for calves at two different bleedings in Experiment 2

| Bleeding period | Sex | Genotype | No. of calves | Grams percent hemoglobin | Percent packed red cells | Fragility of erythrocytes | Total white cell count | Mean corpuscular concentration |
|---|---|---|---|---|---|---|---|---|
| First | Male | Carrier | 20 | 8.2 | 38.8 | 256 | 9,580 | 21.1 |
| | | Clean | 12 | 8.5 | 38.9 | 345 | 9,945 | 21.6 |
| | Mean | | 32 | 8.3 | 38.8 | 289 | 9,717 | 21.3 |
| | Female | Carrier | 8 | 6.9 | 35.9 | 254 | 9,945 | 19.2 |
| | | Clean | 18 | 7.6 | 38.8 | 523 | 10,695 | 19.6 |
| | Mean | | 26 | 7.4 | 37.9 | 440 | 10,464 | 19.5 |
| | Mean of carrier | | 28 | 7.8 | 38.0 | 255 | 9,684 | 20.5 |
| | Mean of clean | | 30 | 7.9 | 38.9 | 451 | 10,395 | 20.4 |
| | Mean | | 58 | 7.9 | 38.4 | 357 | 10,050 | 20.5 |
| Second | Male | Carrier | 18 | 8.1 | 38.3 | 175 | 11,175 | 21.2 |
| | | Clean | 8 | 7.6 | 36.9 | 197 | 12,200 | 20.8 |
| | Mean | | 26 | 7.9 | 37.9 | 182 | 11,490 | 21.1 |
| | Female | Carrier | 8 | 8.2 | 38.6 | 88 | 9,640 | 21.2 |
| | | Clean | 17 | 8.4 | 41.0 | 316 | 10,325 | 20.7 |
| | Mean | | 25 | 8.4 | 40.0 | 243 | 10,105 | 20.9 |
| | Mean of carriers | | 26 | 8.1 | 38.4 | 149 | 10,700 | 21.2 |
| | Mean of clean | | 25 | 8.2 | 39.7 | 278 | 10,925 | 20.7 |
| | Mean | | 51 | 8.2 | 39.0 | 212 | 10,810 | 21.0 |

Table 22. Mean squares for the second bleeding of calves in Experiment 2

| Source of variation | d.f. | Percent hemoglobin | Percent packed red cells | White cell count | Mean corpuscular concent. |
|---|---|---|---|---|---|
| Sex | 1 | 2.35* | 51.93 | 12,772** | .0009 |
| Genotype | 1 | .11 | 1.92 | 3,225 | 2.16 |
| Residual | 48 | .54 | 15.81 | 1,238 | 2.21 |

*Significant at P = .05

**Significant at P = .01

Table 23. Analysis of variance for the difference between the first and second bleedings of Experiment 2

| Source of variation | d.f. | Fragility of erythrocytex | Percent hemoglobin | Percent packed red cells | White cell count | Mean corp. concent. |
|---|---|---|---|---|---|---|
| Sex | 1 | 48,339 | 24.35** | 114.24* | 7,647 | 41.58** |
| Genotype | 1 | 1,531 | 3.01 | 5.56 | 233 | 5.07 |
| Mean difference | 1 | 900,048** | 4.68 | 45.53 | 11,559 | 17.22 |
| Residual | 47 | 26,863 | 1.00 | 21.47 | 1,776 | 5.36 |

*Significant at P = .05

**Significant at P = .01

tween sex and bleeding period for erythrocyte fragility or white cell count, the difference between the first and second bleedings was tested. A significant difference between bleedings was found for erythrocyte fragility but not for white cell count.

Relation of age to various blood characteristics of calves

The calves in the first bleeding of Experiment 2 averaged 248 days of age with a standard deviation of 27.9 days. The regression and correlation coefficients concerning age of calf and fragility, hemoglobin content, packed red cells and white cell count are shown in Table 24. Only the white cell count seemed significantly related to age of calf. As shown in Table 21, the white cell counts were corrected for age of calf in the first bleeding of Experiment 2 but the difference between genotypes still was not significant.

Table 24. The correlation coefficients and the regressions of blood characteristics on age of calf in days

| Statistic | d.f. | Fragility | Percent hemoglobin | Hemato-crit | White cell count |
|---|---|---|---|---|---|
| b | 53 | -1.48 | .01 | .03 | -.67** |
| r | 53 | - .20 | .26 | .21 | -.37** |

**Significant at P = .01

Relation of hematocrit and percent hemoglobin to erythrocyte fragility

The relationships between percent hemoglobin, hematocrit and erythrocyte fragility were studied since all are characteristics of the erythrocyte. The regression and correlation coefficients between these characteristics are shown in Table 25.

Table 25. The correlation coefficients and regressions between erythrocyte fragility, percent hemoglobin and hematocrit

| Dependent variable | Statistic | D.f. | Independent variable: Fragility | Independent variable: Percent hemoglobin |
|---|---|---|---|---|
| Hematocrit | b | 53 | 11.99 | .23** |
| | r | 53 | .22 | .78** |
| Percent hemoglobin | b | 53 | 56.01** | |
| | r | 53 | .30** | |

**Significant at P = .01

The regression of fragility on percent hemoglobin indicated that as hemoglobin went up one percent, the colorimeter reading for erythrocyte fragility went up by 56. This regression was significant. As hematocrit increased one percent, the colorimeter reading for fragility also increased by 12 but this was not statistically significant. Hemoglobin content increased 0.23 percent as hematocrit increased one percent and this regression was significant.

Repeatability of observations taken at different periods

Two methods were used for estimating repeatabilities of the blood characteristics on the same calf. The first method was to correlate the observations obtained in the first bleeding period with the observations obtained in the second period. These product-moment correlations were calculated on a within-group basis in order to eliminate the effects of differences between sex and genotype means. Thus these estimates rest on 47 degrees of freedom. These correlations were fragility, .53; hemoglobin, .44; hematocrit, .23; white cell count, .45 and mean corpuscular concentration, .01. The repeatability from one bleeding to another bleeding six weeks later was significant but only moderately high in the case of fragility, hemoglobin content, and white cell count. The correlation between hematocrit values and between mean corpuscular concentration values obtained six weeks apart was low and not significant.

Some calves were in Experiment 1 and also in both bleedings of experiment 2; this made it possible to compare three observations on a single animal. Whether the calves occurred in all three periods had nothing to do with sex or genotype or the hematological values obtained on the animals. A total of 19 calves including seven carrier males, six carrier females, four clean males, and two clean females were

bled three times each.

The components of variance due to sex, genotype, and the sex-genotype interaction were computed as one value for ease of calculation. The variance due to different times of bleedings and factors associated with times, such as the interactions of sex and genotype with time, were removed so that the error term included the interaction between animals and time. The variances for the three times for each blood characteristic were tested by Bartlett's test and found to be homogeneous. The method of calculating the sums of squares and expected means squares was as follows:

| Source | d.f. | Sums of squares | Expected mean squares |
|---|---|---|---|
| (1) Sex + genotype + sex x genotype | 3 | $\frac{Y_{ij..}^2}{n_{ij..}} - \frac{Y_{....}^2}{N}$ | |
| (2) Times and interactions associated with times within (1) | 8 | $\frac{Y_{ij.1}^2}{n_{ij.1}} - \frac{Y_{ij..}^2}{n_{ij..}}$ | |
| (3) Animals within (1) | 15 | $\frac{Y_{ijk.}^2}{n_{ijk}} - \frac{Y_{ij..}^2}{n_{ij..}}$ | $\sigma_D^2 + 3\sigma_A^2$ |
| (4) Determinations within (3) | 30 | $y_{ijkl}^2 - \frac{Y_{ijk.}^2}{n_{ijk.}}$ | $\sigma_D^2$ |

Where $y_{ijkl}$ represents the $l^{th}$ observation on the $k^{th}$ animal in the $j^{th}$ genotype and $i^{th}$ sex; n is the number of ob-

servations in the subgroup; N is the total number of observations and Y represents sums for the appropriate group. $\sigma^2_D$ represents the variance component for determinations within and $\sigma^2_A$ represents the variance component for animals. The repeatability estimate is the ratio of animal component of variance to the sum of the animal and determination component of variance. The repeatability estimates calculated in this way were fragility, .37; hemoglobin content, .05; hematocrit value, .07; and white cell count, .07. These repeatabilities are, in every case, lower than those obtained by correlating two values obtained six weeks apart.

## Experiment 3 - Response to Adrenalin

Experiment 3 was conducted to study the response of animals of different ages and genotypes to injections of adrenalin. The ages studied were cows and calves and the genotypes were carrier and clean. This experiment had equal sub-class numbers and was analyzed as shown in Table 26. The characteristics of the blood studied were fragility of the erythrocytes, percent hemoglobin, percent packed red cells and total white cell count. Differential white cell counts were made only for the cows.

### Erythrocyte fragility

The response of cows and calves of both genotypes in fragility of erythrocytes following an adrenalin injection is

shown in Figure 6. Carrier calves and the cows of both genotypes increased in fragility from the initial sample to the 20 minute sample. The clean calves decreased in this interval and all four groups decreased from 20 minutes to 2 hours. A time-genotype interaction would indicate a different response, from one time to another, for the two genotypes. The two genotypes did not show a significant change in the two time intervals as is shown in Table 26, by the non-significant interaction between genotype and time. None of the interactions was significant. The average fragility of the clean animals was significantly higher than the average of carrier animals. These distributions are shown in Table 27. Although the difference between the means of the genotypes was significant, the overlap of these groups makes it impossible to discriminate individually between carriers and cleans. The difference between the means of the two age groups was significant.

The cows and calves were analyzed separately as shown in Table 28. The carrier and clean animals did not differ in time trends following adrenalin. The difference between the two genotypes in total fragility levels was significant in both age groups.

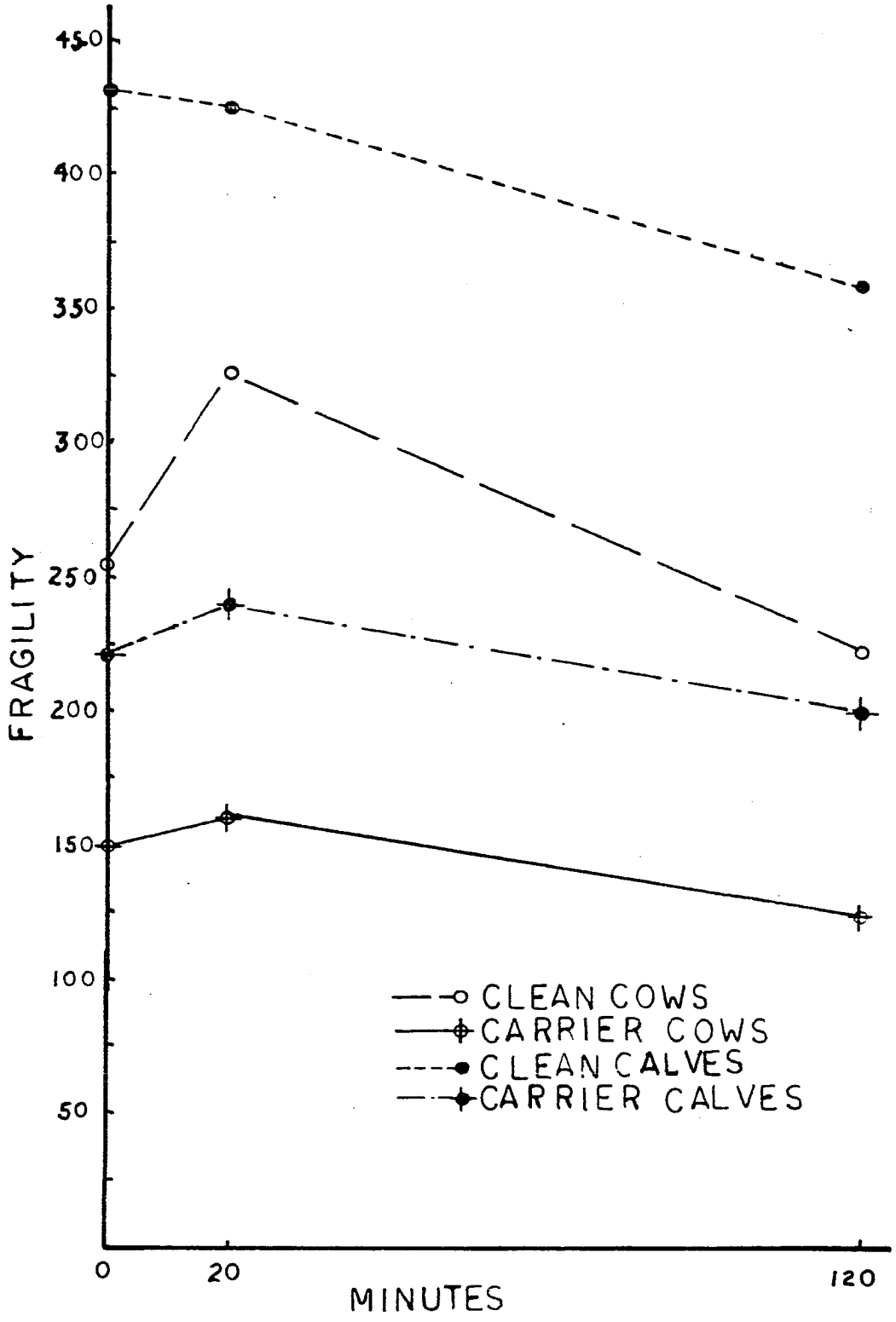



Figure 6. Average response for erythrocyte fragility following an injection of adrenalin.

Table 26. Mean squares for four components of the blood studied after injection of adrenalin

| Source of variation | d.f. | Mean squares | | | |
|---|---|---|---|---|---|
| | | Fragility | Percent hemoglobin | Percent packed red cells | Total white cells |
| Ages | 1 | 405,662** | 114.66** | 930.25** | 43,229** |
| Genotype | 1 | 854,546** | 1.26 | 115.56* | 6,227 |
| Time | 2 | 46,607 | .45 | 28.91 | 13,972* |
| Age x genotype | 1 | 35,941 | .09 | 8.02 | 451 |
| Age x time | 2 | 3,718 | .24 | 16.72 | 1,246 |
| Genotype x time | 2 | 7,499 | .03 | 1.63 | 1,969 |
| Age x time x genotype | 2 | 5,618 | .09 | 4.12 | 1,545 |
| Residual | 132 | 21,221 | .76 | 13.51 | 3,039 |

*Significant at P = .05

**Significant at P = .01

Percent hemoglobin

As shown in Figure 7, percent hemoglobin showed little response to adrenalin in either cows or calves. Both clean and carrier calves decreased from the initial bleeding to the 20

Table 27. Distribution of erythrocyte fragility following an injection of adrenalin

| Fragility | Cows | | | | | | Calves | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Initial | | 20 min. | | 120 min. | | Initial | | 20 min. | | 120 min. | |
| | Carrier | Clean | Carrier | Clean | Carrier | Clean | Carrier | Clean | Carrier | Clean | Carrier | Clean |
| 800 | | | | 1 | | | | | | | | |
| 775 | | | | | | | | | | | | 1 |
| 750 | | | | | | | | | | | | |
| 725 | | | | | | | | | | 1 | | |
| 700 | | | | | | | | 1 | | | | |
| 675 | | | | | | | | | | | | |
| 650 | | | | | | | | | | | | |
| 625 | | | | | | | | | 1 | | | |
| 600 | | 1 | | | | | 1 | | | | 1 | |
| 575 | | | | | | | | 1 | | 1 | | |
| 550 | | | | | | | | 2 | | 2 | | |
| 525 | | | | | | | | | | 1 | | |
| 500 | | | | 2 | | | | | | | | |
| 475 | | | | | | | | 1 | | | | |
| 450 | | | | | | | | 1 | 1 | | | 3 |
| 425 | | 1 | | | | | 1 | | | 1 | | |
| 400 | | 1 | | | | | | | | 1 | | 1 |
| 375 | | | | | | | | 1 | | | | 2 |
| 350 | | | | 2 | 1 | 1 | 1 | 2 | 1 | 1 | | |
| 325 | | | 1 | 1 | 1 | 2 | | | | 1 | 2 | |
| 300 | | 2 | | | | | | 1 | 1 | | 1 | 1 |
| 275 | | | 1 | 2 | | | | 1 | | 1 | | |
| 250 | 2 | | | | | 1 | | | 1 | 1 | | |
| 225 | | 2 | 1 | | | 1 | 2 | | | | | 2 |
| 200 | 2 | | 1 | | | 1 | | 1 | 1 | | | 1 |
| 175 | 2 | | 2 | 1 | 1 | | | | | 1 | 1 | |
| 150 | 1 | 1 | 1 | 1 | 1 | 2 | 4 | | 3 | | 1 | |
| 125 | | 2 | 1 | 1 | | 1 | | | | | 1 | 1 |
| 100 | 2 | 2 | 2 | | 2 | | 2 | | 3 | | 2 | |
| 75 | 2 | | | 1 | 3 | 2 | | | | | 2 | |
| 50 | | | 2 | | 2 | | 1 | | | | | |
| 25 | 1 | | | | 1 | | | | | | 1 | |
| Mean | 150 | 255 | 160 | 327 | 127 | 222 | 222 | 432 | 240 | 426 | 199 | 359 |

Table 28. Mean squares for fragility of erythrocytes for cows and calves

| Source of variation | d.f. | Cows | Calves |
|---|---|---|---|
| Genotype | 1 | 269,990** | 620,497** |
| Time | 2 | 29,323 | 21,002 |
| Genotype x time | 2 | 9,427 | 3,690 |
| Residual | 66 | 16,669 | 25,773 |

**Significant at P = .01

minute sample and returned to their original values at two hours after injection. The cows of both genotypes showed little change. These interactions of age x time and genotype x time were non-significant, as shown in Table 26. It is apparent from Figure 7 that the animals of different ages and different genotypes did not respond differently at the various times. This failure to respond differently is indicated by the non-significant interaction of age x genotype x time. Carrier and clean animals did not differ significantly in percent hemoglobin in this experiment. There also was no significant difference among the three times they were bled. The overlap between genotypes is indicated in Table 29. The overlap between ages is less than the overlap for genotypes and the difference between the means of the cows and calves is highly significant.

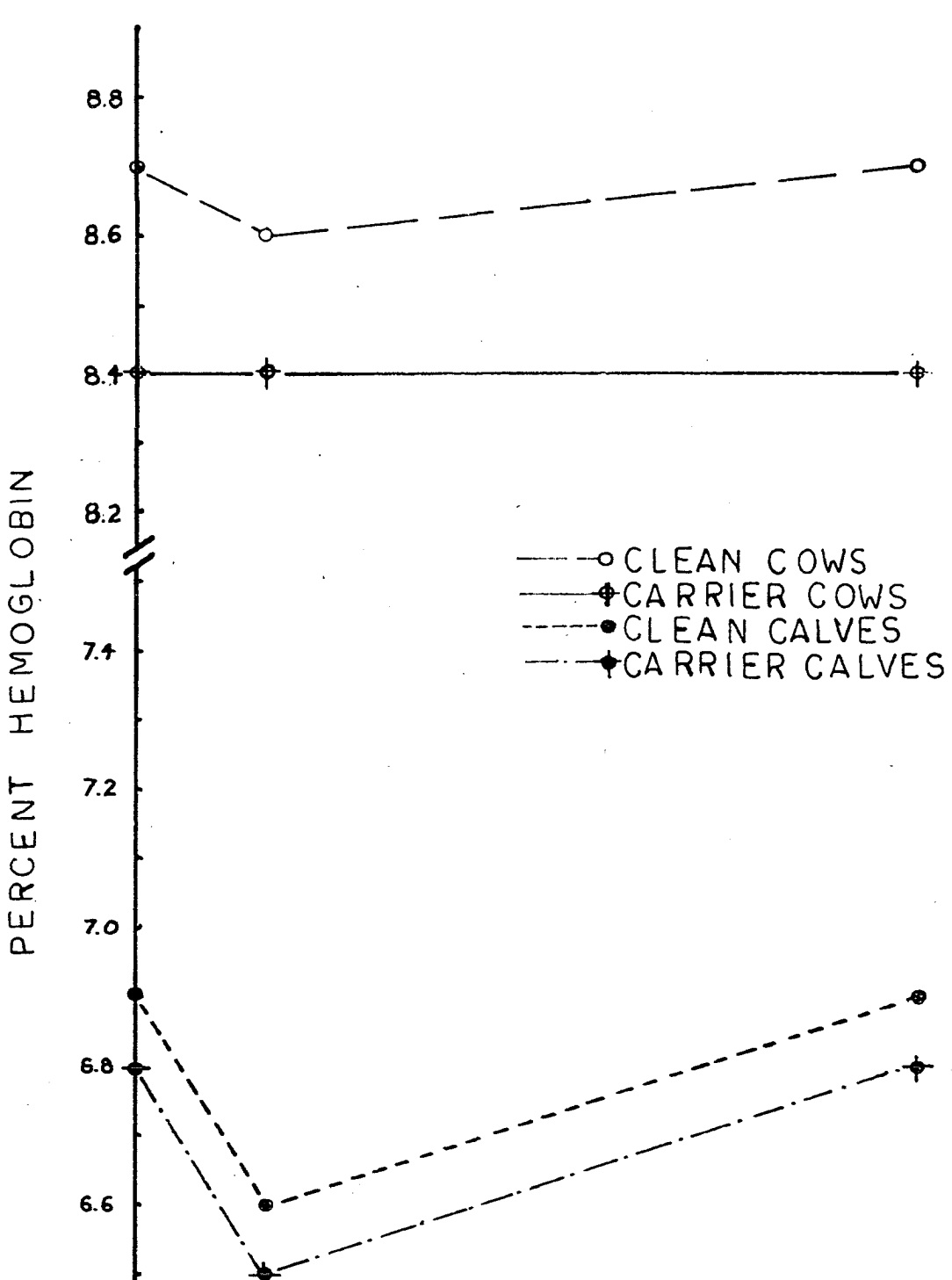



Figure 7. Average response in percent hemoglobin following an injection of adrenalin

Table 29. Distribution of percent hemoglobin following an injection of adrenalin

| Percent hemoglobin | Cows | | | | | | Calves | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Initial | | 20 min. | | 120 min. | | Initial | | 20 min. | | 120 min. | |
| | Carrier | Clean | Carrier | Clean | Carrier | Clean | Carrier | Clean | Carrier | Clean | Carrier | Clean |
| 11.4 | 1 | | | | | | | | | | | |
| 11.2 | | | | | | | | | | | | |
| 11.0 | | | | | | | | | | | | |
| 10.8 | | | 1 | | | | | | | | | |
| 10.6 | | 1 | | | | | | | | | | |
| 10.4 | | | | 1 | | 1 | | | | | | |
| 10.2 | | | | | | | | | | | | |
| 10.0 | | 1 | | | | 1 | | | | | | |
| 9.8 | 1 | | 1 | 1 | | | | | | | | |
| 9.6 | 1 | | 1 | | | | | | | | | |
| 9.4 | | 2 | | 1 | | 2 | | | | | | |
| 9.2 | | 1 | | | | 1 | | | | | | |
| 9.0 | | | | 2 | 5 | | | | | | | |
| 8.8 | 1 | | | | 1 | | | | | | | |
| 8.6 | | | 1 | 1 | 1 | | | 1 | | | | |
| 8.4 | 2 | 1 | 2 | | | 3 | | | | | | |
| 8.2 | | 2 | 3 | | | | | | | | | |
| 8.0 | 2 | 1 | | 3 | 1 | | | | | 1 | 1 | |
| 7.8 | | | 1 | 1 | 2 | 2 | | 1 | | | | 1 |
| 7.6 | 4 | 1 | 3 | | | 1 | 1 | 1 | | | | |
| 7.4 | 1 | 2 | | | 1 | 1 | 2 | 1 | 1 | 1 | | 2 |
| 7.2 | | | | 1 | 1 | | 1 | 2 | | 1 | | 1 |
| 7.0 | | | | 1 | | | 2 | 2 | 2 | 2 | 3 | 2 |
| 6.8 | | | | | | | 2 | | 1 | | 2 | 2 |
| 6.6 | | | | | | | 1 | 2 | 2 | 3 | 1 | 2 |
| 6.4 | | | | | | | 1 | | 2 | | 1 | 1 |
| 6.2 | | | | | | | | 1 | 1 | | | |
| 6.0 | | | | | | | 1 | 1 | | 1 | 2 | |
| 5.8 | | | | | | | | | 1 | 2 | | |
| 5.6 | | | | | | | | | 1 | | | |
| 5.4 | | | | | | | | | 1 | | 1 | 1 |
| 5.2 | | | | | | | 1 | 1 | | 1 | | |
| Mean | 8.4 | 8.7 | 8.4 | 8.6 | 8.4 | 8.7 | 6.8 | 6.9 | 6.5 | 6.6 | 6.8 | 6.9 |

Hematocrit

The response in hematocrit to adrenalin was similar for the two genotypes and for the two ages studied. Average hematocrit values showed an initial decrease at 20 minutes and then an increase between 20 minutes and two hours. These responses are shown in Figure 8. The cows, on the average, were above their initial level at two hours, but the calves failed to return to their initial level at two hours. The drop from the initial sample to the 20 minute sample appeared to be more marked in calves than cows. These responses in level of percent packed red cells from one time to another were non-significant for ages and genotypes, as shown by the age x time and genotype x time interactions in Table 26. The mean of the clean animals was significantly higher than the mean of the carriers. The average level of packed red cells was significantly higher for cows than calves. The hematocrit values for the two genotypes overlapped so much that no attempt in discriminating between individual carriers and cleans by this criterion could be made. The distribution and means for hematocrit level are shown in Table 30.

Total white cell count

The average response of animals to adrenalin, as measured by the total white cell count, is shown in Figure 9. The average responses indicated the tendency for the white cell

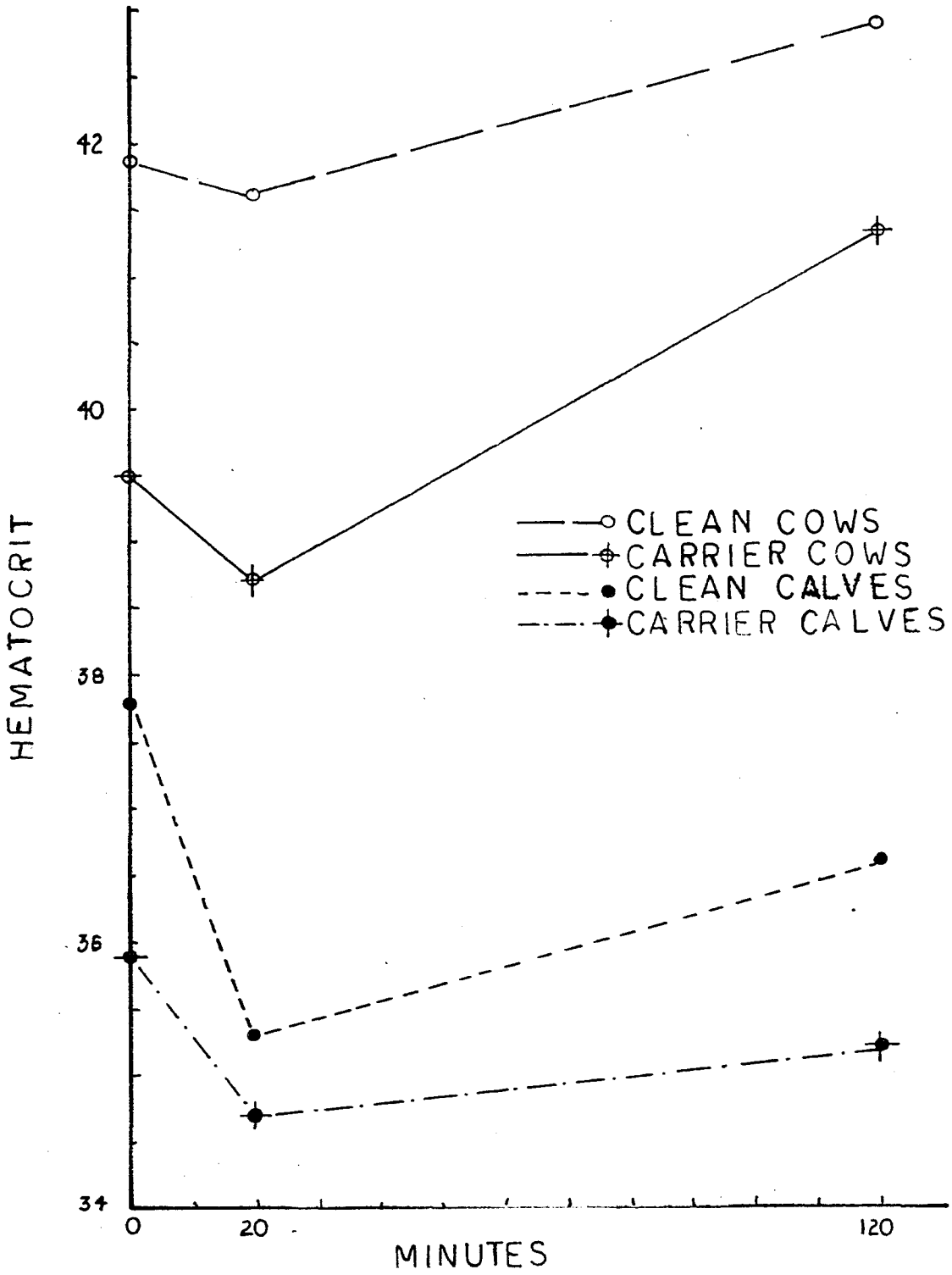



Figure 8. Average response in hematocrit following an injection of adrenalin

Figure 9. Average response in white cell count following an injection of adrenalin

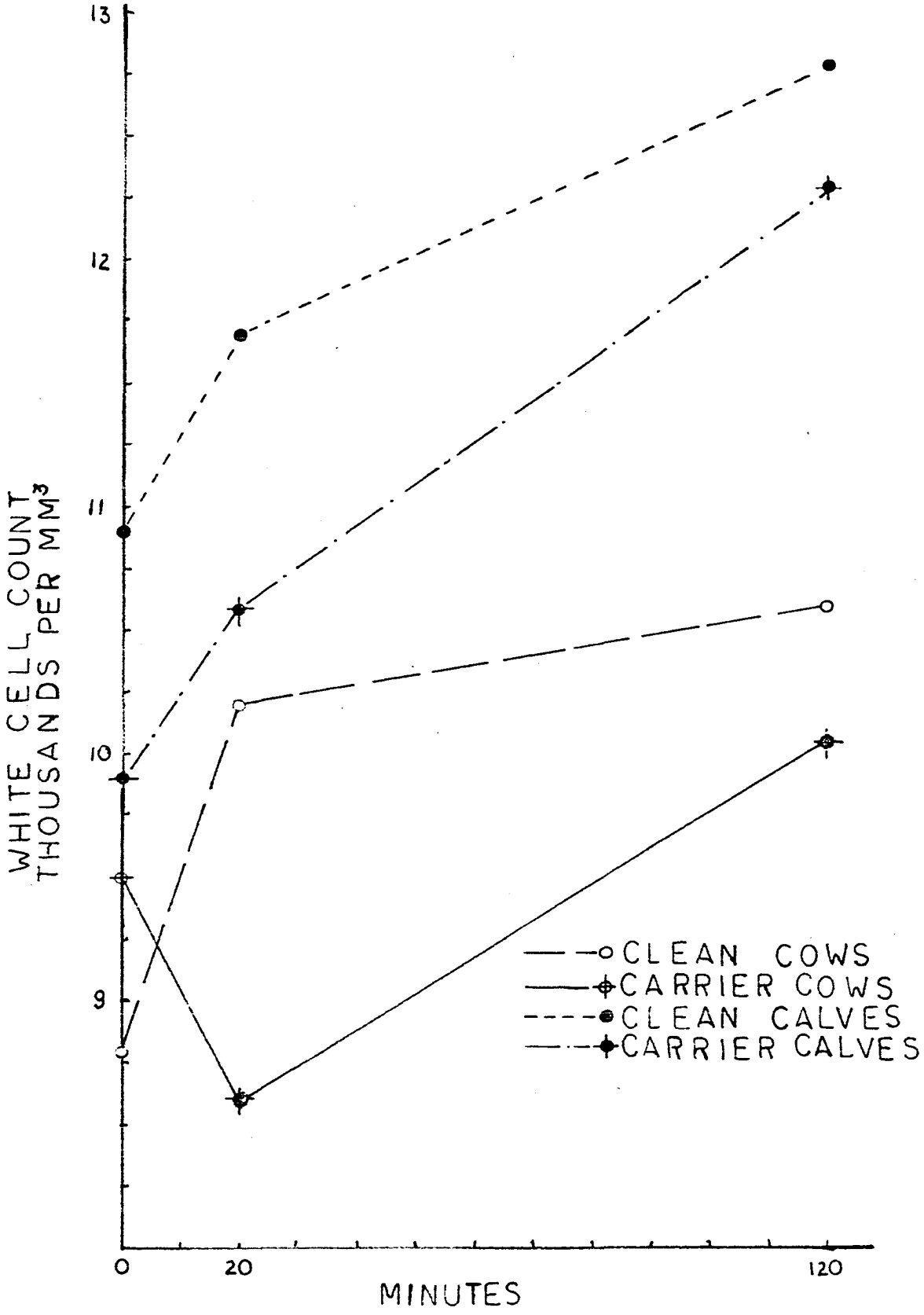

WHITE CELL COUNT
THOUSANDS PER MM³
13
12
11
10
9
0
20
120
MINUTES
CLEAN COWS
CARRIER COWS
CLEAN CALVES
CARRIER CALVES

Table 30. Distribution of percent packed red cells following an injection of adrenalin

| Hem-ato-crit | Cows | | | | | | Calves | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Initial | | 20 mins. | | 120 mins. | | Initial | | 20 mins. | | 120 mins. | |
| | Car-rier | Clean | Car-rier | Clean | Car-rier | Clean | Car-rier | Clean | Car-rier | Clean | Car-rier | Clean |
| 53 | | 1 | | | | | | | | | | |
| 52 | | | | | | | | | | | | |
| 51 | | | | | | | | | | | | |
| 50 | | | | 1 | | 1 | | | | | | |
| 49 | | 1 | | 1 | | | | | | | | |
| 48 | | | | | | 2 | | | | | | |
| 47 | | | | | | | | | | | | |
| 46 | | | | | 1 | 1 | | | | | | |
| 45 | | 1 | | 2 | 1 | | | | | | | |
| | | | | | 1 | 1 | | | | | | |
| 44 | 1 | 1 | | | | 1 | | | | | | |
| | | | | 1 | | | | 1 | | | | |
| 43 | 1 | 1 | 1 | | 3 | | | 1 | | | | |
| | | 1 | | | | | | | | | | |
| 42 | | | 1 | | 1 | 1 | | | | | | |
| | 1 | | | 1 | 1 | | 1 | | | | | |
| 41 | 1 | | 1 | | | | | 1 | | 1 | | 1 |
| | 1 | | | | | | | | | | | |
| 40 | 1 | 1 | 2 | | | 2 | | 1 | | | | |
| | | | | 1 | | | 1 | 1 | | | | |
| 39 | 1 | 1 | 2 | 1 | 1 | | 1 | | 1 | 1 | 2 | 2 |
| | 1 | 1 | | | | | | | | | | |
| 38 | 2 | 1 | 1 | 1 | 1 | | 1 | 1 | | | 1 | 1 |
| | | | | | | 3 | | 1 | | | 1 | |
| 37 | | | 3 | 2 | | | 1 | 1 | | 1 | | 2 |
| | | | | | | | | | | 1 | | 2 |
| 36 | 1 | 1 | | | 1 | | 2 | 2 | 4 | 1 | 1 | |
| | | | | | 1 | | | | | 1 | 1 | 1 |
| 35 | 1 | 1 | | 1 | | | 1 | 1 | 2 | 1 | | 2 |
| | | | | | | | 1 | | 1 | | 3 | |
| 34 | | | | | | | | | 1 | 3 | 1 | |
| | | | | | | | | | 1 | 1 | | |
| 33 | | | | | | | 1 | | | | | |
| 32 | | | 1 | | | | 1 | | 1 | | | |
| 31 | | | | | | | | | | | | |
| 30 | | | | | | | | | | | 1 | |
| | | | | | | | | | | | | 1 |
| 29 | | | | | | | 1 | | 1 | | 1 | |
| 28 | | | | | | | | 1 | | 1 | | |
| Mean | 39.5 | 41.9 | 38.7 | 41.6 | 41.3 | 42.9 | 35.9 | 37.8 | 34.7 | 35.3 | 35.2 | 36.6 |

count to rise following an injection of adrenalin in all classes except carrier cows. These tended to decrease from the initial sample to the 20 minute sample. However, the interaction between age x genotype x time is not significant. The difference between times of bleeding was significant but, as indicated by the genotype x time interactions, genotypes do not differ in response following adrenalin. Genotypes did not differ significantly in total white cell count, but calves had a significantly higher white cell count than the cows. The means and distributions for ages, times and genotypes are shown in Table 31.

The response of the cows to an adrenalin injection, as indicated by the actual number of lymphocytes and neutrophils, is shown in Figure 10. The clean cows tend to increase from the initial sample to the 20 minute sample in both lymphocyte count and neutrophil count, while the carrier animals tend to decrease in these counts. The lymphocytes show a later decrease in both genotypes and the neutrophils show a large increase. The variation within these periods is large, as shown in Table 32 and the difference between times is non-significant. The genotypes did not differ in neutrophil count, but did differ significantly for total lymphocyte count. The analysis of variance for lymphocyte and neutrophils is shown in Table 33. At all three times the clean animals averaged higher

Table 31. Distribution of white cell count following an injection of adrenalin

| Thousands of white cells | Cows | | | | | | Calves | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Initial | | 20 mins. | | 120 mins. | | Initial | | 20 mins. | | 120 mins. | |
| | Carrier | Clean | Carrier | Clean | Carrier | Clean | Carrier | Clean | Carrier | Clean | Carrier | Clean |
| 20.0 | | | | | | | | | | | | 1 |
| 19.5 | | | | | | | | | | 1 | | |
| 19.0 | | | | | | | | | | | | |
| 18.5 | | | | | | | | | | 1 | | |
| 18.0 | | | | | | | | | | | | |
| 17.5 | | | | | | | | 1 | | | | |
| 17.0 | | | | | | | | | | | | |
| 16.5 | | | | | | | | | | | 1 | |
| 16.0 | | | | | | 1 | | | | 1 | | |
| 15.5 | | | | | | | | | | | | 1 |
| 15.0 | | | | | | | | | | | 2 | 2 |
| 14.5 | | 1 | | | | | | | | | | |
| 14.0 | | | | 1 | 1 | | | | | 1 | | 1 |
| 13.5 | | | | 1 | | | | 1 | | 1 | | |
| 13.0 | | | | 1 | | 1 | | 2 | | | | |
| 12.5 | 1 | | | | | 1 | 1 | | 1 | 1 | 1 | 1 |
| 12.0 | | | | 1 | 1 | | | 2 | 1 | | 2 | |
| 11.5 | | 1 | 2 | 1 | 1 | | 2 | 1 | 1 | | 1 | 1 |
| 11.0 | | | | | 2 | 1 | | | 1 | 1 | 2 | |
| 10.5 | 1 | | 1 | 2 | | 2 | 1 | | 5 | 1 | 3 | 2 |
| 10.0 | 4 | 1 | 1 | | 1 | 1 | 3 | 1 | 1 | | | 1 |
| 9.5 | 1 | 2 | 1 | | 1 | | 1 | | | | | |
| 9.0 | 1 | | 2 | 1 | | 3 | 1 | | 2 | | | |
| 8.5 | 2 | 1 | 1 | | 3 | 1 | 2 | | | | | |
| 8.0 | 1 | 2 | | 1 | 1 | 1 | | | | | | |
| 7.5 | | 1 | | 1 | 1 | | | 1 | | | | 1 |
| 7.0 | 1 | 1 | 1 | 1 | | | 1 | 2 | | 2 | | |
| 6.5 | | | 1 | | | | | | | 1 | | |
| 6.0 | | 1 | | 1 | | | | | | | | |
| 5.5 | | 1 | | | | | | 1 | | | | |
| 5.0 | | | 1 | | | | | | | | | |
| 4.5 | | | 1 | | | | | | | 1 | | |
| 4.0 | | | | | | | | | | | | |
| Means | 9.50 | 8.80 | 8.60 | 10.20 | 10.05 | 10.60 | 9.90 | 10.60 | 12.30 | 10.85 | 11.70 | 12.80 |

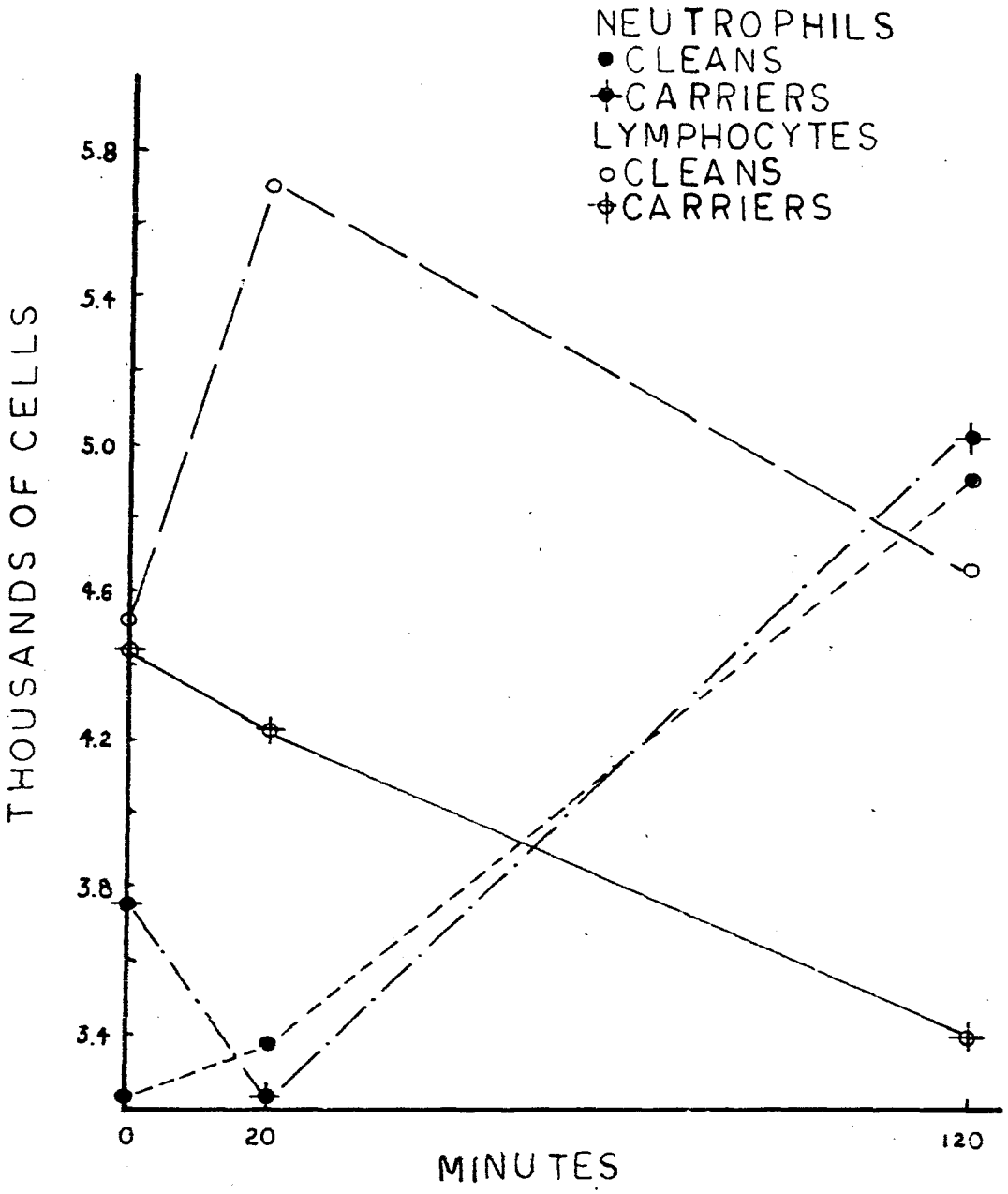



Figure 10. Average response in lymphocytes and neutrophils for cows following an injection of adrenalin

Table 32. Distribution of lymphocyte and neutrophil numbers following an injection of adrenalin

| Thousands of cells | Lymphocytes | | | | | | Neutrophils | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Initial | | 20 mins. | | 120 mins. | | Initial | | 20 mins. | | 120 mins. | |
| | Carrier | Clean | Carrier | Clean | Carrier | Clean | Carrier | Clean | Carrier | Clean | Carrier | Clean |
| 9.25 | | | | 1 | | | | | | | | |
| 9.00 | | | | | | 1 | | | | | | |
| 8.75 | | | | | | | | | | | | |
| 8.50 | | 1 | | | | | | | | | | |
| 8.25 | | | | 1 | | | | | | | | |
| 8.00 | | | | | | | | | | | | |
| 7.75 | | | | 1 | | | | | | | 1 | |
| 7.50 | | | | | | | | | | | | |
| 7.25 | | | | 1 | | | | | | | | |
| 7.00 | | 1 | | | | | | | | | | 1 |
| 6.75 | | | | | | | | | | | | |
| 6.50 | | | | | | 1 | | | | | 1 | |
| 6.25 | | | | 1 | | | | | | | 2 | |
| 6.00 | 1 | | 1 | | | 1 | | | | | | 2 |
| 5.75 | 1 | | 1 | 1 | | 1 | | | | | 1 | 1 |
| 5.50 | | | 1 | | | | 1 | | | | | 1 |
| 5.25 | 2 | | 2 | | 1 | | | 1 | | | 1 | 1 |
| 5.00 | | 2 | 1 | 1 | | | | | | | 1 | |
| 4.75 | 1 | | | | 1 | 1 | 1 | 1 | 1 | | 1 | 1 |
| 4.50 | 1 | 1 | 1 | 1 | | | 2 | 1 | 2 | 1 | | |

Table 32. (Continued)

| Thousands of cells | Lymphocytes | | | | | | Neutrophils | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Initial | | 20 mins. | | 120 mins. | | Initial | | 20 mins. | | 120 mins. | |
| | Carrier | Clean | Carrier | Clean | Carrier | Clean | Carrier | Clean | Carrier | Clean | Carrier | Clean |
| 4.25 | 2 | 1 | 1 | 1 | | | | | | 2 | 1 | 2 |
| 4.00 | 2 | 2 | | 1 | 4 | 1 | 2 | 1 | 1 | 1 | | 1 |
| 3.75 | | 1 | | | 3 | 1 | 2 | 1 | | 2 | | |
| 3.50 | | | | | 1 | 1 | 1 | | | 2 | | 1 |
| 3.25 | 1 | 1 | | 1 | 1 | 2 | | 1 | 2 | | 1 | |
| 3.00 | | 1 | 1 | | | 2 | 1 | 1 | 2 | | 1 | |
| 2.75 | | | 1 | 1 | | | | | | | | 1 |
| 2.50 | | 1 | | | 1 | | 1 | 1 | 1 | 2 | 1 | |
| 2.25 | 1 | | | | | | | 1 | 1 | 1 | | |
| 2.00 | | | 2 | | | | | 1 | 1 | 1 | | |
| 1.75 | | | | | | | | 1 | 1 | | | |
| 1.50 | | | | | | | 1 | | | | | |
| 1.25 | | | | | | | | 1 | | | | |
| 1.00 | | | | | | | | | | | | |
| .75 | | | | | | | | | | | | |
| Mean | 44.53 | 45.37 | 42.39 | 56.77 | 38.99 | 46.82 | 37.60 | 32.11 | 32.33 | 33.80 | 50.16 | 49.16 |

Table 33. Mean squares for lymphocytes and neutrophils for cows in adrenalin experiment

| Source of variation | d.f. | Lymphocytes | Neutrophils |
|---|---|---|---|
| Genotype | 1 | 5,256* | 201 |
| Time | 2 | 11,256 | 7,974 |
| Genotype x time | 2 | 1,101 | 299 |
| Residual | 66 | 960 | 572 |

*Significant at P = .05

in lymphocyte count than the carriers. The response to adrenalin as measured by monocytes, basophils, and eosinophils indicated no trends or differences between genotypes.

## Experiment 4 - Response to Hemorrhage

The response of four carrier and four clean cows to hemorrhage was measured by the changes that occurred in fragility of erythrocytes, percent hemoglobin, percent packed red cells, erythrocyte numbers and total leukocyte numbers. The means for each genotype for these blood components are shown in Table 34. The fragility of erythrocytes increased sharply in both genotypes immediately following induced anemia. The higher fragility was maintained for about five days and was followed by a gradual return to normal levels at two weeks (Figure 11). No statistical analyses of these data were made

Table 34. Average response of carrier and clean cows to hemorrhagic anemia

| | 1st day | 2nd day | 3rd day | 4th day | 5th day | 6th day | 10th day | 14th day | 21st day |
|---|---|---|---|---|---|---|---|---|---|
| Erythrocyte fragility | | | | | | | | | |
| Carrier | 219 | 404 | 388 | 375 | 376 | 369 | 291 | 238 | 286 |
| Clean | 301 | 430 | 429 | 420 | 422 | 363 | 342 | 293 | 392 |
| Percent hemoglobin | | | | | | | | | |
| Carrier | 9.1 | 6.8 | 6.4 | 5.9 | 5.9 | 6.4 | 6.4 | 7.0 | 7.6 |
| Clean | 9.0 | 7.1 | 5.8 | 6.1 | 5.9 | 6.4 | 6.4 | 6.7 | 6.9 |
| Hematocrit | | | | | | | | | |
| Carrier | 42.2 | 30.4 | 30.0 | 28.7 | 30.0 | 33.1 | 32.0 | 35.8 | 38.9 |
| Clean | 43.0 | 32.0 | 31.1 | 30.4 | 29.6 | 33.4 | 31.2 | 34.9 | 36.4 |
| Red cell count | | | | | | | | | |
| Carrier | 7.31 | 5.45 | 5.54 | 5.35 | 5.50 | 6.68 | 5.65 | 5.97 | 6.84 |
| Clean | 6.83 | 5.68 | 5.48 | 5.28 | 5.28 | 5.97 | 5.72 | 5.55 | 6.41 |
| White cell count | | | | | | | | | |
| Carrier | 9750 | 9150 | 6850 | 7050 | 7150 | 7600 | 6700 | 7300 | 9800 |
| Clean | 9000 | 8850 | 7300 | 6850 | 7850 | 7450 | 7200 | 7800 | 7900 |

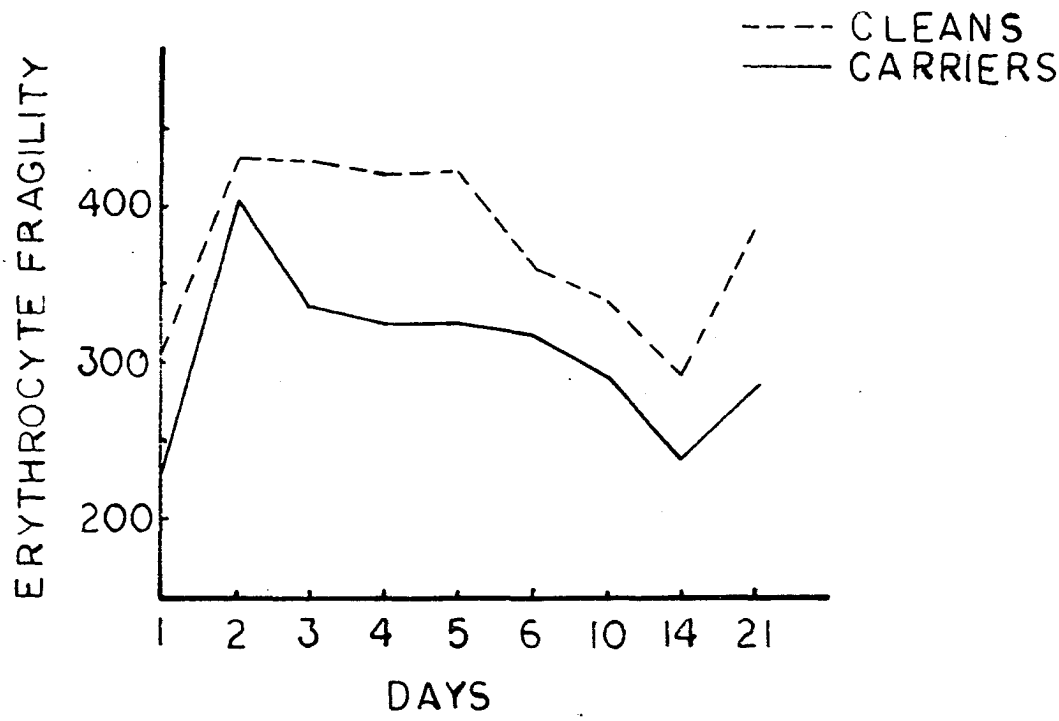



Figure 11. Average response of carrier and clean animals to hemorrhage for erythrocyte fragility

because the numbers in each genotype were so few, but it is apparent from Figure 12 that the overlap of genotypes is so great that no classification of animals as to genotype could be made.

The response to hemorrhage is strikingly similar in percent hemoglobin, hematocrit, number of white cells and number of red cells as shown by the averages of the four animals in each genotype in Figures 13, 14, 15 and 16, respectively. These four characteristics of the blood decreased until the fourth day, and then showed a gradual increase back toward the original levels. In most instances the animals returned completely to the original levels of these various characteristics in 21 days. The responses of the individual animals are shown in Figures 17-18, 19 and 20. These graphs illustrate the overlap between genotypes in these blood components.

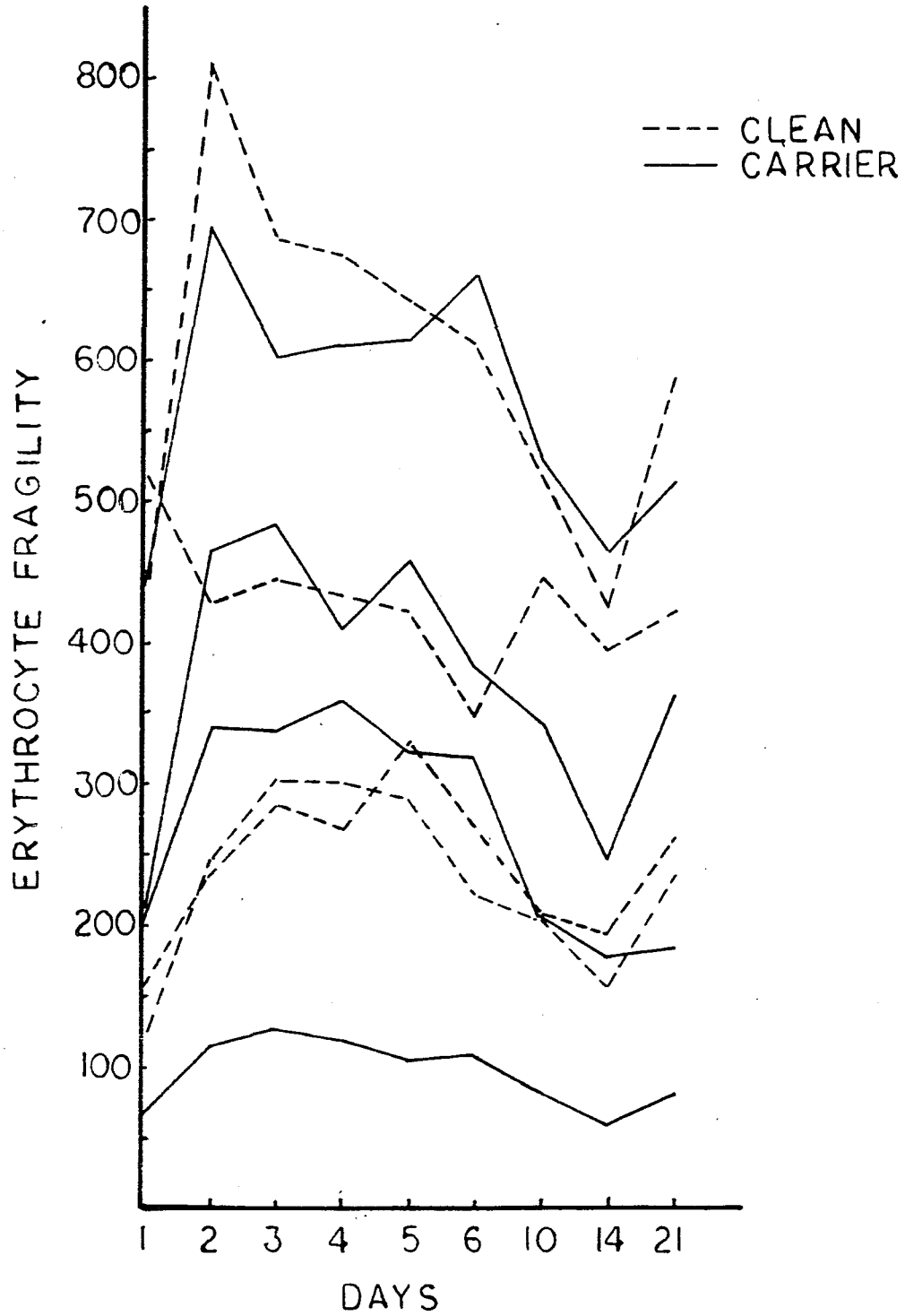



Figure 12. Individual responses of carrier and clean animals to hemorrhage for erythrocyte fragility

Figure 13. Average response of carrier and clean animals to hemorrhage for percent hemoglobin

Figure 14. Average response of carrier and clean animals to hemorrhage for hematocrit

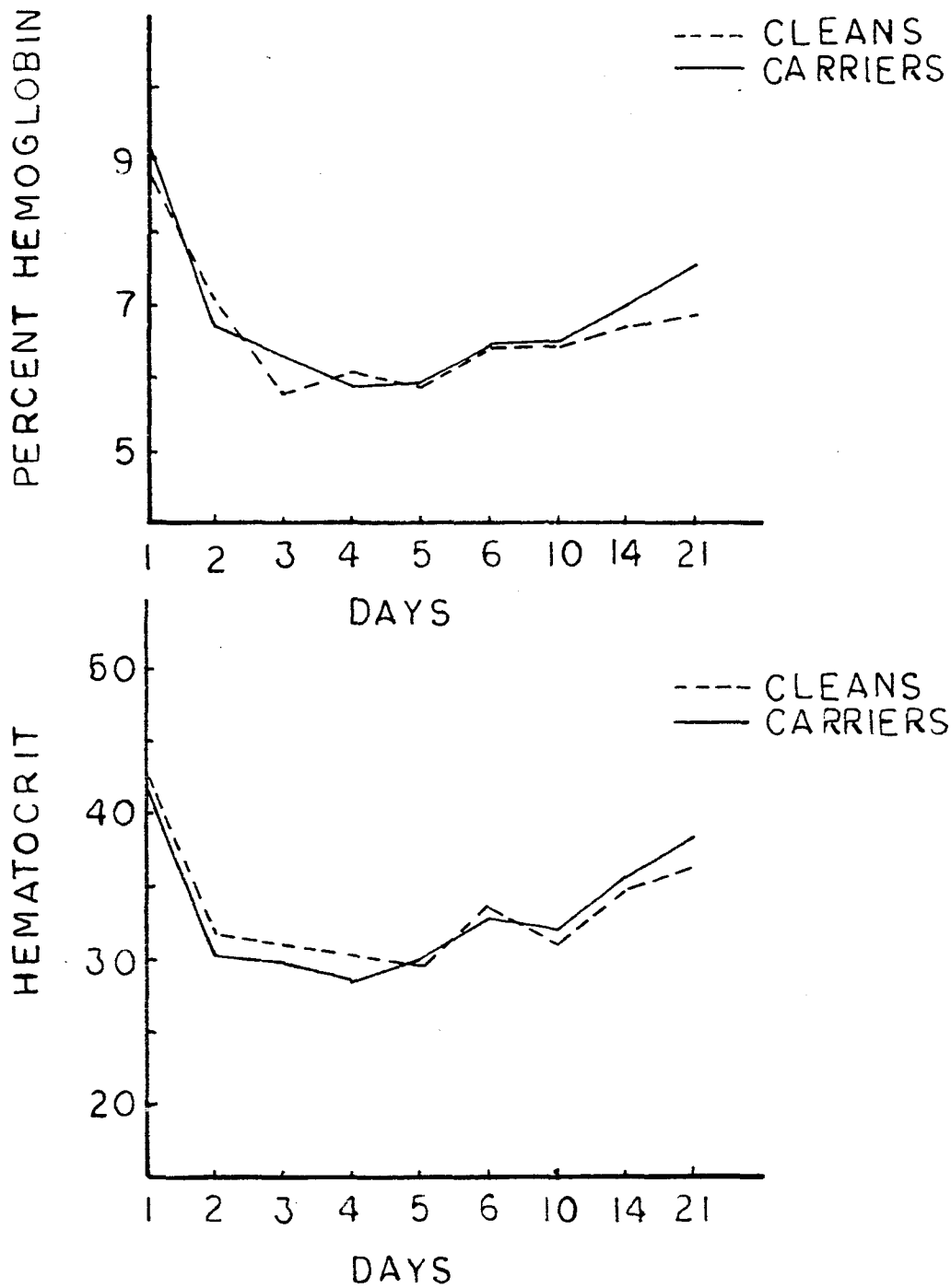
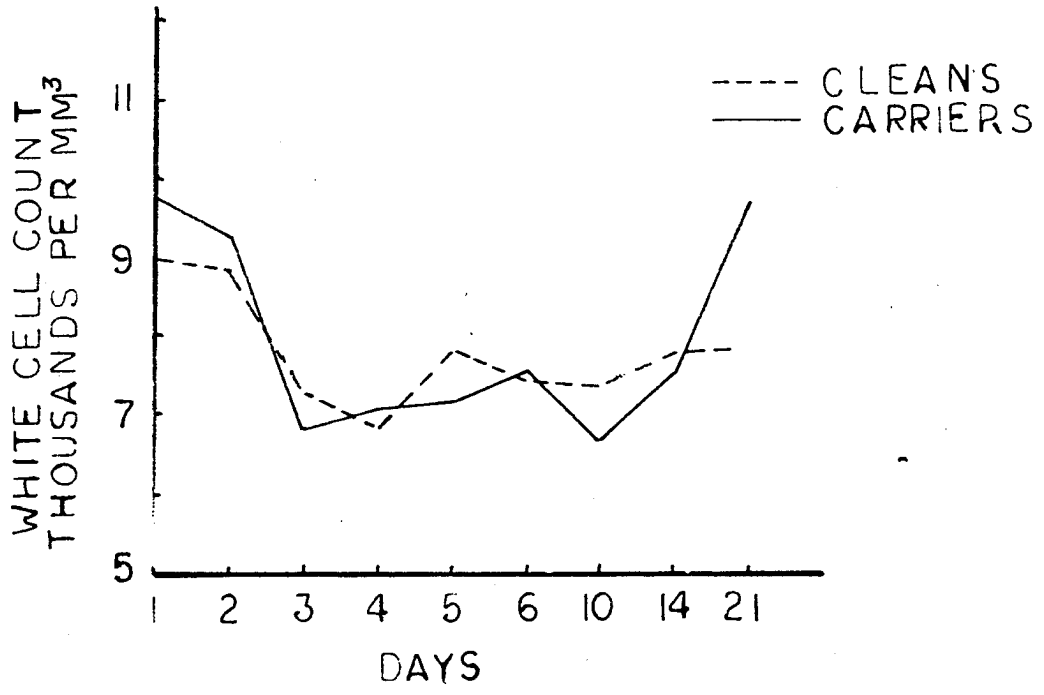

PERCENT HEMOGLOBIN
9
7
5
1 2 3 4 5 6 10 14 21
DAYS
---- CLEANS
—— CARRIERS
HEMATOCRIT
50
40
30
20
1 2 3 4 5 6 10 14 21
DAYS
---- CLEANS
—— CARRIERS

Figure 15. Average response of carrier and clean animals to hemorrhage for total numbers of white cells

Figure 16. Average response of carrier and clean animals to hemorrhage for numbers of red cells


WHITE CELL COUNT
THOUSANDS PER MM³
11
9
7
5
1
2
3
4
5
6
10
14
21
DAYS
---- CLEANS
—— CARRIERS

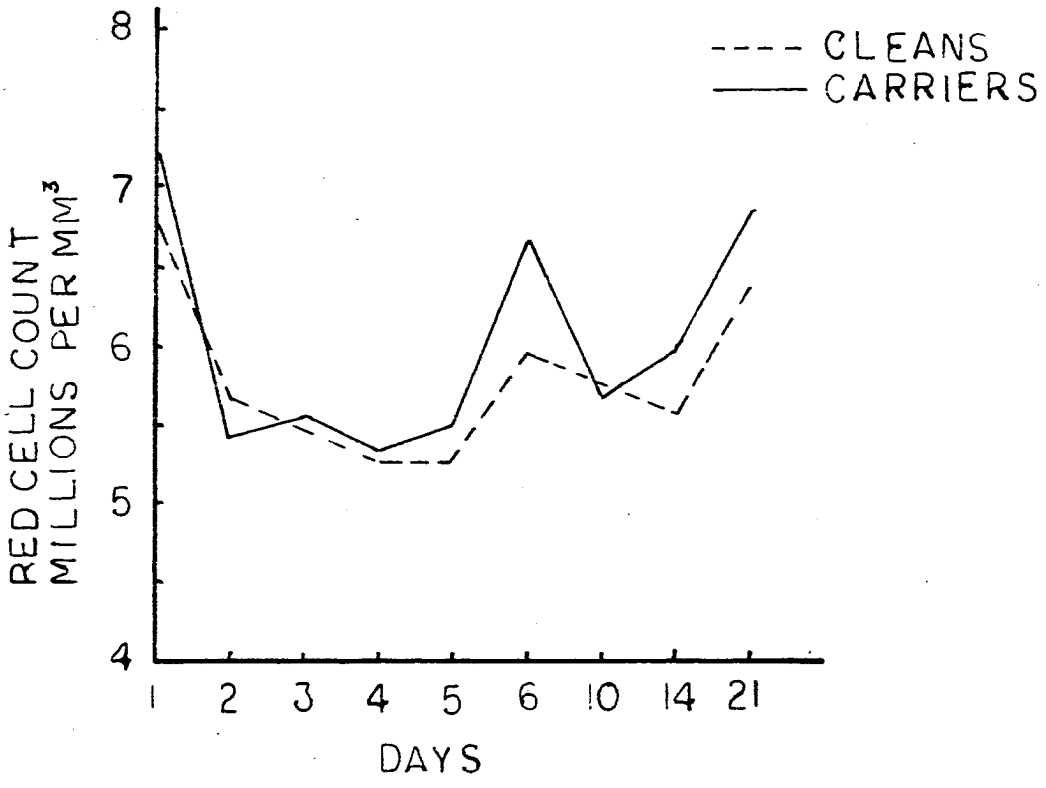
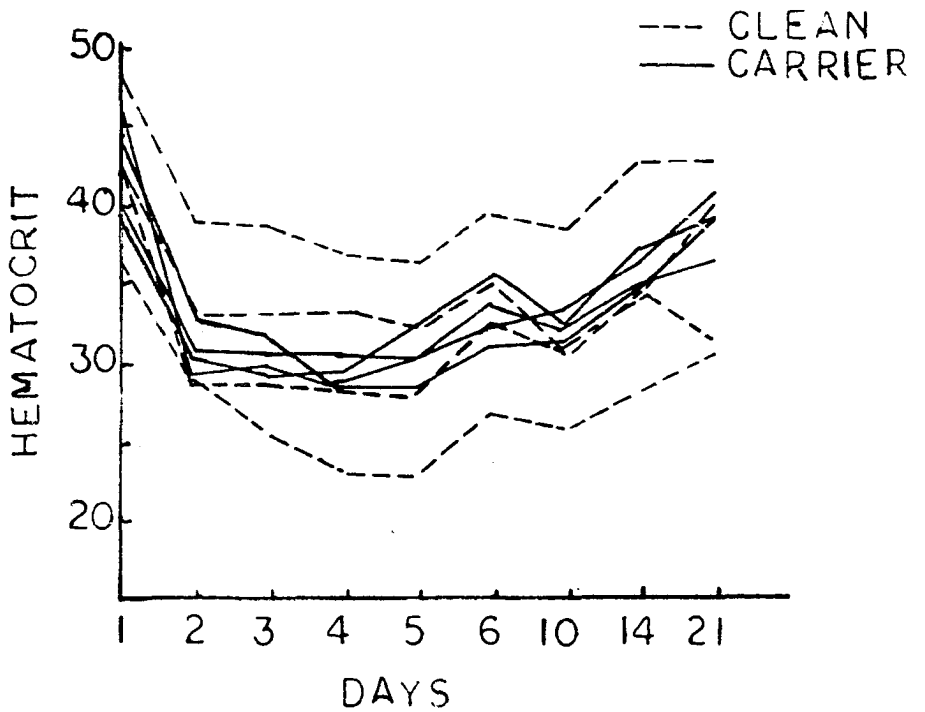

RED CELL COUNT
MILLIONS PER MM³
8
7
6
5
4
1
2
3
4
5
6
10
14
21
DAYS
---- CLEANS
—— CARRIERS

Figure 17. Individual response to hemorrhagic anemia for hematocrit

Figure 18. Individual response to hemorrhagic anemia for percent hemoglobin


--- CLEAN
— CARRIER
HEMATOCRIT
50
40
30
20
1 2 3 4 5 6 10 14 21
DAYS

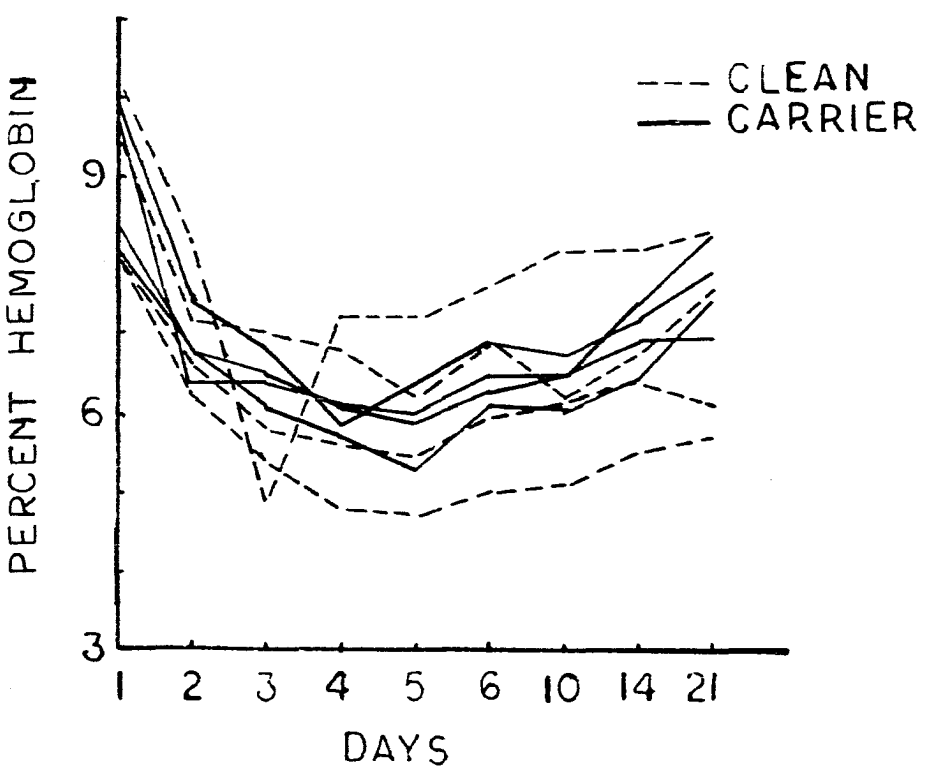
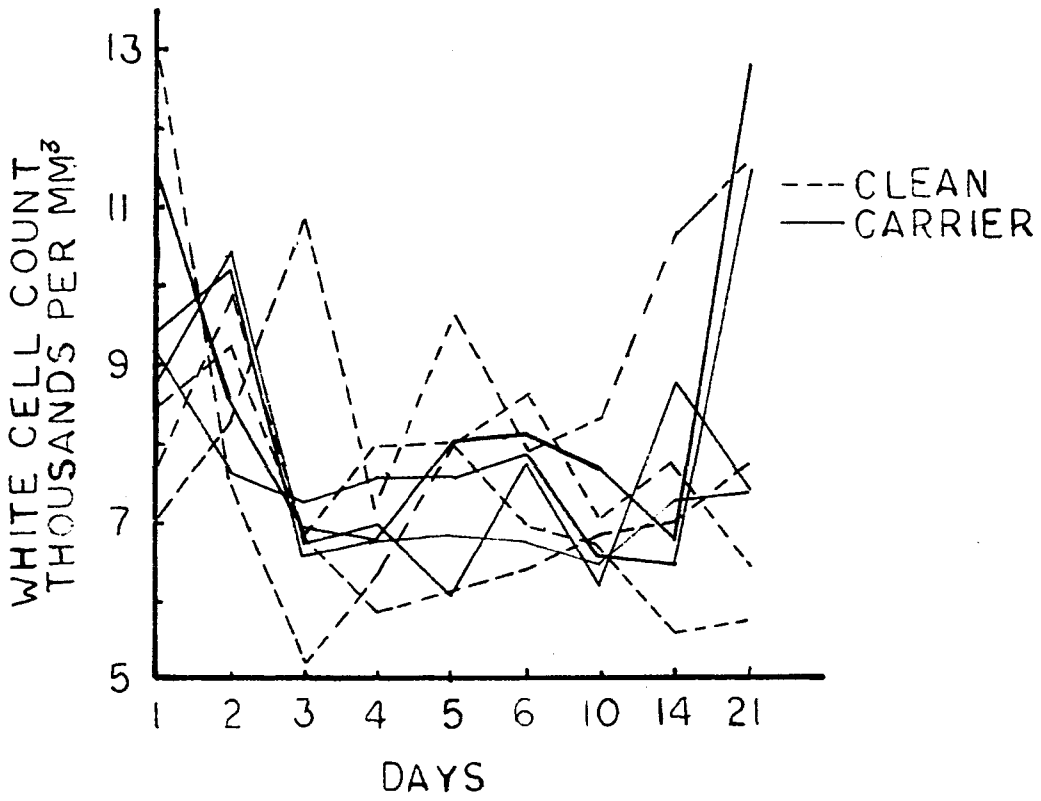
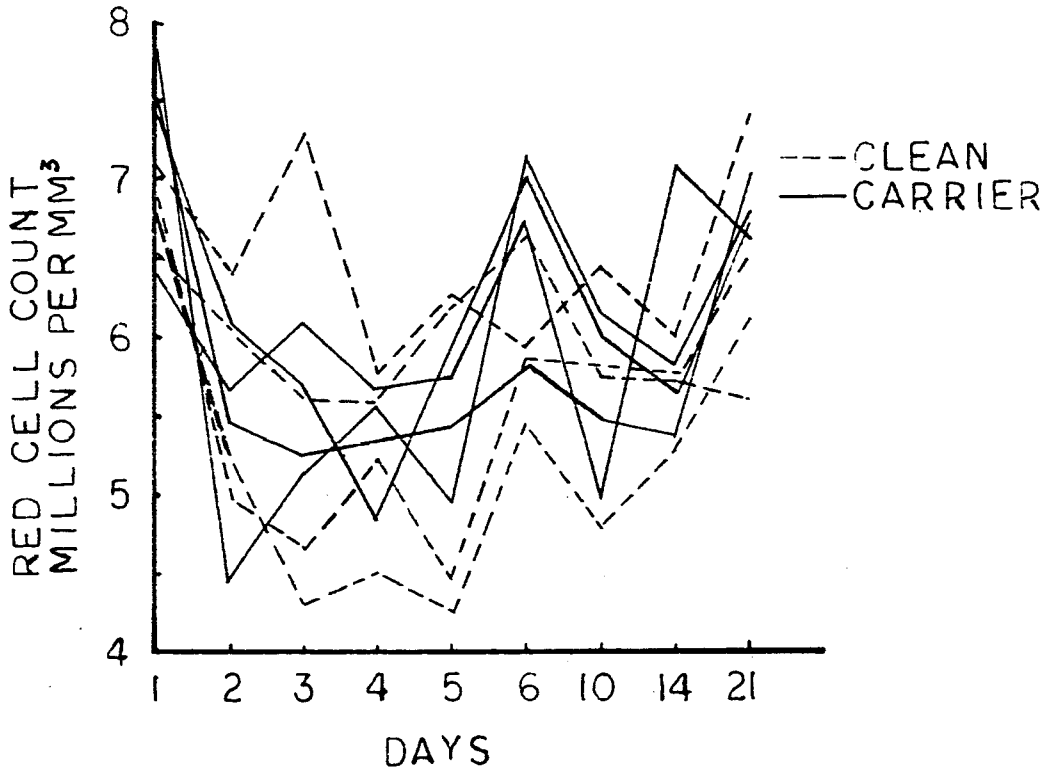

--- CLEAN
— CARRIER
PERCENT HEMOGLOBIN
9
6
3
1 2 3 4 5 6 10 14 21
DAYS

Figure 19. Individual response to hemorrhagic anemia for total numbers of white cells

Figure 20. Individual response to hemorrhagic anemia for numbers of red cells


WHITE CELL COUNT
THOUSANDS PER MM³
13
11
9
7
5
1 2 3 4 5 6 10 14 21
DAYS
---CLEAN
—CARRIER


RED CELL COUNT
MILLIONS PER MM³
8
7
6
5
4
1 2 3 4 5 6 10 14 21
DAYS
---CLEAN
—CARRIER

# DISCUSSION

## Erythrocyte Studies in Discriminating Carrier and Clean Animals

The study of erythrocytes in relation to the dwarfism problem arose from the study of leukocytes. Massey et al. (1956) and Swiger (1957) related that small particles or objects were noticed in the total white cell counts; the number of these particles increased to some extent following injections of insulin and adrenalin. Speculation arose as to the origin and nature of these particles. If the particles in the white cell counts were erythrocyte cell walls, as thought by some workers, then a study of erythrocytes would give more reliable information. An increased number of these "ghosts" or erythrocyte cell walls, in a hypotonic solution, such as that used in making total leukocyte counts, may be caused either by an increase in number of erythrocytes or a decrease in the resistance to osmotic fragility.

### Fragility

Fragility readings obtained on the colorimeter are a measure of and are proportional to, the optical density of the solution as determined by the photoelectric cell. Since the optical density is theoretically proportional to the concentration of the solution (Beer's law, as stated in the Klett Sumerson clinical manual) the scale readings obtained are likewise propor-

tional to the concentration. The manual also states that the most satisfactory portion of the scale for colorimetric measurement is the range from 0 to 400 and that readings above 600 should not be used as a basis for calculating results. Samples with readings above 600 were diluted and the concentration value was calculated on the basis of dilution.

The color of the solution after centrifuging is a measure of the quantity of material contained in the ruptured erythrocytes. The color, then, is determined by the number of red cells and the material contained in the individual red cells, assuming that the cell walls were removed by centrifuging. The color obtained when a sample of blood is completely hemolyzed in distilled water may be an indication of hemoglobin content. As indicated in Figure 21, this relationship was rather high on eight calves in a preliminary experiment. When these blood samples were only partially hemolyzed in 0.60 percent saline solution, the color obtained still seemed closely associated with the hemoglobin content of the samples as shown in Figure 22. However, when these values were compared on 58 calves, a correlation of only 0.30 was found. This indicates that the hemoglobin content and the color obtained in a saline solution are not as closely related as shown in Figure 22. Wintrobe (1956) stated that the strength of the cell membrane in resisting the osmotic pressure from within the cell varies between

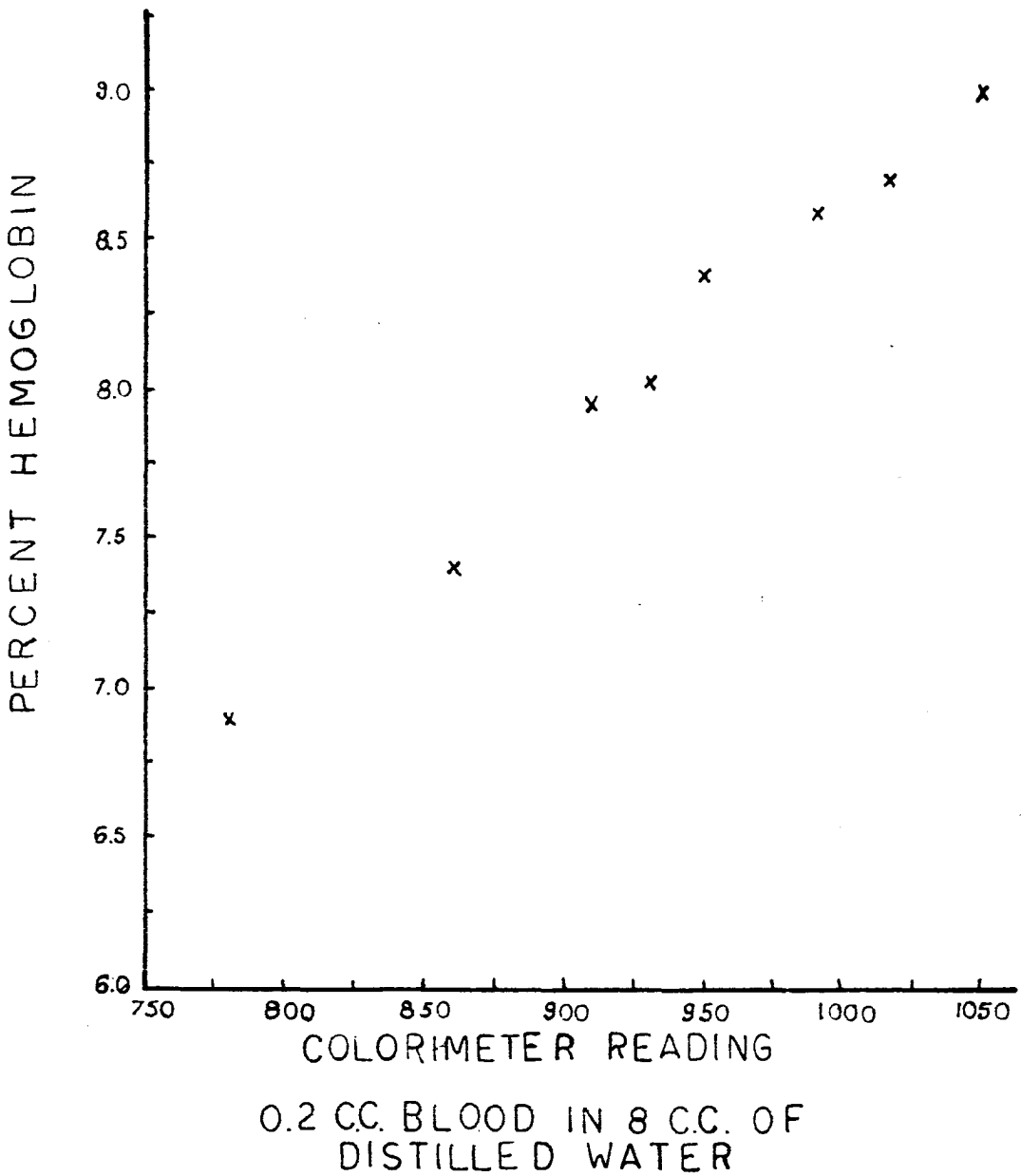



Figure 21. Relationship between percent hemoglobin and calorimeter readings for eight calves

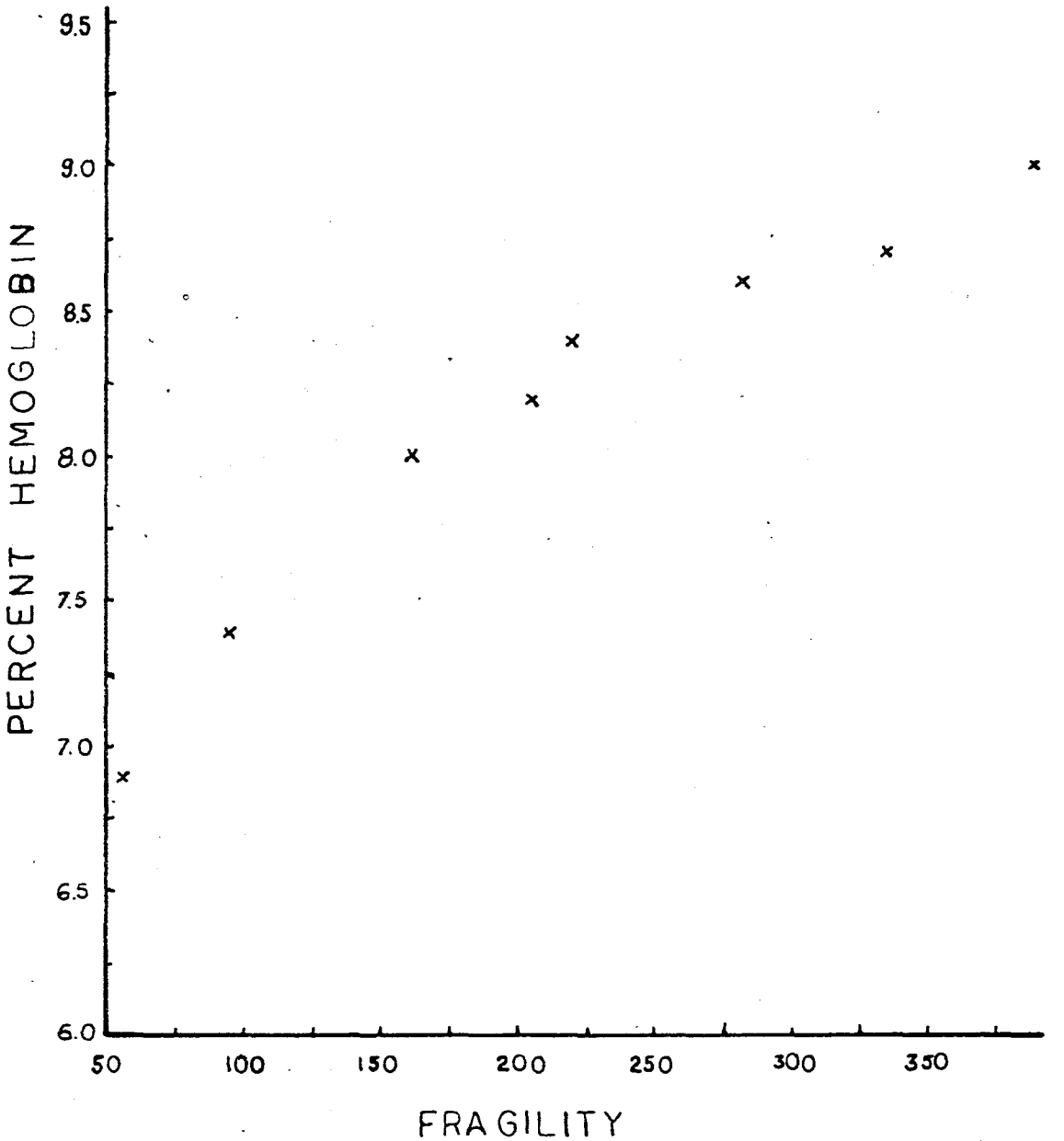



Figure 22. Relationship between percent hemoglobin and erythrocyte fragility as measured by 0.4 cc. blood in 8 cc. of 0.60 percent saline solution for eight calves

animals and also between times and conditions within an animal. Therefore the color obtained in a saline solution is to some extent, a function of fragility of the cell membrane and the amount of hemoglobin contained within the cells, since the unhemolyzed cells and cell membranes are centrifuged out.

The discrimination between carrier and clean animals by using these fragility readings was quite poor. The significant differences obtained between genotypes of calves indicated that differences between the groups may exist, but the overlap prohibits accurate classification of the genotypes of individuals. The limited information on yearling steers in this study and the results obtained by Hazel (1958) indicate that similar differences between genotypes may exist for yearlings also. Significant differences existed in total fragility between genotypes of cows in the adrenalin experiment, even though the response to adrenalin showed little difference between genotypes. It appears from the data in these experiments that significant differences between genotypes can be found for any age if a sufficient number of animals is used or, in the case of the adrenalin work, if the number of observations on an animal is increased.

The negative correlation of age and fragility for calves, although not statistically significant, agrees with the fact that calves have higher fragility of red cells than either

yearlings or cows. Also, in Experiment 2, the first sample of blood from the calves was significantly higher in fragility than the second sample. In fact this difference is too large to be accounted for by age alone. The regression of weight on age accounts for a difference of 62 whereas the observed difference is 145. However, the difference between the two six-week samples may not have any bearing at all on age since the 0.60 percent saline solution was not of the same batch for both experiments. Also, effects of season of the year, feeding regime that the calves were on, and the possibility of physiological changes in the digestive and metabolic processes may overshadow a single source of variation such as age. This may be evidenced by the fact that a repeatability of 0.53 was obtained when two samples six weeks apart were taken but three such samples had a repeatability of only 0.37.

The distribution of fragility values closely resembles a Poisson distribution (Figure 4). Closer examination suggests that perhaps calves, yearlings and cows follow different distributions but numbers in these age groups are inadequate to establish this. The variances for the age groups were not homogeneous. The square root transformation of the fragility data in the first experiment was used in an effort to obtain normal distributions and equal variances. The transformation helped little in obtaining either normality or homogeneous

variances. The transformed data are more nearly normal than the raw data, as indicated by the data plotted on probability paper (Figure 3), but the curve still indicates non-normality. The literature revealed little information concerning the distribution of erythrocyte fragility in animals of any species. Bolton (1949) pointed out that hemolysis of a single sample of blood in varying strengths of saline solution followed a normal distribution but this is a different situation than that confronted here.

Since equal variances of age groups can not be assumed in this study, it appears that genotypes should be compared within age groups. Possibly each age group in itself might be normally distributed but, because greater variability occurs in calves than in cows or yearlings, these ages should be examined separately.

The fragility of erythrocytes generally increased following the administration of adrenalin or the induction of hemorrhagic anemia. In a preliminary experiment samples were taken 10, 20, 30, 40, 50 and 60 minutes following adrenalin. These samples suggested the duration of this increase was about 20 minutes following an adrenalin injection. Samples taken every six hours up to 48 hours following hemorrhage indicated that the increase in fragility peaked at about 24 hours. The responses of the animals to either of these stress factors did not seem

to be affected by their genotypes.

An increased fragility value may result from an increase in the number of erythrocytes being exposed to the saline solution, from a decrease in the strength of the outer membrane of the cells that are exposed, or from an increased amount of hemoglobin per cell. The first of these theories can be nearly repudiated from the data obtained on hemorrhagic anemia. A comparison of Figures 11 and 16 indicates that following hemorrhage, fragility increased during the first 24 hour period in all animals but one, while the number of red cells in all animals decreased during this period. These observations are on only eight animals but the relationship between fragility and red cell numbers appears obvious. Since hemoglobin content also decreased during this period, owing principally to the decrease in erythrocytes, it appears that the increased color obtained in the fragility test was not due to an increase in either cell number or increased hemoglobin per cell but to some other cause. Perhaps the erythrocytes become more fragile after the onset of anemia. The term "fragility" seems well suited to describe the hemolysis which takes place when blood is pipetted into a saline solution.

Hemoglobin and hematocrit

Hemoglobin and hematocrit content of the blood offered little help in differentiating between carriers and cleans.

Differences between genotypes were small and an interaction between age group and genotype existed in the first experiment for both components. No explanation can be given for these interactions except that the yearlings were on a full feed of grain while the cows and calves were on good grass pasture. The genotypes in each age group were not fed or managed differently, however. The age-genotype interaction was not present for either hemoglobin or hematocrit in Experiment 3 where adrenalin was administered. Steers were not included in this study. However, the cows in this experiment were considerably higher than the calves, while in Experiment 1 cows were only slightly higher on the average in hemoglobin content and percent packed red cells. Change in feeding and management could again be responsible since in Experiment 1 the calves and cows were together on grass and during Experiment 3 the cows were on winter feed and the calves, being weaned, were in the dry lot.

The significant differences in hemoglobin content between sexes in the two bleeding periods of Experiment 2 needs further examination. Table 21 shows that in the first bleeding period the male calves had the higher hemoglobin content and in the second period, six weeks later, the females were the higher. Of the seven calves that appeared in the first

bleeding but did not appear in the second, six were males and averaged 0.5 percent higher in hemoglobin content than the remaining 26 male calves. This may have had a bearing on the switch in rank of males and females as far as hemoglobin is concerned but it is doubtful and of little importance in this study.

Red cell count

As in both hematocrit and percent hemoglobin, the interaction between age and genotype in the numbers of red cells was significant in Experiment 1. There was no indication in this experiment that animals of different genotypes could be differentiated by their red cell count. Dukes (1955) states that the volume of packed red cells and red cell count are directly related and that red cell count varies widely between individuals and even within an individual. Reasons for this variability, among others, are environment, exercise, nutritional status and climate. It was for these reasons, along with the fact that red cell counts are quite tedious and time consuming to make, that they were not made in the other experiments, except in the anemia experiment. However, this work showed that the relationship between hematocrit and red cell count was not as high as supposed. Within age and genotype the correlation was .42 between these two components in Experiment 1. If the effects of age and genotype were not

removed, this correlation was .39. Both of these correlations were highly significant.

## Leukocyte Studies in Discriminating Carrier Animals

None of the data in this thesis indicates that carrier and clean animals can be differentiated by total white cell count. In fact, no significant differences between means of the two genotypes were found in any of the four experiments. Swiger (1957) found that the best means of prediction was by the change in neutrophils from one to two hours following an injection of adrenalin. He obtained a correlation between actual and predicted genotypes of .71. However in the present work it was not possible to draw a line of discrimination for the response from 20 minutes to 120 minutes in neutrophil count. Interestingly enough, in Table 18, i.e. Experiment 1 with no treatment, a discrimination line was drawn for calves at 2875 neutrophils. The prediction of genotypes by this line is correlated 0.61 with the actual genotypes shown in Table 35. However, when all three ages are considered and a line is drawn to make the fewest mistakes, the predicted genotypes and actual genotypes are correlated only 0.10.

## Recommendations for Further Work

The evidence obtained in this study suggests certain recommendations for future work in this area. The data in

Table 35. Correlations between actual and predicted genotype based on the neutrophil count

| | Calves | | All three ages | |
|---|---|---|---|---|
| | NN | Nn | NN | Nn |
| Predicted NN[a] | 6 | 4 | 13 | 18 |
| Predicted Nn[b] | 0 | 12 | 12 | 25 |
| Correlation | .61*** | | .10 | |

[a]NN - Homozygous normal

[b]Nn - Heterozygous normal

*** - Significant at P = .01

this study and those reported by Swiger (1957) and Turman, (Stillwater, Oklahoma, 1957), indicate that further work on total white cell counts and differentials would not be rewarding. The volume of packed red cells and hemoglobin content of the blood need not be studied for their value in discriminating genotypes, although they are beneficial in trying to explain the results obtained concerning fragility. If red cell counts are not made, either of these criteria help in diagnosing anemia and provide a basis for reference. Further work should be conducted on the fragility of the erythrocytes. Fragility should be studied more in relation to different strengths of saline solution and should be extended to other hypotonic solutions as well.

# SUMMARY

A hematological study involving 118 animals was made in an effort to discriminate between homozygous normal animals and those heterozygous for the snorter dwarf gene. The characteristics of the blood studied were red cell count, percent packed red cells, percent hemoglobin, fragility of the red cells, total white cell count and differential white cell count. The effect of age, sex, temperature, adrenalin chloride and hemorrhagic anemia on the above characteristics of the boood were studied. Techniques for studying erythrocyte fragility and for causing hemorrhagic anemia were worked out. Standard techniques for the other hematological determinations were used.

All animals studied were of known genotype with reference to the "snorter" dwarf gene in that carrier animals had one dwarf parent or had produced a dwarf, while the clean animals had no history of "snorter" dwarfism. The clean cows and some of the clean calves were of one inbred line and were quite excitable upon handling. The remainder of the clean calves resulted from an outbreeding system where no intensification of a particular sire or inbred lines was made.

The data on percent hemoglobin, hematocrit, red cell count, total white cell count and differential white cells

appeared to follow the normal distribution. The limited data on erythrocyte fragility did not seem to follow the normal distribution nor were the variances homogeneous for the age groups studied. The fragility data appeared to be distributed as the Poisson. The square root transformation was used but it failed to normalize the data completely or to make the variances homogeneous.

Calves were higher in red cell fragility, total white cell count and lymphocyte count than were cows and yearlings. The regression of total white cell count on age in calves was significant. When injected with adrenalin chloride, cows were significantly higher than calves in volume of packed red cells and in percent hemoglobin but were lower than calves in fragility of red cells and in total white cell count. The response to an adrenalin injection was approximately the same in calves and cows.

Atmospheric temperature affected percent hemoglobin and volume of packed red cells but not any of the other characteristics of the blood studied. Percent hemoglobin and packed red cells decreased, on the average, with an increase in temperature.

Homozygous or clean animals have erythrocytes which tend to be less resistant to a hypotonic salt solution than carrier

animals. Carrier and clean calves differed significantly in their fragility values but no accurate classification of individual genotypes was possible. None of the other characteristics of the blood studied seemed to offer any accurate means of discriminating between genotypes. Discriminating between genotypes by fragility of erythrocytes seemed more promising in yearling cattle than in either of the other age groups but this classification was still highly inaccurate.

Genotypes seemed not to differ significantly in their response to an adrenalin injection, as measured by percent hemoglobin, volume on packed red cells, red cell count, fragility of red cells and white cell count. Also, following hemorrhagic anemia, little difference between genotypes was noted in these components of the blood.

Repeatabilities for three samples six weeks apart were low for all characteristics studied. However, the repeatability was higher when only two periods were studied.

The small differences that exist between groups of heterozygous and homozygous animals in these hematological characteristics indicate that average physiological differences probably do exist between these two genotypes. However, the large total variation in these characteristics and the small average difference between genotypes make discrimination between individual genotypes impossible.

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