THE EFFECTS OF STRESS UPON ERYTHROCYTE RESISTANCE IN PEDIGREE CLEAN, KNOWN CARRIER AND DWARF BEEF CATTLE

A Thesis

Presented to

the Faculty of the Graduate School

University of Missouri

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

---

by

Arthur Guy Johnson

August 1958

The undersigned, appointed by the Dean of the Graduate Faculty, have examined a thesis entitled

THE EFFECTS OF STRESS UPON ERYTHROCYTE RESISTANCE IN PEDIGREE CLEAN, KNOWN CARRIER AND DWARF BEEF CATTLE

presented by Arthur Guy Johnson

a candidate for the degree of Master of Science

and hereby certify that in their opinion it is worthy of acceptance.

John F Lasley

W. L. Naumann

Merle Mohrer

ACKNOWLEDGEMENT

The author would like to express his indebtedness and sincere appreciation to Dr. John F. Lasley, Professor of Animal Husbandry, under whose direction this research was conducted. Dr. Lasley's sound advice and assistance as well as his continuous encouragement and sincere interest were invaluable during the preparation of this thesis and throughout the period of graduate study.

Acknowledgement is given to Dr. Dennis T. Mayer, Professor of Agricultural Chemistry, for his timely comments and suggestions which aided the author in this work and to Mr. Cecil L. Gregory, Assistant Professor of Rural Sociology, for the assistance which he gave in calculating the data used in this research.

Deep appreciation is expressed to Dr. V. B. Reddy, Assistant Professor of Animal Husbandry, and to Mr. John W. Massey, Mr. Lawrence L. Kramer, Mr. Mike Milicevic, Mr. Charles W. Foley and other graduate students in the Department of Animal Husbandry who contributed their time and efforts to help make this investigation possible.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

INTRODUCTION

Has the fight against beef cattle dwarfism progressed? Are methods now available whereby the breeder can enjoy a "dwarf-free" production program? These questions are examples of those frequently encountered by the animal husbandry scientist who is engaged in the ever growing battle against this problem. Although increased numbers of inquiries are comforting in one sense they are not uncommon when a situation reaches the magnitude now assigned to dwarfism. It is through reference to them, however, that a portion of the expanding concern over this problem can be pictured and the expressed desire for established facts should be looked upon with favor. While the above type of enthusiasm stems from various sources additional and more specific interests can be illustrated by noting the enlarged efforts of many universities and independent research firms, and the increased willingness of many breeders to supply animals for experimental purposes.

This somewhat recent acceleration of dwarfism research is obviously not without reason. Although many factors continue to be involved, two which merit notation at this time are the economic losses consistently endured by the producer and the encouraging advances which have been made within the past months.

In a study of the major beef cattle breeds it was found that more than one type of dwarf individual was in existence. As a result

the terms dwarf and dwarfism take on a somewhat abstract definition and should be qualified as to meaning. In order to eliminate classes of lesser importance and deal directly with that group which is of greatest economic concern at the present time, the foregoing terms will be used almost entirely in connection with dwarfs of the "snorter" type. This animal's appearance has been described many times, but some general characteristics which are frequently observed include difficulty in breathing, abnormal development of the skull, unusually short legs, an undershot jaw, a shaky walk and a tendency to be rather paunchy. Some of the traits are present at birth while others develop as the dwarf becomes older.

To further explain some of the fundamental factors concerning dwarfism a brief description of how the condition is transmitted might be helpful. Rather conclusive data are now available to show that this abnormality is inherited and is the result of a simple recessive gene. Genes occur in pairs in the body cells, but in the reproductive cells only one member of each pair is present. Of each gene pair, the individual receives one gene from its sire and one from its dam. Since only two different genes are involved here the inheritance of dwarfism is relatively simple. One gene "D" is for normal size while the other gene "d" is for dwarfism. Gene "D" is called dominant gene because all cattle carrying it are normal in appearance. In a similar manner gene "d" is called a recessive gene because it will not express itself when the "D" gene is present. With this in mind it becomes apparent that three genetically different individuals are possible within a breed.

The "clean" members are homozygous dominant and have a genetic makeup of "DD". These animals, which appear normal, can pass only the "D" gene and therefore cannot produce a dwarf calf. "Carrier" animals also appear normal, but are heterozygous and have the genetic composition of "Dd". These individuals are capable of producing a dwarf and upon doing so are termed "known carriers". The homozygous recessive animals are symbolized by the genotype "dd" and are dwarfs. Since most of the work in this study was centered around these three classifications, reference to them will be found throughout the text of the discussion.

Until recent months the breeding test was considered the most satisfactory method for determining whether or not a normal appearing animal was a carrier of the dwarf gene. This test required at least one year to check a bull after he reached sexual maturity and was initiated by mating him to thirteen "known carrier" cows. If no dwarf calves were produced from these crosses the chances were 95 out of 100 that he was a "clean" animal. In cows, however, it was almost impossible to reach this level of probability because of the large number of matings required. Although employed successfully in some cases, the obvious disadvantages of this test limited its use and thereby prevented it from significantly effecting the number of dwarfs found in many herds. Still, the realization that dwarfism was of major concern to many producers stimulated and encouraged various new approaches to the problem. Among the most successful of these was the recent study conducted with blood. In that research stressed representatives from the three different genotypes showed statistically significant variations

in their leukocyte counts at one and two hours after the administration of the stress.

Upon observation and analysis of the preceding data a question arose as to whether variations might also be observed in the other major blood cell group, the erythrocytes. The method chosen to determine this was erythrocyte resistance and the resulting investigation was conducted with two primary thoughts in mind. First, would the red blood cells of "clean", "carrier" and "dwarf" individuals become more fragile when the animals were put under stress, and was there a significant variation in resistance shown by the three different genotypes? Several different types of pituitary, adrenal and pancreatic hormones were administered in nonphysiological doses to induce stress or conditions similar to those found in animals under stress. The results were analyzed by examining red blood cell resistance to hemolysis in a hypotonic saline solution.

The trials were conducted mainly on female Hereford cattle during the various seasons of 1956 and 1957. Some data are also present on members of the Aberdeen Angus breed, however, a considerably smaller number of these individuals were used.

Primarily this study was not intended to develop into another diagnostic test for dwarfism, but mainly to supplement present data and to contribute additional information which might be of aid to future research along this line.

CHAPTER II

REVIEW OF LITERATURE

Extensive hematological studies have been carried out which illustrate various aspects of the blood picture in cattle. Some of these have been instrumental in the development of certain concepts at the Missouri laboratories. However, the author has no knowledge of previous investigations of genotypic differences in erthrocyte resistance to hemolysis.

EARLIER STUDIES OF BOVINE DWARFISM

According to Seligman (1904) the first official record of dwarfism in cattle was made in 1860, but Craft and Orr (1924) were the first to describe a dwarf in the United States. Later Lush (1930) reported on a herd of duck-legged cattle in Texas.

Further observations have aided in substantiating the belief that several different types of dwarfism may be in existence; each possessing its own characteristics and mode of inheritance. According to Gregory (1956) there are at least six different variations in the condition. Although breed is not the exclusive factor in determining the type of individual produced, it does seem to exert some influence. Roubicek, Clark and Pahnish (1955) stated that some kind of dwarf had been reported in all of the major breeds.

Among the several different kinds of dwarfs the short headed or snorter type seems to show up most frequently and therefore appears to

be responsible for a great deal of the trouble. It is now generally accepted that this kind of dwarfism is the result of a single pair of autosomal recessive genes. The problem that confronts the scientist and the practical animal breeder centers around the inability to diagnose the dwarf gene in a carrier animal and to confirm beyond any doubt the absence of such a "dirty" gene in a normal individual.

Due to the frequent occurrence of death soon after birth the condition might possibly be considered sub-lethal. Johnson et al. (1950) described a type of dwarfism which could be classed as latent lethal, that is, the animals seldom reached the age of reproduction. The presence of this condition was originally noted in one herd of purebred Hereford cattle, but later reports proved that it was occurring in numerous other herds of the same kind. A study of the factors indicated that this dwarfism was being transmitted as a monofactorial, autosomal recessive characteristic, and that known carriers of the gene were rather intermediate in type.

In a different and somewhat more specific approach, Carroll et al. (1951) found dwarf pituitaries to be active only at subnormal rates in the secretation of thyrotropic hormone. These investigators then suggested that this condition might account for the dwarfism observed. Johnson et al. (1950), however, did not find this situation to be present when they carried out a similar study. Marlowe and Chambers (1954) also disagreed with the work reported by Carroll et al. (1951) and they found no significant differences in the thyrotropic hormone potency of dwarf and normal calf pituitaries. In a recent

investigation by Crenshaw et al. (1957) beef cattle dwarfism was explored with the idea that it might be due, in part, to a dysfunction of the pituitary-thyroid gland axis. They concluded that the condition was not a result of any abnormal relationship between these two endocrine glands.

Gregory et al. (1953) suggested that dwarfism in beef cattle and cretinism in human infants were identical due to the predominantly bulging frontal bones noticed in both of these abnormal conditions. It was also mentioned that a measurable, but less pronounced expression of this defect was present in carriers.

A great variety of techniques have been employed in an effort to detect the carrier or heterozygous animal. One of the oldest of these is progeny testing, however, this method has decreased in popularity due to its many limitations.

During more recent years the investigations have been focused toward establishing a quicker and simpler means for detecting the carrier animals. Gregory et al. (1952, 1953) in their anatomical studies indicated three valuable points for diagnosing the heterozygous individual. These were the parietal junction, the region of the midforehead and the nasal frontal junction. As a result of the work done by Gregory et al. (1952, 1953) an instrument called the "profilometer" was developed. It was designed to aid in the detection of carriers of the dwarf gene, and was to be used on clean, carrier and dwarf animals.

Tyler, Julian and Gregory (1956) conducted two types of studies in an attempt to determine the nature and location of the process

responsible for the short-headed dwarf. In a comparison of the skulls of dwarf and normal cattle they found that the sphenooccipital synchondrosis of the dwarf closed within a week after birth, while that of the normal animal did not close until twenty-six months of age. In addition to this some other irregularities were observed and the cumulative knowledge possibly explains the abnormal skull structure encountered in this kind of dwarfism.

In another aspect of their work, Julian, Tyler and Gregory (1956) recorded measurements of the appendicular skeleton of dwarf and normal animals. Proportional differences between the two genotypes were observed. Of the differences, one of the most outstanding was in the metacarpal bone of young dwarfs, which was reduced in diaphyseal length. They concluded that a mild form of hypoplastic achrondroplasia was present, and that it was responsible for the snorter type individual. Emmerson and Hazel (1956) showed that the radiographic technique might be a promising method for carrier detection, with x-ray interpretation as a limiting factor. Employing this technique Bovard, Hazel and Emmerson (1956) studied the differences in the spinal column of dwarf and normal appearing calves. Variation between the groups was obtained, but limited progeny testing illustrated that some vertebral classifications did not agree with the genotypes of the animals. Buchanan et al. (1956) also carried out an investigation which showed that the dwarf gene effected various skeletal changes, primarily in the vertebrae and skull.

The so called "chemical" tests for dwarfism have been rather

successful and certainly more promising than most other methods when considering the probability of distinguishing the carrier individual from other genotypes. In some initial studies Heidenreich, Lasley and Comfort (1955) pointed out a difference in the ability of dwarfs and normal appearing animals to regain their normal blood sugar following stress. Foley, Massey and Lasley (1956) then demonstrated that after insulin injection, dwarf beef cattle had a more rapid and acute drop in blood sugar level and required a longer time to return to normal than did the clean individuals. This group further illustrated that clean, carrier and dwarf animals had significantly different white blood cell counts at one and two hours after insulin induced stress. These differences were thought to be associated with some type of adrenal cortical insufficiency.

Crenshaw et al. (1957) stated that dwarfism in beef cattle showed symptoms of hypothyroidism, but was not due to a dysfunction of the pituitary-thyroid gland axis.

In spite of considerable efforts to eliminate or at least reduce the frequency of the dwarf gene, this physiological phenomenon has become rather common in registered herds of the major beef breeds, and a noticeable increase in its frequency has also been observed by commercial producers.

SOME CHARACTERISTICS OF THE ERYTHROCYTE

The red blood cell has long served as a favorite subject for the study of cellular physiology and as an index of pathological

changes within the organism. It has also been the item of prime interest in various other types of investigations and many hematologists have discussed its characteristics in great detail.

General Appearance. Mammalian red cells are nonnucleated and are believed to exist normally in the blood stream as biconcave discs. Generally they make up about 45 per cent of the blood by volume and are responsible for the opacity of the fluid.

Structure. Dukes (1955) stated that the minute structure of the red cell was still unsettled. He believed that erythrocytes consisted of some type of elastic or spongelike stroma containing hemoglobin, and that they were surrounded by some type of cellular membrane. Best and Taylor (1955) reported that the red cell was bounded by a membrane made of protein in association with lipid and steroid materials, chiefly lecithin and cholesterol. They also mentioned that this membrane had not been demonstrated histologically, but that indirect evidence indicated its presence and that it consisted of an outer and inner layer.

In regard to the body of the cell, these authors stated that it was constructed of a spongelike stroma made of the same or similar materials probably in the form of a gel. They also felt that in the meshes of the stroma, or more likely actually bound up in the stroma substance itself, the respiratory pigment hemoglobin was held. An important fact in the support of this theory was that mechanical division of the cell, even into the finest particles, failed to liberate

the hemoglobin.

Size. According to Hawk, Oser and Summerson (1954) mammalian erythrocytes vary in size from species to species, ranging from two microns to about nine microns in diameter. Fraser (1930) using a micrometer eyepiece, recorded an average of 5.0 microns with a common range from 4.0 to 6.0 and an extreme range of 3.6 to 9.6 microns. Blount (1939) employed a cover slip technique to lessen corpuscular shrinkage and recorded an average diameter of 6.5 microns with a common range from 5.0 to 7.0 and an extreme range of 4.0 to 8.0 microns in diameter. Canham (1930) reported that cells of 6.0 microns were frequently seen and that cells of 7.0 microns were present. Wintrobe (1951) quotes Kushner for the range of 5.0 to 5.9 microns in diameter, but this included calves which had a smaller cell size. Holman (1955) then stated that an average diameter of about 5.7 microns would be applicable to most individuals provided that they had a normal erythrocyte count of about 6 million cells per cubic millimeter. Dukes (1955) was in close agreement with this figure and placed the mean diameter at 5.6 microns.

Another way in which erythrocyte size may be estimated is that of corpuscular volume. This measurement, as the name might suggest, is simply a determination of the volume of the cell. Since it is obtained by using more than one measurement it may have more descriptive value and it is frequently quoted in preference to cell diameter. The relationship noted by Holman (1952) between mean corpuscular volume

and total red cell count seemed to imply that when a high erythrocyte count was recorded a low mean corpuscular volume would be present. Coffin (1953) reported a range for mean corpuscular volume of 50.0 to 61.0 cubic micra. Canham (1930) stated that the mean corpuscular volume was nearer 47.0. The range given by Price et al. (1957) for beef cattle at 500 pounds of body weight and under uniform environmental conditions was from 43.9 to 59.5 cubic microns. These animals had a mean corpuscular volume of 51.8. Holman (1955) found that when the total erythrocyte count was near 6 million, a useful working average for mean corpuscular volume would be approximately 57.0 cubic micra. Price-Jones (1931) set the value for this measurement at 57.5 ± 0.216 cubic micra.

To include an analysis of all the factors which could effect corpuscular size would necessitate a lengthy discussion and is certainly a problem within itself. Holman (1946) reported some interesting data which illustrated the effects of age upon erythrocyte dimensions. He first observed that the erythrocyte had a mean corpuscular volume of about 52.0 cubic micra at birth, but that it rapidly decreased in size over the first week and then decreased less rapidly to average about 40.0 cubic micra at two months. After two months the cell slowly increased in size to reach 57.0 cubic micra at the end of about two years. He also found that at four months the erythrocyte count fell as the corpuscular size increased. In a more specific discussion of maturation time, it was noted by him that the red cell count reached the adult figure at 22 months while corpuscular size obtained

this level in 29 months.

Canham (1930), working on South African cattle, supplied figures which confirm this work and Greatorex (1954) also indicated that such changes do take place.

Composition. Dukes (1955) reported that the erythrocyte contained, in different species, 62.0 to 72.0 grams of water per 100 milliliters of cells. In addition, this author stated that the pigment hemoglobin constituted much the greater part (about 95 per cent) of the total solids. He believed that the stroma itself was composed of proteins, the lipids lecithin, cholesterol, and cephalin; and of inorganic substances. Hemoglobin, the pigment of the erythrocyte, was found to be a complex, iron-containing, conjugated protein composed of a pigment and a simple protein. The pigment was ferroheme and the protein fraction was globin, a histone. Hawk, Oser and Summerson (1954) stated that hemoglobin was the most abundant protein in the blood, being usually found to the extent of about 14.0 to 16.0 grams per 100 milliliters of whole blood. Under normal conditions it was observed to exist almost entirely within the erythrocyte, but a wide range of hemolytic agents may cause its release.

Hawk et al. (1954) dealt with the subject more specifically and introduced some additional information about the constituents of a red blood cell. In their study they found that the erythrocyte contained approximately 65.0 per cent water and 35.0 per cent solids. Of the solids, the red chromoprotein hemoglobin comprised about 32.0 of the

35.0 per cent. Most of the remaining 3.0 per cent formed the stroma of the cell and consisted largely of proteins, phospholipids and cholesterol. Inorganic ions found in the erythrocyte included potassium, chloride, bicarbonate and phosphate. Of these, potassium was present in the largest amount, being comparable quantitatively, and in certain respects physiologically, to the sodium of the plasma. Organic constituents were found to include various phosphate esters and certain enzymes, such as phosphatases and carbonic anhydrase. This latter constituent was of particular significance in connection with the function of erythrocytes.

The brief discussion of hemoglobin which preceded this information did not mention the fact that all hemoglobins were not identical compounds, even among the mammalian species. Dukes (1955) believed that the variation was due to a difference in the globin part of the molecule. Beach et al. (1939) stated that the incompatability was apparently related to the chemical composition of the globin fraction, which seemed to vary in amino acid content. Reichert and Brown (1909) also demonstrated that hemoglobin from different animals was not the same. In their work they employed the process of crystallography and showed that the hemoglobin from most species could be crystallized, but that the ease of crystallization and the shape and size of the crystals often varied greatly.

Function. Dutcher, Jensen and Althouse (1951) reported that erythrocytes were highly specialized cells, responsible for the transfer of oxygen and carbon dioxide between the lungs and tissue. In a

rather similar description Hawk, Oser and Summerson (1954) stated that the major function of the red cell appeared to be the transportation of oxygen and carbon dioxide, and that this function could be explained on a purely physiochemical basis in terms of the role of hemoglobin and other substances which were present. Additional comments concerning the function of erythrocytes were made by other investigators and generally they seemed to be in agreement with the information presented here.

THE ORIGIN OF THE ERYTHROCYTE

When considering the origin of erythrocytes one factor that warrants attention is the age of the individual. Best and Taylor (1955) stated that the first signs of blood and blood vessels appeared very early, but that during this stage they were present only in the mesenchyme of the wall of the yolk-sac. Following this it was found that the embryonic and extra-embryonic vessels formed communications with each other to allow the primitive blood plasma and erythrocytes to enter the body of the embryo. The mesenchyme cells of the general connective tissues of the embryo's body formed some of the blood components at this time. It was suggested that this type of blood formation was of short duration and that the process soon became localized in the liver, spleen, bone marrow and lymph glands. In addition to this it was noted that during the latter part of the pre-natal life of most animals, the liver and spleen lost the power to produce the necessary cell types and therefore ceased to serve as blood forming organs.

Erythrocyte formation was then located primarily within the red bone marrow.

The bone marrow has frequently been called hematopoietic tissue and is responsible for the normal production of erythrocytes throughout post-natal life. In the adult animal this tissue was found to be limited almost entirely to the flat bones, such as, the sternum, ribs, diploe of the skull and the bodies of the vertebrae. In young individuals small amounts were often observed in the ends of the femur and humerus, but little was usually present in such long bones as the tibia. Dukes (1955) confirmed this process and reported, that in the fetus, the liver, spleen and lymph nodes were jointly concerned with the formation of red blood cells, but that in post-natal life the bone marrow was, under normal conditions, the only area of erythropoietic activity. Additional mention was made to the fact that if certain abnormal or pathological conditions were present, it was possible for all the organs to reassume the function of red blood cell production. Grant and Root (1952) also believed that, under ordinary circumstances, adult mammals formed their erythrocytes in the red bone marrow.

SOME FACTORS WHICH STIMULATE THE PRODUCTION OF ERYTHROCYTES

Grant and Root (1952) found that red cell formation occurred under the conditions of anoxia irrespective of whether the functional state was one of anoxic or anemic anoxia. As a result, they regarded anoxia as the fundamental or primary stimulus for erythropoiesis.

Dukes (1955) was in agreement with this work and reported that anoxia was a potent stimulus for erythrocyte formation.

The mechanism by which anoxia or some similarly acting agent stimulates erythropoiesis has not definitely been established, but Bert (1882) suggested that anoxia of the bone marrow was the specific requirement for the initiation of the process. Dallwig et al. (1915) also believed that this condition was important in the cycle of events which were necessary for increased red cell production. In a later study of bone marrow oxygen saturation, Reznikoff et al. (1935) introduced partial evidence to show that an anoxia was present in the marrow of individuals with polycythemia. Further work by Schwartz and Stats (1949) illustrated that the percentage of oxygen saturation in the marrow blood of such patients was greater than normal, and that the marrow blood of anemic individuals was not abnormal in relation to oxygen content. Grant and Root (1952) concluded that most evidence offered for the anoxic marrow mechanism was indirect and was derived from the association of accelerated erythropoiesis with low arterial oxygen pressure, present under some conditions.

The lack of convincing evidence to demonstrate that local anoxia of the bone marrow was the fundamental stimulus for red cell production led many investigators to believe that anoxia must act at some site other than the bone marrow and produce an effect upon erythroid tissue secondarily. As a result, several workers began to study the effects of the nervous system and carbon dioxide tension upon erythrocyte production, but they could establish no significant relationship

between the two. Numerous other investigators assumed an alternative possibility and analyzed the effects of various endocrine gland secretions upon the process.

Davis (1942) demonstrated that small or moderate injections of posterior pituitary extract induced a marked increase in the number of erythrocytes and in hemoglobin values. He explained these results on the basis of a stimulation of erythropoiesis caused by anoxia of the bone marrow from the vasoconstricting effect of the extract. In another investigation Grant and Root (1952) reported that feeding of fresh hypophyseal material produced erythropoietic stimulation in normal animals. Van Dyke et al. (1957) also carried out an extensive experimentation to determine the role of the pituitary in red blood cell production. Their work was designed to establish the existence of a specific erythropoietic hormone which would have as its target organ the red cell producing portion of the bone marrow. Results from the administration of pituitary extracts showed that no preparation would stimulate red cell production unless it contained ACTH, and that no ACTH was free of erythropoietic activity. However, when hypophysectomized or normal rats were fed a diet of anterior pituitary substance, red cell production was increased without adrenal stimulation. They concluded from this that the separate existence of a pituitary erythropoietic hormone seemed very likely.

Dougherty and White (1944) reported that single injections of either adrenal cortical or adrenocorticotropic extracts in mice caused an initial rise in hemoglobin and red cell counts within three hours,

but twenty hours later these values decreased to below those of an untreated animal. They also noted that daily injections of adrenocorticotrophic extract in mice for fifteen days produced an increase in hemoglobin values and red cell counts to above normal standards. In addition, the administration of whole adrenal cortical extracts to adrenalectomized rats was found to be effective in preventing anemia and was observed to cause increased resistance of the red cells to hypotonic saline solutions. This substance did not influence any bone marrow changes.

Garcia et al. (1951) and Hudson et al. (1952) reported that the administration of ACTH caused increased red cell production in man and experimental animals, and Gordon et al. (1947), Summers and Sheehan (1951) and White and Dougherty (1945) stated that hypofunction or extirpation of the adrenals resulted in bone marrow hypoplasia.

Although these methods and results appeared promising, other workers sought to determine the effects of various hormones upon red cell production by the process of hypophysectomy. Vollmer et al. (1939) found that anemia developed in rats after the removal of the pituitary gland. Crafts (1941) was in agreement with this work and stated that a 30 per cent reduction in red cells was a common finding in the post-hypophysectomized state. Crafts (1946) also reported that after the pituitary gland had been removed the bone marrow became hypoplastic and exhibited a decrease in erythroid elements, while the spleen partially decreased in size. In addition to the changes in the blood constituents, hypophysectomy was reported by Levin (1943) and

Lee et al. (1936) to produce a 20 to 30 per cent loss in body weight and about a 30 per cent reduction in metabolic rate. This decrease in body weight suggested that a reduced food intake occurred and that post-hypophysectomy anemia was related more directly to nutritional factors. Vollmer et al. (1942) and Meyer et al. (1940) also believed that the anemia following hypophysectomy was the result of an upset in general metabolism rather than a consequence of the removal of any specific hematopoietic factor. Grant and Root (1952) commented that it was apparent that hypophysectomized animals had a decreased number of red cells and a reduced hemoglobin concentration. Such alterations indicated no deficit in erythropoiesis, but rather were a part of the generalized change in physiological status. In addition, the integrity of the erythropoietic mechanism in hypophysectomized animals was demonstrated by a normal response to an adequate lowering of the barometric pressure and to hemorrhage.

The major hormone of the adrenal medulla was also investigated in an effort to determine its effect upon red cell production. Gordon et al. (1951) reported that adrenal demedullation was without influence upon the formed elements of the blood, but many other workers have recorded a transient increase in red cell counts, in hematocrit values and in hemoglobin concentrations after the injection of epinephrine. Such increases were interpreted by Knisely (1934) to be the consequence of splenic contraction and not the result of any erythropoietic stimulation. Davis (1942) also found that the administration of epinephrine produced temporary changes in plasma volume and in red cell

concentration, but stated that there was little evidence to support the belief that this or similar substances stimulated red cell formation.

Although the fundamental stimulus for erythropoiesis in the adult mammal has generally been considered to be anoxia, another important means of inducing red cell production is the administration of cobalt. Grant and Root (1952) reported that there was no evidence to support the fact that it was related to anoxia, but that the common use of the element as a means of producing polycythemia suggested that its mode of action could provide some clue to mechanisms concerned. Moreover, Marston (1952) felt that cobalt was an agent of physiological importance since it was found to be present in a concentration of 0.4 per cent in the vitamin B_{12} complex. Dukes (1955) stated that this vitamin was regarded as being identical with the extrinsic factor and the antipernicious anemia factor, and was necessary for the process of red cell formation. He also mentioned that a cobalt deficiency in ruminants was considered to be largely a vitamin B_{12} deficiency, since the omission of this element from their diet was usually accompanied by a greatly decreased production of the complex. Morrison (1956) reported that cobalt was required by the bacteria of the rumen in order that they could digest feed and synthesize the B-complex vitamins. He believed that the chief cause of cobalt-deficiency trouble in ruminants was the lack of vitamin B_{12}, and indicated that one of its frequent characteristics was a serious anemia.

Kato (1937) reported that cobalt polycythemia could be produced

in a wide range of animals. Stanley et al. (1947) were in agreement with this work and felt that the increase in red cell counts and hemoglobin values represented the action of cobalt upon erythropoietic tissue. Barron and Barron (1937) ascribed the production of cobalt polycythemia to an inhibition of the respiratory function of immature erythrocytes in the bone marrow and their subsequent early release into the general circulation. Burk et al. (1946) reported that cobalt's interference with the respiration of certain bacteria was probably due to the formation of only partially reversable complexes. They also felt that since these complexes were not readily dissociated in the body, it pointed out the possibility that their formation created, in effect, anoxia of the erythroid cells of the bone marrow and the characteristic increase in erythropoiesis. Orten (1935) suggested that vasodilation caused by cobalt slowed the blood flow and resulted in a local anoxia with erythropoietic stimulation. Davis (1941) found, however, that most vasodilator drugs were effective in reducing red cell production. He assumed that these agents dilated the bone marrow arteries, improved the blood supply to the tissue, and thereby diminished the local anoxia which was presumably the stimulus for polycythemia. Grant and Root (1952) reported that cobalt was regarded as a potent erythropoietic agent, but exactly how the element produced its polycythemia was still unknown. In addition, these authors felt that true erythropoietic stimulation could be achieved by only three fundamental conditions; anoxic anoxia, anemic anoxia and excessive cobalt. The existence of more than one mechanism of stimulation was also con-

sidered to be a source of misconception, since the inhibition or removal of one could favor an alternate route of action.

THE NORMAL RED BLOOD CELL COUNT

Holman (1955) stated that the average total erythrocyte count for cows was 5,950,000 cells per cubic millimeter, however Coffin (1953) reported that the total cells per cubic millimeter could range from 5,400,000 to 9,000,000. Fraser (1930) mentioned that the erythrocyte counts for Shorthorn cows in milk averaged 5.6 million, but averaged 6.6 million for those cows which were dry. In an investigation by Burnett (1917) it was reported that the average red cell count for American cattle was 6.0 million. This work was supported by Ferguson, Irwin and Beach (1945) and by Miller (1934) who both recorded averages of 6.2. Dukes (1955) was also in close agreement with this figure and gave an average for the cow of 6.3 million cells per cubic millimeter.

Price, Bogart, Alexander and Kruger (1957) carried out some experiments with beef cattle which showed that at 500 pounds body weight red cell counts ranged from 6.0 to 9.8 million per cubic millimeter. The mean value of this range was 7.73 million. When body weights were increased to 800 pounds the average total red cell count for Angus males was 8.5 million per cubic millimeter, while Hereford males averaged 8.0 million. The range in average total erythrocyte count for heifers from these lines was from 8.0 to 8.8 million cells.

SOME FUNDAMENTAL CAUSES OF VARIATION IN THE NORMAL RED BLOOD CELL COUNT

Holman (1955) reported that a well-chosen sample of about 30 individuals gave a good indication of the mean and distribution of the population in question. However, Rusoff and Piercy (1946) stated that blood values from one well managed dairy herd could vary significantly from those of another in the same district or from those of the same herd during a different month. It was thought that in addition to the commonly encountered factors, some important but relatively unknown causes of variation were in existence and were establishing a rather uncontrollable source of error.

Age. Age seems to influence the normal red cell count to some extent. Holman (1956) reported that the count was extremely variable for individual calves, but on the average kept at a high level for four months and then decreased in number as the corpuscles increased in size. The average total counts obtained by him included 9.82 million per cubic millimeter on the day of birth, 9.6 million at two months, 10.62 million at four months, and 8.43 million at eight months. Fraser (1930) also recorded a definite trend in this direction for young animals, and Greatorex (1954) has published results which fully confirm these findings. In addition, Canham (1930) reported a gradual drop in red cell count with the increase in age of bull calves in South Africa. Mullick and Pal (1943) also found a similar decrease in Hissar cows in India.

Holman (1956) has stated that the measurements for size and number of corpuscles in calves reached the same level as the cow in about two years. Some variation was noted between the two factors since red cell counts reached the adult figure at 22 months and corpuscular size reached the peak at 29 months.

Sex. Although sex has been regarded as a possible source of variation for many years, some investigators feel that it is a relatively unsubstantiated belief and is not too important in animals.

Canham (1930) compared a group of male and female cattle in an effort to illustrate sexual differences in the erythrocyte count, but average figures from the two groups showed no significant variation. Wintrobe (1951) stated that the difference in the human male and female was not evident until puberty, and in mammals in which menstrual loss did not occur, the values were the same for both sexes. Holman (1956) reported that from the time it was first shown that men had a higher hemoglobin value than women, many workers recorded the same sexual differences in animals. Because this fact was not basically confirmed he felt that the conditions of their measurements were hard to find. It was also stated that when higher erythrocyte counts were present in the male it was probable that some confounding factor, possibly environment, caused this variation.

Price et al. (1957) showed some differences due to sex in both hemoglobin level and total red cell count. In their investigation male calves had lower levels of hemoglobin and lower red cell counts than female calves at both 500 and 800 pounds body weight. Canham (1930)

recorded a higher erythrocyte count about the time of calving and Ferguson, Irwin and Beach (1941) reported an increase in total erythrocyte count immediately after calving, followed by a fall over three weeks to below pre-calving levels. In addition, Holman (1955) stated that there was an increase in red blood cells from about 12 hours before calving to about 12 hours after calving.

Breed. Most investigations carried out to determine the effects of this variable upon the blood picture in cattle have illustrated that little difference between breeds exists. Holman (1955) reported that there appeared to be little variation among European breeds as recorded in Europe and in the temperate zones of America. Among African breeds Canham (1930) showed close similarity to various European breeds in the same district. Mullick and Pal (1943), who recorded results for Indian breeds, reported a somewhat higher erythrocyte count, but also mentioned that the red cells were of a smaller size. The average leucocyte count for these breeds was in agreement with counts obtained for European animals.

Price et al. (1957) reported that at 800 pounds body weight Angus males had an average total red cell count of 8.5 million, while Hereford males averaged only 8.0 million. Rusoff, Johnston and Branton (1954) also suggested that differences between breeds were in existence, but their conclusions were incomplete due to the small number of individuals examined. Based on previous investigations, Holman (1955) concluded that breed differences were in most cases limited to erythrocyte size.

Environment. The many factors which go together to comprise a given environment undoubtedly are responsible for a large part of the variation commonly observed between groups of cattle. In a discussion of this subject Holman (1955) reported several cases in which red cell counts were effected by nutritional defects. Morrison (1956) also indicated that proper amounts of copper, iron and cobalt were necessary in order to maintain normal hemoglobin and red cell levels. Other investigators have commented on some of these requirements, but their work was included in the previous discussion of cobalt.

Another variable which has often been overlooked is the effect of altitude. Bert (1882) first reported the presence of increased concentrations of hemoglobin and red blood cells in men and animals living at high altitudes. Hurtado, Merino and Delgado (1945) and Schneider (1921) confirmed this observation and stated that the concentration of hemoglobin and erythrocytes in the blood of an individual living at a high altitude was noticeably greater than at sea level. In a more recent investigation Merino (1950) showed that residence of one to five weeks at altitudes of 11,000 to 14,000 feet produced an increased red cell volume of about twenty per cent, when compared to determinations made at sea level. Canham (1930) compared the red cell count of cows living at a level of 3,500 feet with cows under similar conditions and of the same age living at 4,400 feet. His results showed that the average total erythrocyte count was one to two million greater in the higher altitude. In addition, Grant (1951) recorded an increased reticulocyte percentage in the circulating blood of man and animals

living in high altitudes.

Neser (1923) suggested that much of the fluctuation in the red cell count was due to the amount of exercise taken, and believed that active animals retained a higher hemoglobin level. In his work he cited instances in which calves had a higher erythrocyte count at three to four weeks than they had shown a day or two after birth. Age was not completely discounted as a cause of this variation.

Apparently little work has been done to determine the direct effect of temperature upon the total red cell count. The literature reviewed for this investigation revealed no definite statements regarding the subject and only a few remarks indicating a relationship were found.

Best and Taylor (1955) stated that low temperatures caused a reduced blood volume and an increased blood concentration, while high temperatures influenced a reversal of these reactions. Manresa, Reyes, Gomez, Zialcita and Falcon (1940) reported that due to tropical conditions in the Philippines, Holsteins and Herefords had hemoglobin values as low as 6.8 grams, while native cattle maintained a level of about 9.4. In some later work Manresa and Orig (1941) observed a double cycle in hemoglobin during each year. Their results illustrated that the levels rose during cooler periods, but were independent of the values for relative humidity or total leucocyte counts. Garner and Unsworth (1953) also reported a definite fall in the hemoglobin values of Zebu bulls during June and July of each year.

Although the above work was concerned largely with hemoglobin

levels, Price et al.(1957) reported that the values for blood hemoglobin and the red cell count were highly interrelated.

In regard to environmental conditions, Holman (1955) concluded that there was a great variation in the erythrocytic picture of the cow due to its environment. He also believed that much uncertainty was present and that many of the differences were, often by surmise, assigned to several factors.

Disease. Hadley (1949) reported that many infections were reflected by erractic red cell counts. Likewise, Dukes (1955) stated that some pathological conditions could influence marked variations in the erythrocyte number. Hawk, Oser and Summerson (1954) also acknowledged the relationship between disease and certain blood abnormalities. The effects of various pathological conditions upon the total red cell count were discussed somewhat by Holman (1950) in a report on anemia and polycythemia. In this work he stated that anemia was usually due to an abnormal blood loss or deficient blood production, and was usually accompanied by a lower red cell count. He further mentioned that a certain degree of inflammation or toxemia in the body inhibited erythrocyte production and therefore caused a fall in the red cell count during almost any infection. Conditions noted to be closely associated with reduced erythrocyte counts were wide spread tumour formations, chronic infections such as tuberculosis and heavy infestation with various types of intestinal parasites.

Although infections were reported to influence a general decrease in the red cell count, Grant and Root (1952) and Holman (1950)

observed some conditions in which the total erythrocyte count was elevated to above normal standards. Holman (1950) stated that an absolute increase in erythrocytes could occur due to an over-compensation for some types of infection, but Grant and Root (1952) reported that many of the increases were relative and not of the absolute type. The mechanisms which they suggested to account for this phenomenon were a decreased plasma volume with an unaltered cell mass, unequal distribution of red cells with the introduction of sampling errors and the emptying of reservoirs of sequested cells throughout the body. Despite the fact that all of these were possible, Holman (1950) believed the primary cause of this relative condition to be a loss of plasma from the circulation so that the blood became more concentrated and gave an apparent increase in the number of corpuscles. This type of rise was observed to be present in states of continual vomiting, marked diarrhea, and in any condition which produced shock. Hahn et al. (1943) also mentioned the effects of hemodilution and hemoconcentration as possible sources of error in the total red cell count.

The Individual. Holman (1950) stated that in domestic animals there was a considerable variation in the blood picture between healthy individuals. Such a difference, however, was not considered unusual since in all biological measurements there was no single correct value but a normal range. In an effort to establish standards by which an individual could be classified this author reported that animals giving measurements two standard deviations above or below the average were considered questionable, while those showing values three standard

deviations either way were definitely abnormal.

THE ERYTHROCYTE'S RESISTANCE TO HEMOLYSIS

Dukes (1955) stated that hemolysis was characterized by the discharge of hemoglobin from the erythrocyte in such a manner that it became free in the plasma or other surrounding media. Kurnick (1941), Dukes (1955) and Best and Taylor (1955) cited a wide range of both internal and external factors which were active in promoting this condition, if present. Of the ways, alternate freezing and thawing and excess agitation were generally believed to cause hemolysis by rupturing the cell's stroma. On the other hand, various chemical substances such as ether, acetone and alcohol were found to bring about cellular destruction by dissolving the lipid constituents present in the envelope and stroma. The venoms of certain poisonous snakes, spiders and insects were also noted to be potent hemolytic agents, but their mode of action was varied within the animal's body. Dacie (1950), Holman (1950) and Best and Taylor (1955) suggested several other means for inducing hemolysis which were designed to assist clinical and experimental investigation. It was agreed, however, that lowering the osmotic pressure around the cell by introducing hypotonic saline solutions had the most diagnostic value and was therefore the most suitable method for this type of work. No results were present to indicate that this mechanism was important in the normal breakdown of erythrocytes within the body, and many investigators have reported that large amounts of water could be injected into the blood stream without produc-

ing significant amounts of hemolysis. In an experiment on horses, Roberts (1943) found that injections of over 4000 milliliters of distilled water or tapwater regularly produced a temporary hemolysis and hemoglobinuria, but that injections of less than 2000 milliliters had no effect. It then became apparent that the extent to which the osmotic pressure of plasma could be lowered without causing significant hemolysis was considerable. Further investigation showed that the normal red cell was mainly responsible for this effect, since it offered a definite resistance to the disintegrating action of hypotonic solutions.

Additional studies of this resistance in relation to specific concentrations of saline demonstrated two dilutions to be of basic analytical importance. In a consideration of these, Dukes (1955) stated that the point in terms of strength (per cent) of sodium chloride solution at which hemolysis just began indicated the osmotic resistance of the weakest corpuscles (minimum resistance), while the point at which complete hemolysis occurred indicated the resistance of the strongest corpuscles (maximum resistance).

Since these values were found to be useful in most determinations of erythrocyte resistance, several workers established standard ranges for various species of animals. Holman (1950) reported that cattle and horses showed initial hemolysis at 0.55 per cent sodium chloride, but did not exhibit complete breakdown until the solution reached 0.40. Dukes (1955) found the overall range for cattle to be slightly larger than that mentioned by Holman (1950) and recorded the

respective values of 0.59 and 0.42 per cent for the two levels. His data for hemolysis in the horse illustrated that cellular destruction began at 0.59, but terminated very naar the previous value given for this species at 0.39. Swine showed a somewhat decreased resistance in comparison to most other animals with a range of only 0.74 to 0.45 per cent. As a note of caution, it was reported by Best and Taylor (1955) that some cells show a reduced resistance to hypotonic solutions, but maintain a normal resistance to other hemolytic agents such as lysolecithin. The antogonistic nature of this reaction was considered to be the result of two different hemolytic conditions, each effecting the cell in a somewhat opposite manner.

Up to this point red cell resistance has been approached from the standpoint of responses given by the healthy adult individual. Such responses, however, are not always present and the onset of various abnormalities can be the result of several factors.

Holman (1950) found disease to be one of the most significant causes of variation in the red cell resistance. In his report it was illustrated that erythrocyte resistance could be appreciably reduced, even to the point of hemolysis within the body, by the action of several types of bacteria and protozoa. In addition, various poisons and certain types of allergies were shown to possess potent hemolytic effects. In a somewhat similar report, Best and Taylor (1955) found that the toxins of many pathogenic bacteria, such as streptococcus, staphylococcus and tetanus bacillus, could cause lowered erythrocyte resistance as well as complete breakdown of the cell. They also noted

that intense hemolysis took place during some of the more virulent types of infectious fevers and in the presence of severe burns. Likewise, Hawk, Oser and Summerson (1954) mentioned that certain kinds of disease definitely reduced the red cell's resistance to hemolysis.

In addition to the effects of disease, Holman (1956) reported that age exhibited some influence on erythrocyte resistance. In an investigation on cattle he found that corpuscles were most resistant on the day of birth, followed by a sharp decrease in resistance up to about four months. At this age resistance began to increase gradually, until at two years, it was equal in degree to that of an adult individual. In a rather similar study Greatorex (1954) found that during the first month calves did not show complete hemolysis until the osmotic pressure was reduced to approximately 0.3 to 0.35 per cent sodium chloride. Throughout the next four months these values increased to between 0.4 and 0.45 per cent, and then were observed to remain near 0.5 for the remainder of the year.

The presence of a vitamin E deficiency was considered by Hawk, Oser and Summerson (1954) to be another cause of lowered erythrocyte resistance. The results of in vivo and in vitro experiments partially substantiated this fact, since the administration of alpha tocopherol in both cases influenced the resistance to return to normal levels.

Although most of the previous reactions have been concerned with the factors which cause a drop in red cell resistance, Holman (1950) reported that in several instances erythrocyte resistance increased with the appearance of infection. Best and Taylor (1955) were in

agreement with this and stated that during pernicious anemia red cells have been found to exhibit a greater resistance to many hemolytic agents. Non-physiological doses of certain pituitary and adrenal cortical hormones have also been reported to increase red cell resistance in a variety of hemolytic conditions, but their specific actions were considered in a different section of this review.

THE EFFECTS OF INSULIN AND EPINEPHRINE UPON ERYTHROCYTE RESISTANCE

Insulin. Cutler (1934) and Petersen et al. (1931) recorded the characteristic hypoglycemia which occurs in normal animals as a result of non-physiological administration of insulin. A condition such as this was reported by Long (1952) to stimulate the secretion of epinephrine and eventually cause the release of ACTH. That insulin can augment this response, is of definite importance when considering the maintenance of normal blood sugar levels, and it may possibly be significant when contemplating the effects of this hormone upon erythrocyte resistance. However, despite the fact that insulin was accredited with the ability to alter various blood components, no statement illustrating a direct relationship between insulin and red cell resistance could be found.

Epinephrine. In addition to its hyperglycemic function, Barcroft (1925) and Taylor (1943) found that epinephrine injections caused transient increases in red cell counts, hematocrit values and hemoglobin concentrations. These results were explained on the basis

of splenic contraction and other physical changes within the body, but in no instance were the effects of epinephrine discussed with reference to erythrocyte resistance.

THE EFFECTS OF CORTISONE AND ADRENOCORTICOTROPHIC HORMONE (ACTH) UPON ERYTHROCYTE RESISTANCE

Although several of the other hormones under consideration have not been demonstrated to effect red cell resistance, ACTH and cortisone have frequently been reported to possess the capacity to alleviate various hemolytic conditions.

The Effects of ACTH and Cortisone upon Acquired Hemolytic Anemias. Idiopathic, acquired hemolytic anemia is an interesting and perplexing hematological syndrome. Until recent reports of remission following administration of pituitary corticotropin (ACTH), treatment was unsatisfactory in a large percentage of the cases. Gardner (1950) and Dameshek (1950) reported on the early use of corticotropin in this disorder, but Dameshek et al. (1951), Gardner et al. (1951), Young et al. (1951) and Unger (1951) carried out similar investigations within the next year. A rapid change in the blood picture toward normal was uniformly noted by these investigators, however, some cases remained in remission for weeks to months while others required maintenance doses to prevent relapse.

Thorn (1950) reported that most studies of corticotropin effect on normals failed to show significant alteration of circulating antibodies. Singer and Motulsky (1949) pointed out that some of the

hemolytic anemia cases exhibited falling titers in the developing Coombs test. Worgley et al. (1948) reported that reticuloendothelial cells, particularly of the spleen, were active in coating the red cells with a protein which could be detected by employing this test. Evans and Duane (1949) indicated that the amount of globulin present, when measured by the quantitative Coombs test, correlated roughly with the evident degree of hemolysis. Wright et al. (1951) did not completely agree with this idea and stated that some individuals with weakly positive reactions to the test had no evident hemolysis. In regard to erythrocyte destruction these workers believed that adsorption of certain abnormal globulins at the cell surface shortened the life span of the erythrocyte by increasing mechanical fragility and susceptibility to the destructive and phagocytic activity of the reticuloendothelial system.

The abnormal globulin which coated the red cells was probably an immune globulin, but this had not yet been proven. There were also several theoretical mechanisms by which an immune globulin might involve erythrocytes. An antibody to an environmental allergen might, for some reason, become fixed to the cell surface. Hemolysis would then follow exposure to that antigen. Luisada (1941) reported that the hemolytic anemia which, in sensitized patients, followed ingestion of the ripe fava bean or inhalation of its pollen probably represented this sort of mechanism. This reaction began within hours after exposure, reached a maximum in a few days and subsided rapidly thereafter. Acquired hemolytic anemia, on the other hand, was more chronic in its

course and had no obvious allergic etiology. That red cells could also become coated upon mixture with appropriate type-specific hemagglutinins, was demonstrated by Jakobowiez, Krieger and Simmons (1948) in an investigation on erythroblastosis fetalis. They found that when maternally produced anti-Rh antibodies crossed the placenta and combined with the Rh antigen inherent in the fetal cell, a hemolytic anemia occurred which differed from the acquired variety only by the presence of a type-specific reaction and an externally produced anti-red cell antibody.

Best, Limarzi and Poncher (1951) reported that whatever the nature of the adsorbed globulin, the action of corticotropin appeared to inhibit its production or adsorption to the red cell. Likewise, it was proposed by Thorn et al. (1950) that the cell membrane was an important site of corticoid activity in the response of a wide spectrum of diseases to corticotropin therapy. In addition, Best et al. (1951) found that the adrenal corticoids, stimulated by corticotropin, increased the resistance of red cells to mechanical fragility, despite a coating of globulin. In accordance with this it was observed that marked hematological remissions were repeatedly induced by corticotropin therapy with no significant alteration in circulating antibodies. In such cases hemolysis returned each time the drug was withdrawn.

The Effects of ACTH and Cortisone on Various Types of Induced Hemolytic Anemia. It was illustrated in the work of Best, Limarzi and Poncher (1951), Dameshek et al. (1951), Unger (1951) and Wintrobe and Cartwright et al. (1951) that cortisone and ACTH would mitigate the

hemolysis in many cases of acquired hemolytic anemia and often arrest the disease. White and Dougherty (1945) indicated that the adrenal cortex was, to some extent, associated with the maintenance of normal red cell levels in the peripheral blood. In addition to this, Garcia et al. (1951), Gordon and Charipper (1947), Gordon et al. (1951), Lowenstein et al. (1951), Palmer et al. (1951) and Summers and Sheehan (1951) showed that the administration of ACTH increased the red cell volume and hemoglobin values in the intact animal. Bethell et al. (1951), Hill and Hunter (1951), Hovermark and Nordenson (1950) and Whitelaw (1951) believed that ACTH and cortisone could have beneficial effects in other types of anemia, but Feldman et al. (1953) first conducted the investigation to determine the role of the adrenal cortex during periods of induced red cell destruction, in which phenylhydrazine was employed as the hemolytic agent. The results of this work clearly illustrated that in adrenalectomized rats, anemia produced by phenylhydrazine was significantly greater than in the intact animals. It was also shown that the administration of cortisone prevented this increased destruction and maintained a red cell count comparable with the level found in control animals. Under the conditions of this experiment, however, ACTH was more effective than cortisone and almost completely prevented the red cell destruction induced by phenylhydrazine.

As previously mentioned, the adrenalectomized animals receiving phenylhydrazine showed an increased destruction of red cells. This response was not believed to be the result of an additive anemia consequent to adrenalectomy, since Gordon and Charipper (1947) and White

and Dougherty (1945) reported a fall in red cells of between twelve and twenty-five per cent following adrenalectomy alone. It was also unlikely that the effects of phenylhydrazine in adrenalectomized animals included blood volume changes, as it was reported by White and Dougherty (1945) that there were no changes in the blood volume in rats following adrenalectomy. In addition, Frost and Talmage (1951) demonstrated that in adrenalectomized rats fluid shifts effected the cell, but did not alter extracellular volumes. However, Gordon and Charipper (1947) and White and Dougherty (1945) referred to work which stated that hemoconcentration occurred in species other than the rat following this operation. Gordon et al. (1951) noted that there was a decreased red cell resistance in adrenalectomized rats, but that the condition was transient and disappeared approximately four weeks after the initial surgery.

Feldman et al. (1953) postulated that the adrenal cortex influenced the integrity of the red cell, either directly or indirectly, by alterations of the plasma, by governing red cell breakdown in the reticuloendothelial system, or by controlling red cell production in the bone marrow and extramedullary sites. It was also felt that cortisone or ACTH increased the resistance of the red cells to phenylhydrazine destruction. Gordon et al. (1951) observed conditions which point to the fact that the adrenal cortex could effect the structure of the red cell or its surrounding milieu. Also Cruz, Hahn and Bale (1942) demonstrated that the products of red cells, destroyed by acetylphenylhydrazine, were immediately utilized for the production of new cells.

Feldman and Rachmilewitz (1954) conducted an investigation to determine the role of the adrenal cortex during a period of induced red cell destruction, in which immune serum was employed as the hemolytic agent. Their results showed that after an intravenous injection of immune serum, there was a progressive fall in the red cell level from minus twenty-eight per cent at twenty-four hours, to minus fifty-eight per cent at four days. In another group, which received both immune serum and ACTH, there was a comparable decline in red cells from minus thirty-three per cent at twenty-four hours, to minus sixty-two per cent at four days. The differences obtained here were not statistically significant and it was apparent that ACTH was ineffective against the anemia induced by immune serum.

The Effects of the Adrenal Cortex upon the Peripheral Blood Cell Reactions which Commonly Accompany Various Types of Hemolysis. Feldman et al. (1953) and Feldman et al. (1954) reported that in the course of studying the effect of the adrenal cortex and its hormones upon hemolysis, a rather uniform pattern of leukocyte response was noted. Although minor variations existed, leukocytosis and neutrophilia were immediate and lasted up to four days; eosinophilia was marked and persistent after the first six hours; reticulocytosis appeared two to three days after the inception of hemolysis and increased rapidly for the remaining days of the experiment. It was found, however, that the peripheral blood cell response associated with hemolysis occurred in the presence or absence of the adrenals and with

or without the administration of ACTH. As a result it was concluded that hemolysis caused a distinct pattern of cellular response in the peripheral blood, which was not mediated through the adrenal cortex.

CHAPTER III

MATERIALS AND METHODS

Experimental Animals

Data for this investigation were obtained from animals donated by several breeders throughout the State of Missouri and from the University of Missouri's herd of Hereford cattle. Studies were carried out during the various seasons of 1956 and 1957.

In this work a total of seventy-eight different animals were examined. This number included twenty-three pedigree clean, twenty-seven known carriers and twenty-eight dwarfs.

Seventy-four Hereford cattle comprised the majority of the animals used. They ranged in age from three months to approximately ten years and represented both sexes. All three of the genotypes believed to be associated with dwarfism were present in this group. The four members of the Aberdeen Angus breed which were available for this study ranged from three months to eight years of age and included only females of the dwarf and carrier genotypes.

Each different phase of this investigation was not conducted upon the entire seventy-eight animals, but in some cases the same individuals were run on more than one type of test.

The Total Erythrocyte Count As a Method of Determining Red Blood Cell Resistance

In this technique four fundamental steps were involved.

1. Collection:

A blood sample to be analyzed by this method was collected in the following manner. Using a 16 gauge bleeding or hypodermic needle the animal's jugular vein was punctured and approximately 5.0 cc.'s of venous blood were obtained in a standard blood collection tube containing an anticoagulent. Throughout this study the primary anticoagulents used were potassium oxalate tablets or sodium oxalate powder. Thorough mixing of the blood with the anticoagulent was obtained by rolling each sample gently. This was important in order to avoid excess hemolysis due to agitation.

2. Diluting the Blood:

Using a standard erythrocyte diluting pipette the sample was diluted 1 to 200 with a 0.48 per cent sodium chloride solution. This was accomplished by filling the pipette up to the 0.5 mark with blood and by adding diluter (in each case 0.48 per cent saline) until the mixture reached the 101 mark. In order to obtain a uniform distribution of cells the sample was shaken for two minutes. The saline solution was prepared by dissolving chemically pure sodium chloride in distilled water. The correct amount of solute was obtained by weighing with a standard laboratory balance.

3. Counting the Erythrocytes:

The red cell counts were made using a regular hemocyto-

meter and cover-slip. Immediately after shaking, but before the counting chamber was filled, two or three drops of diluted blood were discarded from the stem of the pipette. The purpose of this was to eliminate the nonrepresentative portion of the sample composed primarily of diluting fluid. The solution was placed on the hemocytometer by putting the tip of the pipette on the edge of the ruled area and by allowing a drop of the fluid to run underneath the cover-slip by capillary action. If any of the substance spilled over into the dividing spaces (the moat) or if an even distribution was not obtained the procedure was repeated. The loaded hemocytometer was then placed under the microscope, brought into focus with the high power lens and allowed to stand until the cells settled (approximately one minute).

The section of the hemocytometer designed for counting erythrocytes is in the center. This area is composed of twenty-five large squares each of which contain sixteen smaller squares. The procedure followed was to count the cells in five of these large squares by moving diagonally across the field. Those cells observed to be on the border of a square were included in the count when touching the upper and left-hand margin, but excluded when they were in contact with the lower and right-hand boundaries. The final number obtained was multiplied by a factor of 10,000 to give the total red cell count per cubic millimeter.

In this analytical method an error may be encountered if the blood samples are not well mixed immediately before using. Therefore the observation of grossly abnormal results should always be rechecked for accuracy. A minimum of error can also be obtained by using fresh blood samples.

4. Calculations:

Dilution = 1:200

Height of Chamber = 1/10 mm.

Total area of the 25 large squares = 1 sq. mm.

Area of the 5 squares counted = 1/5 sq. mm.

200 X 10 X 5 = 10,000

10,000 X the total count of five large squares = red blood cells per cubic millimeter.

Phase I: Differences in Initial Red Blood Cell Resistance

In the determination of initial resistance a single blood sample was collected from each individual to be tested. After the blood had been obtained it was analyzed by the total red cell count.

Seventy-eight animals have been studied during this aspect of the investigation. This sum is comprised of twenty-three pedigree clean, twenty-seven known carriers and twenty-eight dwarfs. All individuals belonged to the Hereford breed except three Angus dwarfs and one Angus known carrier.

Since this was a study of initial resistance no hormone was injected before taking a blood sample.

Phase II: Changes in Red Blood Cell Resistance After the Injection of Insulin

Regular zinc insulin was administered intravenously at the rate of 0.8 of a unit of insulin per kilogram of body weight. An initial blood sample was taken immediately before injection and additional collections were made at 0.5, 1, 2, 6, 10 and 12 hours. At exactly 48 hours after the first injection insulin was again injected into the jugular vein at the rate of 0.3 of a unit of insulin per kilogram of body weight. A 48 hour blood sample was taken immediately before this injection and further collections were made at 0.5, 1, 2, 6, 10 and 12 hours after the 48 hour sample was obtained.

The effects of this hormone upon cellular resistance were studied by using the total red cell count.

A total of thirty-one animals have been run on this test. These animals represent twelve pedigree clean Herefords, eleven known carrier Herefords, six dwarf Herefords and two dwarf Angus.

Phase III: Changes in Red Blood Cell Resistance After the Injection of Adrenalin

A single dose of 4.0 cc.'s of 1:10,000 adrenalin was injected intravenously into all animals studied in this phase. An initial blood sample was taken immediately before injection and other samples were obtained at 1, 2, 6 and 10 hours.

Fifteen animals from the Hereford breed have been checked using this procedure. They consisted of five pedigree clean, five known

carriers and five dwarfs. Adrenalin 1:1000 was diluted 9:1 with a 0.9 per cent saline solution to yield the final dilution of 1:10,000, and total red cell counts were used to analyze the effects of this hormone upon erythrocyte resistance.

PHASE IV: Changes in Red Blood Cell Resistance After the Injection of Adrenocorticotrophic Hormone (ACTH)

The work involving the adrenocorticotrophic hormone (ACTH) was divided into two parts. Each animal in the first group received a subcutaneous injection of 0.25 of a unit of ACTH per pound of body weight and an additional intramuscular injection of 0.25 of a unit of ACTH per pound of body weight. An initial blood sample was taken before injection and was followed by collections at 2.5, 5 and 10 hours.

Thirty-seven Herefords have been studied by the procedure described for group one. Of this number, there were ten pedigree clean, ten known carriers and seventeen dwarfs.

In the second phase of the ACTH study the hormone was injected intravenously at the rate of 0.5 of a unit of ACTH per pound of body weight. An initial sample was taken before injection and additional samples were taken at 0.5, 1, 2, 6 and 10 hours.

Data have been collected on sixteen animals from the Hereford breed. This group was comprised of five pedigree clean, five known carriers and six dwarfs.

Armour long-acting veterinary corticotropin was the principle type of ACTH utilized and red cell counts were employed to check the

effects of the hormone upon erythrocyte resistance during both aspects of the work.

Data Calculations

The statistical calculations on the data obtained from this research were carried out on a Burroughs Electronic Computor Type E-102, which is operated by the Department of Rural Sociology and currently under the direction of Mr. C. L. Gregory, Assistant Professor of Rural Sociology.

CHAPTER IV

RESULTS AND DISCUSSION

PHASE I: INITIAL RED BLOOD CELL RESISTANCE

Differences in Initial Red Blood Cell Resistance Determined by the Total Erythrocyte Count. All blood samples were diluted one to two hundred with a 0.48 per cent saline solution in order to induce a partial hemolysis. Following this, an average initial red cell count for each genotype was obtained from twenty-three pedigree clean, twenty-seven known carriers and twenty-eight dwarfs.

The pedigree clean group averaged 3,153,000 cells per cubic millimeter while the known carrier animals had a lower average of 1,708,100. The average red cell count for the twenty-eight dwarfs was much less in comparison to the other two genotypes and was only 475,000 cells per cubic millimeter. A statistical analysis showed the differences between these averages to be significant at the 0.01 level of probability. Table I and Figure 1 illustrate the average initial erythrocyte count for each genotypic group. It is believed that these values indicated each group's initial red cell resistance. At the present time it is difficult to establish a definite reason for these differences, but the reports of several investigators indicate one possible cause. Feldman et al. (1953) and Best et al. (1951) carried out studies which illustrated that ACTH from the pituitary and cortisone from the adrenal cortex had definite beneficial effects in

TABLE I

INITIAL RED BLOOD CELL RESISTANCE* DETERMINED BY THE TOTAL ERYTHROCYTE COUNT

Sample Time	Pedigree Clean			Known Carriers			Dwarfs		
	Thousands of Red Cells Per Cubic Millimeter of Blood	±Standard Deviation	Coefficient of Variation in Percent	Thousands of Red Cells Per Cubic Millimeter of Blood	±Standard Deviation	Coefficient of Variation in Percent	Thousands of Red Cells Per Cubic Millimeter of Blood	±Standard Deviation	Coefficient of Variation in Percent
Initial or 0.0 Hour [2]	3,153.00	± 1,504.20	47.71	1,708.10	± 1,461.20	85.55	475.40	± 480.50	101.07

Pedigree Clean (n = 23)

Known Carriers (n = 27)

Dwarfs (n = 28)

* Erythrocyte resistance measured by diluting the red cells with 0.48 percent sodium chloride solution.

[2] Difference between average counts of the three genotypes was significant (P=.01).

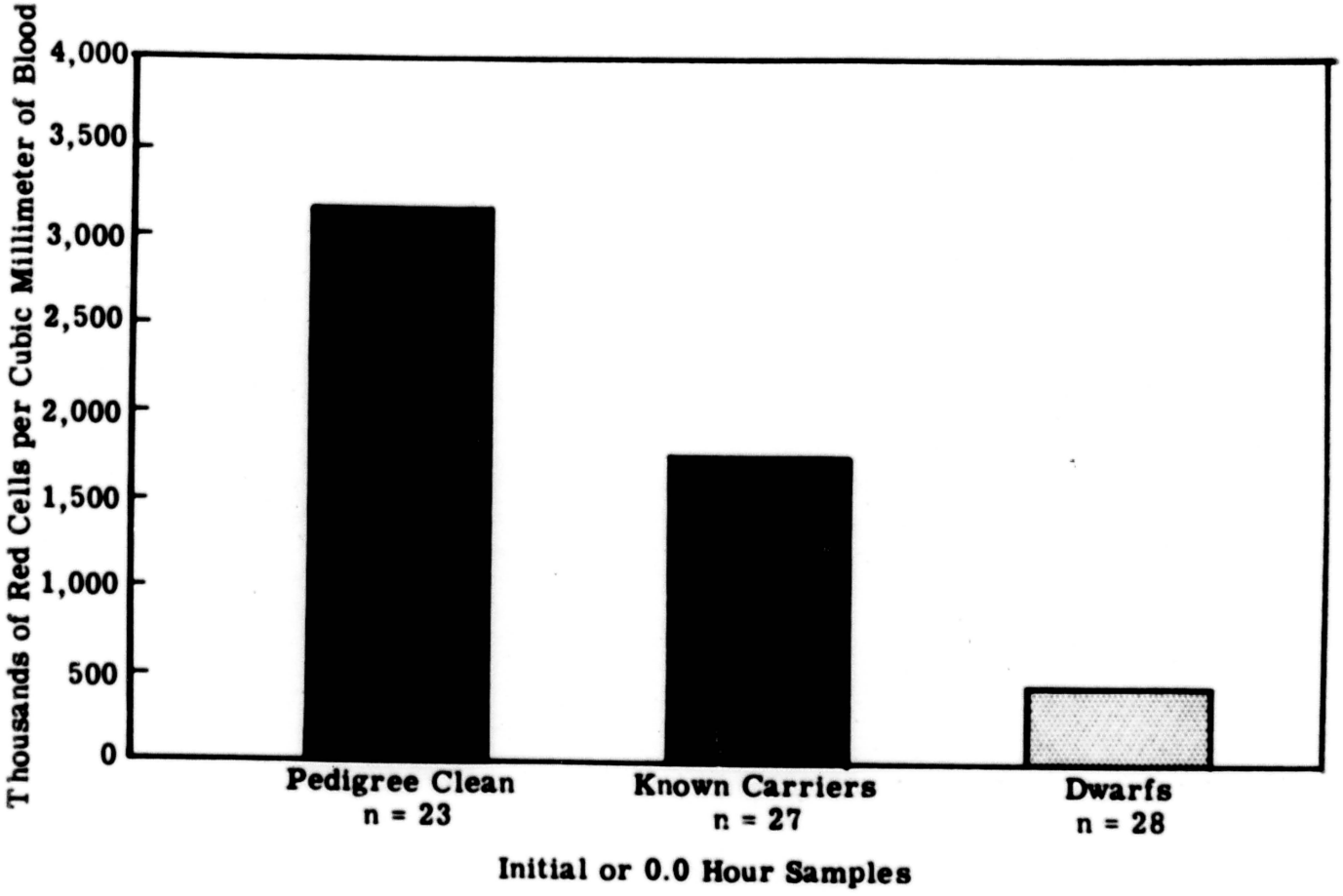

Figure 1. Genotypic Differences in Initial Red Blood Cell Resistance Determined by the Total Erythrocyte Count.

relieving the hemolysis which occurred in several types of hemolytic anemia. Furthermore, it was hypothesized by these workers that these compounds were increasing erythrocyte resistance by some type of reaction which took place at the cell membrane. Although no work was found which stated that these substances would have a similar effect in cattle, it is the opinion of this author that an insufficient amount of either or both of these hormones, as influenced by genotype, might be linked to the variation observed in red cell resistance.

Table I also includes the coefficient of variation for each genotype. This value, a measure of variation within the genotype, was 47.71 for the pedigree clean group, 85.55 for the known carriers and 101.07 for the dwarfs. Differences in age as well as the presence of low grade infections may have been responsible for some of the variation within the classes, but Holman (1950) found that in domestic animals there was a considerable variation in the blood picture between healthy individuals.

Throughout this study the coefficient of variation was usually smallest for the pedigree clean group, intermediate for the known carriers and greatest for the dwarfs. This indicates that genotype influences the amount of variation in red cell resistance within a class. However, further research is needed on this point.

PHASE II: CHANGES IN RED BLOOD CELL RESISTANCE AFTER THE INJECTION OF INSULIN

The Effects of a Single Insulin Injection Upon Red Blood Cell

Resistance. Table II and Figure 2 show the effects of a single intravenous injection of insulin. This hormone was administered to twelve pedigree clean, eleven known carriers and ten dwarfs at the rate of 0.8 unit of insulin per kilogram of body weight. In order to analyze red cell resistance all blood samples were diluted with a 0.48 per cent sodium chloride solution and a total erythrocyte count was made.

Over the twelve hour period the three genotypes showed a varied response to a single insulin injection. The average total erythrocyte count for the pedigree clean animals was, at each sampling time, higher than the values obtained for the other two groups. The average red cell count for the clean individuals began to increase one-half hour after the hormone injection and by one hour averaged 419,170 cells per cubic millimeter greater than the zero hour level. At two hours the mean count had dropped somewhat and approximated the average obtained for these animals prior to insulin administration. This was followed by a second, more gradual, rise in the average erythrocyte count, which was of greater duration and continued until a peak of 4,251,670 cells per cubic millimeter was reached at ten hours. By twelve hours, the count showed a tendency to decrease once again. It was at this time that the largest difference between the average cell counts of the pedigree clean and known carrier groups existed.

In general, the average cell count of the known carrier individuals remained intermediate, but showed an alternating increase and decrease throughout the entire sampling period. At one-half hour after the hormone injection these animals demonstrated an increased average

TABLE II

THE INFLUENCE OF A SINGLE INSULIN INJECTION* UPON RED BLOOD CELL RESISTANCE.**

Sample Time	Pedigree Clean			Known Carriers			Dwarfs		
	Thousands of Red Cells Per Cubic Millimeter of Blood	± Standard Deviation	Coefficient of Variation in Percent	Thousands of Red Cells Per Cubic Millimeter of Blood	± Standard Deviation	Coefficient of Variation in Percent	Thousands of Red Cells Per Cubic Millimeter of Blood	± Standard Deviation	Coefficient of Variation in Percent
Control or 0.0 Hour [2]	3,473.33	± 1,740.22	50.10	2,580.00	± 1,395.00	54.07	875.00	± 1,454.96	166.29
0.5 Hour [2]	3,823.33	± 1,345.04	35.18	2,624.55	± 1,275.63	48.60	1,302.00	± 1,297.99	99.69
1.0 Hour [2]	3,892.50	± 1,249.90	32.11	2,384.55	± 1,334.79	55.98	1,144.00	± 1,413.72	123.58
2.0 Hours [2]	3,535.00	± 1,527.20	43.20	2,966.36	± 1,494.98	50.40	1,376.00	± 1,307.19	94.99
6.0 Hours [2]	3,925.00	± 1,673.90	42.65	2,324.55	± 1,115.09	49.52	1,704.00	± 1,157.98	67.96
10.0 Hours [2]	4,251.67	± 2,245.09	52.80	2,820.91	± 1,548.71	54.90	1,588.00	± 1,434.84	90.36
12.0 Hours [2]	4,154.17	± 2,146.25	51.66	1,989.09	± 1,257.20	63.20	1,980.00	± 1,706.32	86.18

Pedigree Clean (n = 12)
Known Carriers (n = 11)
Dwarfs (n = 10)

* Insulin administered at rate of 0.8 units insulin per kilogram of body weight.

** Erythrocyte resistance measured by diluting the red cells with 0.48 percent sodium chloride solution.

[2] Difference between average counts of the three genotypes was significant (P=.01).

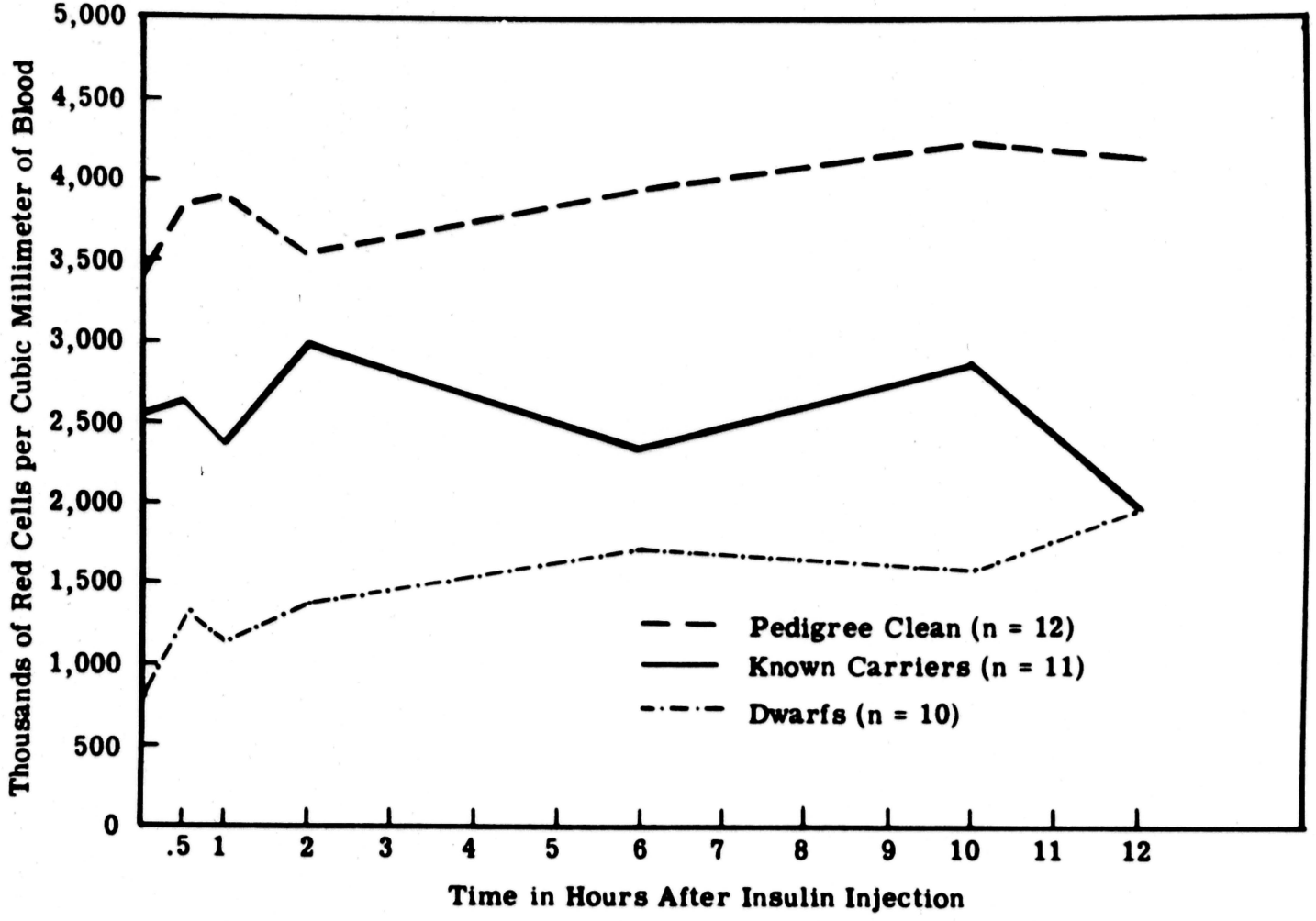

Figure 2. The Influence of a Single Insulin Injection upon Red Blood Cell Resistance.

cell count, but the rise was transient and by one hour the value recorded was 240,000 cells per cubic millimeter lower than their initial level. The known carriers reached a peak at two hours with an average count of 2,966,360 cells per cubic millimeter. This level was not maintained and at six hours the average cell count for these animals had decreased to a point approximately equal to their one hour value. Their mean count increased again at ten hours, but by twelve hours had dropped to its lowest point, and was only 9,090 cells per cubic millimeter greater than the average obtained for the dwarf animals at that time.

Although the average total erythrocyte counts for the dwarf group were considerably lower, they followed somewhat the same pattern as the known carriers' throughout the first two hours of sampling. Both genotypes exhibited a greater average red cell count one-half hour after the hormone was administered and each showed a drop at one hour. This apparent similarity in response ceased at this point due to the fact that the count of the dwarf group increased more and decreased less than the carriers' count during this period. Furthermore, the one hour value of the known carriers fell below their control level while the same sample for the dwarfs was well above this point. Each of the two groups had an increased average erythrocyte count at two hours, but the rise was much more pronounced in the known carriers. The averages recorded for the dwarfs at succeeding collection times were also lower than corresponding mean counts of the other genotypes. However, these animals had an increased average erythrocyte

count at the twelfth hour which closely approached the lowered level of the known carriers.

A statistically significant difference was obtained between the average total erythrocyte counts of the three genetically different groups at all hours of sampling. The most highly significant differences were observed prior to, one-half and one hour after the hormone administration.

In this study insulin was administered intravenously in a non-physiological dosage and was designed to act as a general body stressor, primarily by causing hypoglycemia. Normally, when the body is under stress, adrenocorticotrophic hormone (ACTH) and steroids from the adrenal cortex are released in an effort to combat or relieve the condition. ACTH and cortisone have also been reported to increase red cell resistance. In view of this, it seems likely that these hormones may have been responsible for the changes observed in erythrocyte resistance after the onset of stress.

It was reported by Best *et al*. (1951) and Feldman *et al*. (1953) that the amount of ACTH and cortisone present affected the role which these hormones played in increasing erythrocyte resistance. They believed that lower levels of these compounds were accompanied by lower levels of resistance. Based on these findings, it is this author's opinion that the genotypic differences in erythrocyte resistance give some indication of each group's response to stress. In a report by Foley *et al*. (1956) it was indicated that dwarf animals may be deficient in pituitary and adrenal secretions, or tend to be rather slow

in allowing for the release of these substances. The generally low level of cell resistance demonstrated by the carrier and dwarf animals during this study suggests that these conditions may be present in both genotypes. It is thought by the author that carrier and dwarf animals are unable to respond rapidly to stress induced by insulin because of insufficient amount of the necessary pituitary and adrenal cortical hormones or because they fail to release these compounds as quickly as the normal animal. Neither genotype showed a complete inability to respond to stress since some increase in erythrocyte resistance was noted for each group at several points during the study.

A rise in the average number of erythrocytes per cubic millimeter of blood was demonstrated by all groups one-half hour after the hormone administration, and continued until one hour in the pedigree clean animals. The increase was transient and may have resulted from the introduction of a foreign protein. When a foreign protein is administered, a rapid release of adrenalin is induced in normal animals. The subsequent activity of this compound augments splenic contraction, which in turn produces an absolute increase in the number of circulating red cells. The pedigree clean animals showed a more prolonged rise in their average cell count. This indicated that the magnitude of their response to a foreign protein was greater. These data further suggested that dwarf and carrier animals may be unable to maintain the level of adrenalin necessary to cause the release of stored erythrocytes or were suffering from a true deficiency of reserve cells.

The Effects of Successive Insulin Injection Upon Erythrocyte Resistance. The results obtained in this aspect constitute the second half of a sixty hour insulin study and are presented in Figure 3 and Table III. The procedures employed in analyzing red cell resistance and the sampling times were in accordance with those established during the first section of the insulin work.

Following the initial twelve hour examination period there was a thirty-six hour interval in which the animals were allowed to rest and no blood samples were collected. At the conclusion of this period, exactly forty-eight hours after the first insulin injection, a control blood sample was taken from all animals and insulin was administered at the rate of 0.3 of a unit per kilogram of body weight. The purpose of this injection was to induce a second stress in the animals. It is believed by the author that changes in red cell resistance reflected each genotype's ability to respond to the second nonphysiological administration of the hormone.

In Figure 4 there is a graphical comparison of the average total erythrocyte counts for the three groups at zero and forty-eight hours. The zero hour values illustrate the average counts obtained prior to the first insulin injection. The forty-eight hour levels represent the average counts forty-eight hours after the first insulin administration. The pedigree clean and dwarf genotypes demonstrated a significant increase in their average cell count over this period. The pedigree clean animals obtained the highest total erythrocyte count for either phase of the insulin study at forty-eight hours.

TABLE III

THE INFLUENCE OF SUCCESSIVE INSULIN INJECTIONS* UPON RED BLOOD CELL RESISTANCE.**

Sample Time	Pedigree Clean			Known Carriers			Dwarfs		
	Thousands of Red Cells Per Cubic Millimeter of Blood	± Standard Deviation	Coefficient of Variation in Percent	Thousands of Red Cells Per Cubic Millimeter of Blood	± Standard Deviation	Coefficient of Variation in Percent	Thousands of Red Cells Per Cubic Millimeter of Blood	± Standard Deviation	Coefficient of Variation in Percent
48 Hours [2]	5,133.33	± 1,130.16	22.02	2,281.81	± 1,068.77	46.84	2,493.75	± 1,374.03	55.10
48.5 Hours [2]	4,087.50	± 1,171.66	28.66	1,995.45	± 1,489.91	74.67	2,797.50	± 2,025.11	72.39
49 Hours [2]	4,269.17	± 1,217.25	28.51	2,255.45	± 1,585.84	70.31	2,055.00	± 1,384.38	67.37
50 Hours [2]	4,466.67	± 820.30	18.36	2,077.27	± 1,484.25	71.45	2,428.75	± 1,609.41	66.26
54 Hours	3,334.17	± 1,274.38	38.22	2,413.64	± 1,466.37	60.75	1,805.00	± 1,560.46	86.47
58 Hours [2]	4,287.50	± 1,062.62	24.78	1,020.91	± 1,083.18	106.10	2,321.25	± 1,413.00	60.87
60 Hours [2]	4,418.33	± 1,600.61	36.23	1,603.64	± 1,167.73	72.82	1,960.00	± 1,607.37	82.01

Pedigree Clean (n = 12)
Known Carriers (n = 11)
Dwarfs (n = 8)

* Initial insulin injection at rate of 0.8 units insulin per kilogram of body weight, followed 48 hours later by a second injection at rate of 0.3 units insulin per kilogram of body weight.

** Erythrocyte resistance measured by diluting the red cells with 0.48 percent sodium chloride solution.

[2] Difference between average counts of the three genotypes was significant (P=.01).

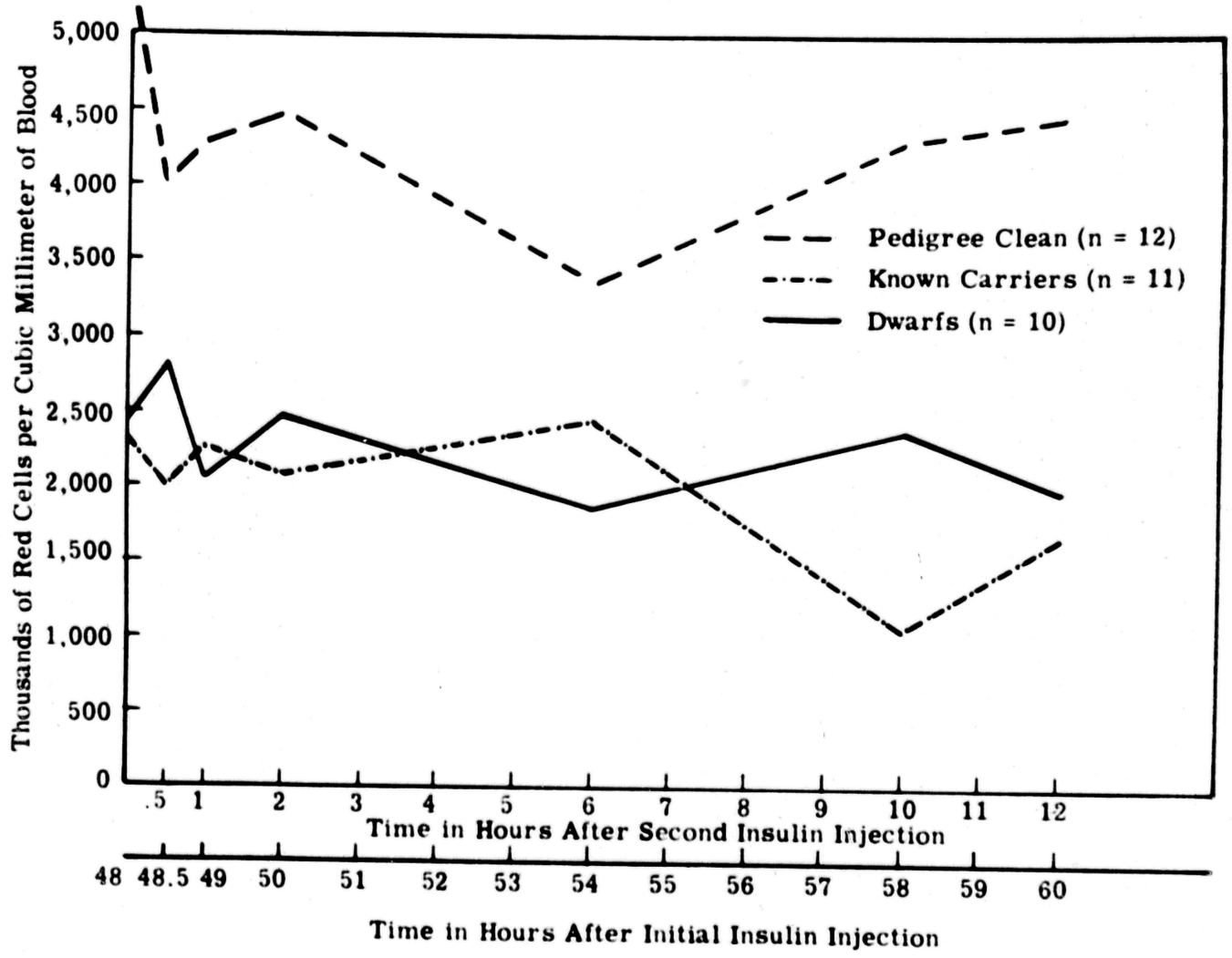

Figure 3. The Influence of Successive Insulin Injections upon Red Blood Cell Resistance.

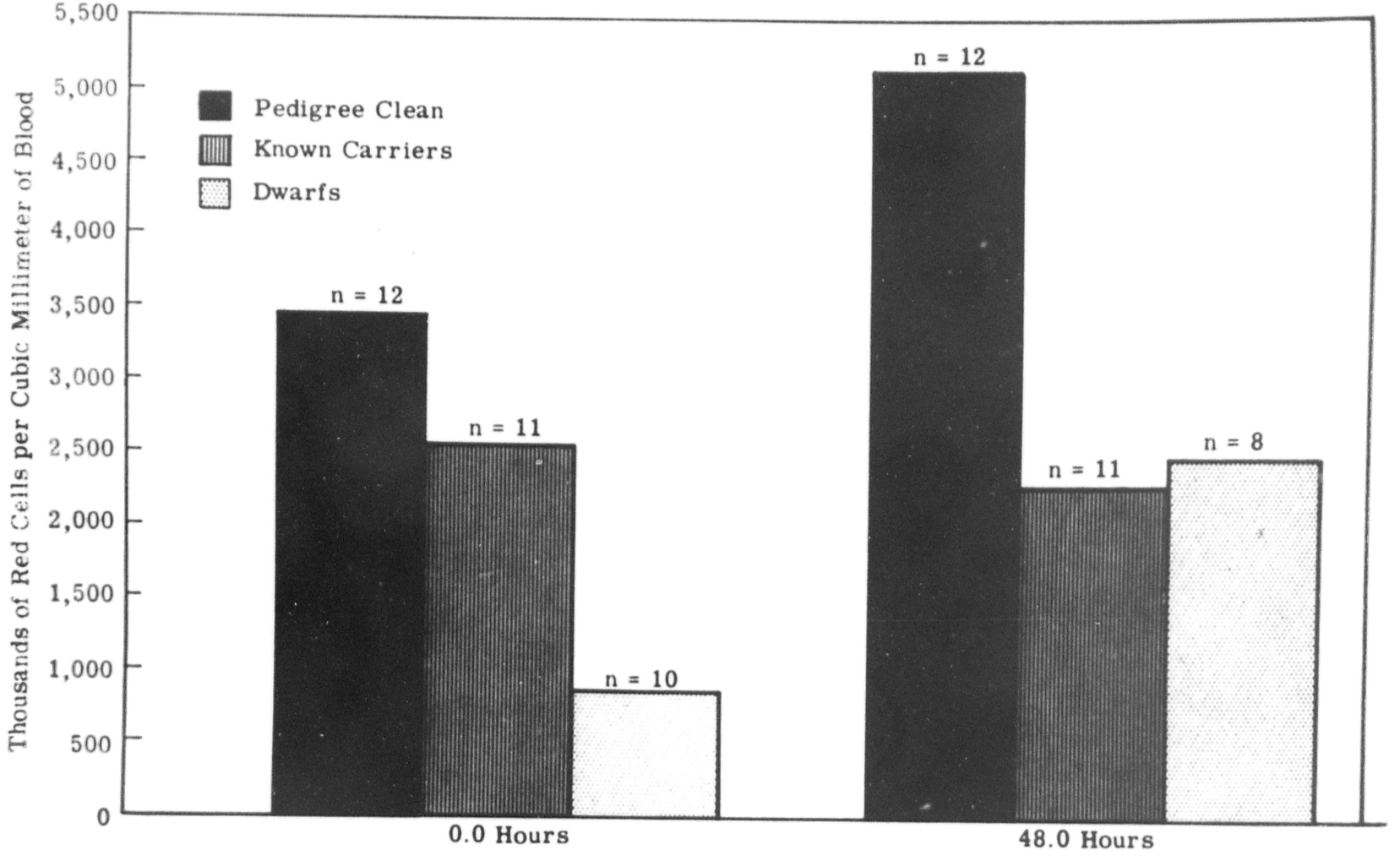

Figure 4. Changes in Red Blood Cell Resistance 48.0 Hours After a Single Insulin Injection.

Figure 3 shows the response of each genotype after the second insulin administration. In general, the pedigree clean and dwarf groups maintained higher average red cell counts during this period. The mean values for the known carrier animals were slightly lower following the second administration of the hormone.

The average count of the pedigree clean animals dropped sharply within one-half hour after the second insulin injection. The level at this time was approximately equal to their previous twelve hour average. After a small increase at one and two hours, the mean count dropped to 3,334,170 cells per cubic millimeter at six hours. This was the lowest level recorded for the clean animals during either aspect of the insulin study. From six to twelve hours the average number of erythrocytes per cubic millimeter of blood showed a uniform rise. The change demonstrated at these times appeared similar to the response observed at corresponding hours after the first insulin induced stress.

The average red cell count of the known carriers showed an alternating decrease and increase throughout the latter twelve hour period. This type of response was similar to the reaction which followed the first injection of insulin. The fluctuation in their mean count remained small for six hours after the second hormone administration. By ten hours, however, their average cell count had decreased 1.3 million cells per cubic millimeter under the six hour value. This was the lowest level recorded for any genotype during the entire insulin investigation. The final average cell count of the

known carriers, at twelve hours, showed approximately a one-half-fold increase over the ten hour value.

For the first two hours after the second insulin injection the mean erythrocyte count of the dwarfs averaged about 1.2 million cells per cubic millimeter higher than corresponding counts from the preceding twelve hour period. The dwarfs reached their maximum peak one-half hour after the second hormone administration. The mean count declined gradually from two to six hours, but returned to approximately the two hour level by ten hours. The average number of erythrocytes per cubic millimeter of blood twelve hours after the second insulin injection was 1,960,000. This value was very close to the level recorded for the dwarfs at the end of the first analysis period.

With the exception of the sixth hour averages, there was a significant variation between the mean erythrocyte counts of the three genotypes at each sampling hour of the latter twelve hour period. The most highly significant differences during the interval were observed immediately prior to and ten hours after the second insulin administration.

An average coefficient of variation was determined for pedigree clean, known carrier and dwarf animals at the conclusion of the first and second insulin studies. These values were obtained for each genotype by taking the arithmetic mean of the coefficients of variation recorded in each period from zero through twelve hours. The results of the analysis after a single insulin injection showed the average coefficient of variation to be 43.96 for the clean individuals, 53.81

for the known carriers and 97.01 for the dwarfs. This indicated that the amount of variation over the twelve hour period in red cell counts, within the genotypes, was smallest among the clean animals, intermediate in the carriers and greatest in the dwarfs. Furthermore, a low average coefficient of variation would suggest a more uniform intragroup response to stress.

Following the second insulin administration the average coefficient of variation for the clean animals showed a marked decrease in comparison to the previous level and was 28.11. The dwarfs also had a lower average coefficient of variation at this time, but their value of 70.07 was considerably higher than that illustrated by the clean animals. The carriers responded in an opposite manner and demonstrated a mean value for this statistic of 71.85, slightly higher than their earlier level.

These data indicated that the response within the clean and dwarf groups was more uniform after the second stress, but that a large amount of variation, not evident among the clean individuals, remained in the cell counts of the carrier and dwarf genotypes.

The results of this investigation suggested that the first insulin injection influenced the pedigree clean and dwarf animals to continue the secretion of pituitary and adrenal cortical hormones during the thirty-six hour recovery period. Evidence to support this belief is provided by the increased erythrocyte resistance which both groups showed forty-eight hours after the initial insulin administration. This condition was not apparent among the known carrier

animals.

In the pedigree clean genotype, the trend toward a higher level of cellular resistance was continued after the second insulin injection. It is thought by the author that this indicated a greater response by the pituitary adreno-cortical mechanism to the second stress.

The red cell resistance of the dwarf group was also greater after the second insulin injection, but tended to remain near that of the known carriers. The resistance of both genotypes, however, was considerably lower than the pedigree clean animals. These data suggested that dwarf animals, when allowed a longer period of time, respond to stress approximately the same as known carriers. In addition, the lower resistance of both groups indicated a deficiency or extremely sluggish release of hormones from the pituitary or adrenal cortex.

PHASE III: THE INFLUENCE OF ADRENALIN UPON RED BLOOD CELL RESISTANCE

Each animal used in this phase of the investigation received a 4.0 cc. intravenous injection of 1:10,000 adrenalin. Erythrocyte resistance was analyzed by making total red cell counts in a 0.48 per cent sodium chloride diluting solution. Data were collected from five pedigree clean, five known carriers and five dwarfs, prior to and at various periods following the hormone administration. The results of this work are shown graphically in Figure 5 and are summarized in

Table IV.

Average control erythrocyte counts were determined immediately before adrenalin was injected. The values recorded for the clean and dwarf animals appeared normal in comparison to their previous zero hour counts. The known carriers demonstrated a level that was much lower than control counts recorded for them during the preceding phases.

In the ten hour period which followed the hormone injection, average cell counts for the three genotypes were generally lower than those observed during the insulin study. This effect was most pronounced in the carrier and dwarf animals and is illustrated in Figure 5. The data presented in this figure also show that the pedigree clean group maintained a considerably higher mean count than either of the other two genotypes, and demonstrated the only marked response to adrenalin.

The average count for the clean animals showed a drop of 378,000 cells per cubic millimeter within one hour after the hormone was injected, but began to increase slightly at two hours. The count continued to rise steadily from two to six hours and showed a subsequent peak of 3,334,000 cells per cubic millimeter at the end of this period. Although this level was not outstandingly high in comparison to some values obtained after insulin stress, it was approximately 2,828,000 cells per cubic millimeter greater than the averages shown by the dwarf and carrier animals at that time. Furthermore, it was one of the largest differences observed between genotypes in the entire

TABLE IV

THE INFLUENCE OF INTRAVENOUS ADRENALIN UPON RED BLOOD CELL RESISTANCE.*

Sample Time	Pedigree Clean			Known Carriers			Dwarfs		
	Thousands of Red Cells Per Cubic Millimeter of Blood	± Standard Deviation	Coefficient of Variation in Percent	Thousands of Red Cells Per Cubic Millimeter of Blood	± Standard Deviation	Coefficient of Variation in Percent	Thousands of Red Cells Per Cubic Millimeter of Blood	± Standard Deviation	Coefficient of Variation in Percent
Control or 0.0 Hour [2]	3,096.00	± 1,005.31	32.47	876.00	± 706.36	80.36	700.00	± 1,043.77	149.11
1.0 Hour [2]	2,718.00	± 328.98	12.10	752.00	± 664.67	88.31	1,260.00	± 1,065.19	84.54
2 Hours [2]	2,764.00	± 1,521.08	55.03	844.00	± 604.48	71.62	858.00	± 920.31	107.26
6 Hours [2]	3,334.00	± 937.88	28.13	478.00	± 347.96	72.79	534.00	± 699.23	130.94
10 Hours [2]	2,396.00	± 455.08	18.99	276.00	± 155.08	58.08	____**	____	____

Pedigree Clean (n = 5)
Known Carriers (n = 5)
Dwarfs (n = 5)

* Erythrocyte resistance measured by diluting the red cells with 0.48 percent sodium chloride solution.

** No sample taken.

[2] Difference between average counts of the three genotypes was significant (P=.01).

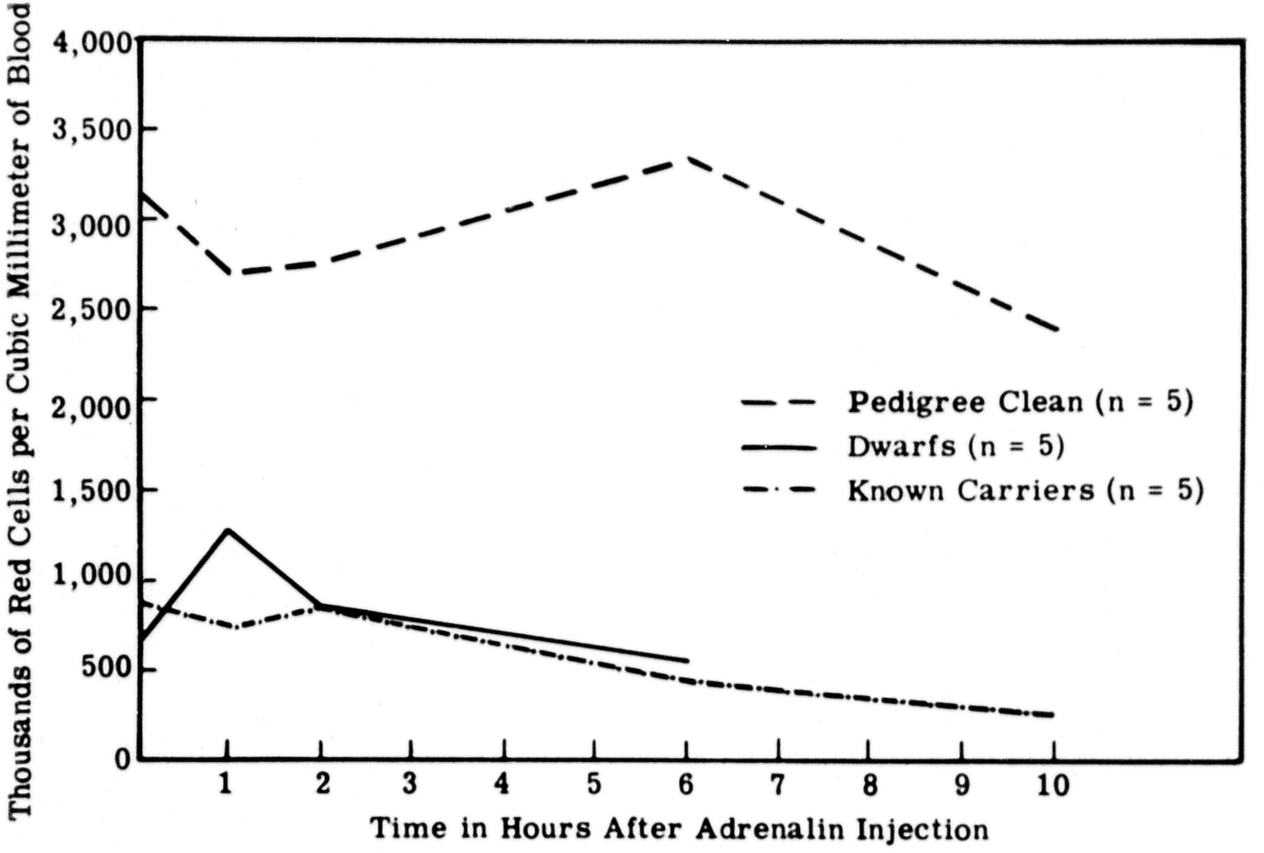

Figure 5. The Influence of Intravenous Adrenalin upon Red Blood Cell Resistance.

study. Figure 6 illustrates this difference and shows the average cell count for each group six hours after the intravenous injection of adrenalin. A significant difference was obtained between the mean counts of the three genotypes at this time. The final average erythrocyte count of the clean individuals showed a drop of nearly one-fold from the previous peak and was 2,396,000 cells per cubic millimeter. This total represented the lowest point for the pedigree clean genotype during the adrenalin study.

The known carriers showed very little response to the adrenalin injection. The group maintained a level approximately equal to their initial value for the first two hours, but thereafter demonstrated a decline at each succeeding erythrocyte count. At the conclusion of ten hours the average count for the carrier animals was exactly 600,000 cells per cubic millimeter below their initial level. This count 276,000 cells per cubic millimeter, was the lowest value recorded for any genotype during the entire study of red cell resistance.

The dwarfs demonstrated an increased average cell count within one hour after adrenalin was administered, but did not continue the rise past that point. At two hours their count had dropped and was slightly higher than the value exhibited by the known carriers. In the period from two to six hours the dwarfs showed a decline in their average cell count that was similar to the response encountered in the carriers. However, the overall decrease was not as great and these animals demonstrated an average six hour count of 534,000 cells per cubic millimeter.

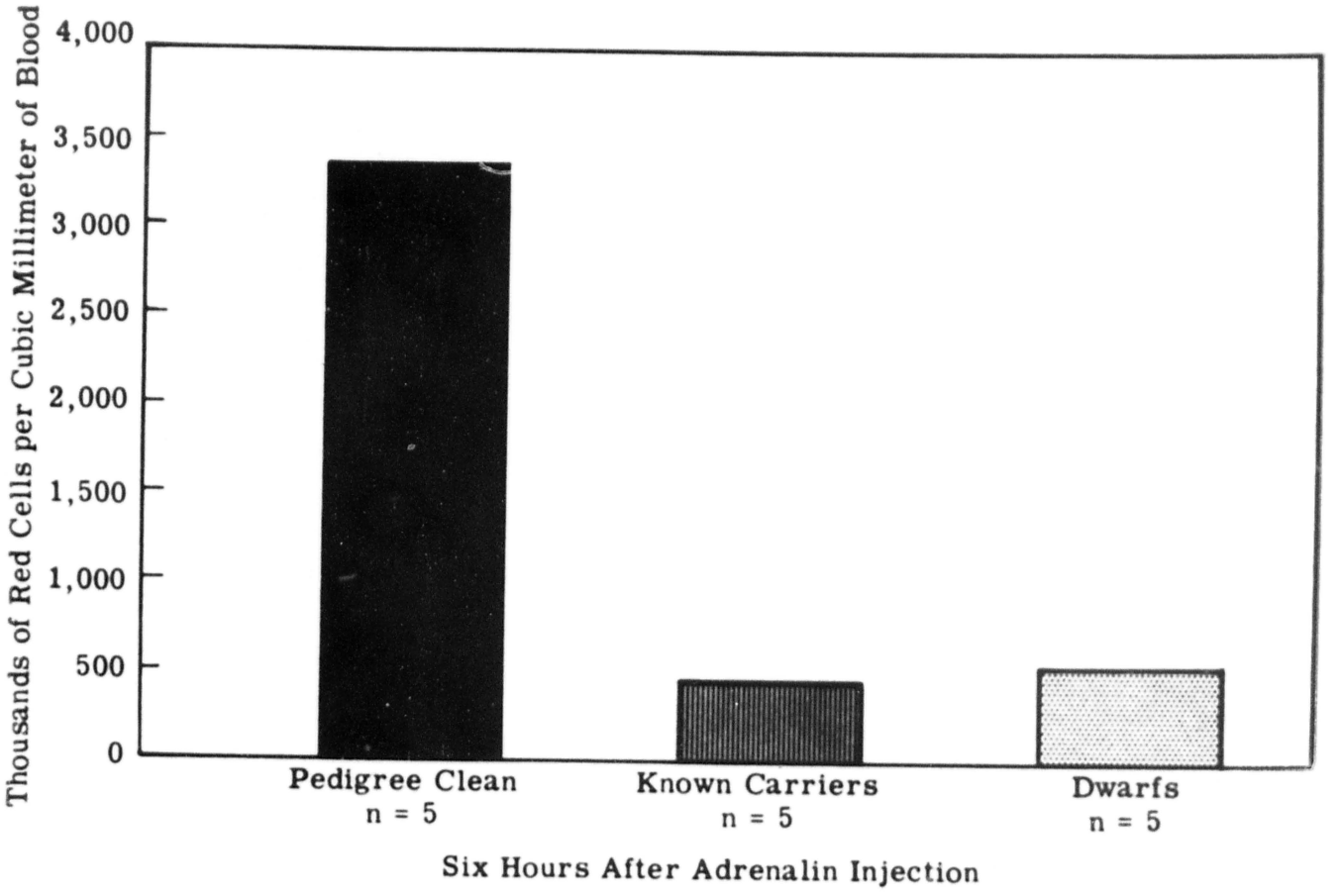

Figure 6. Genotypic Differences in Red Blood Cell Resistance Six Hours After the Intravenous Injection of Adrenalin.

In addition to the difference at six hours, a significant variation was observed between the mean cell counts of the three genotypes prior to, one and two hours after the adrenalin injection. There was a highly significant difference between the average counts of the clean and carrier animals ten hours after the hormone administration.

The hormone, epinephrine, is secreted primarily by the medulla of the adrenal gland and is one of the first substances released by the body during most types of stress. The compound has been accredited with the ability to produce a wide variety of effects, several of which are evident in this study.

The secretion of epinephrine (adrenalin) is normally followed by an immediate and absolute rise in the number of circulating red blood cells. The ultimate capacity of this hormone to bring about such a response is based primarily upon its power to augment splenic contraction. Barde (1950) reported that increases in erythrocyte numbers resulting from adrenalin were transient. Best and Taylor (1955) felt that this was due to the short active life of the compound after its release. Of interest in this study, is the fact that adrenalin can apparently cause a brief increase in the number of red cells in the peripheral blood. Although the dwarfs indicated a reaction of this type at one hour, it is felt by the author that the response may have taken place in the other genotypes and subsided by this time.

Adrenalin has not been reported to increase erythrocyte resistance, but it is a potent activator of the pituitary adreno-cortical mechanism. The ability of the adrenotrophic hormone to increase

adrenal steroid release is believed by this investigator to be the important basis for adrenalin's influence on red cell resistance.

Based on low red cell resistance, it does not appear that adrenalin stimulated, to any extent, the release of ACTH or adrenal cortical hormones in the carrier and dwarf genotypes. This condition may have resulted from an insufficient amount of either ACTH or the cortex substances, or possibly both. However, the investigation was conducted for only ten hours and may not have been of a great enough duration to allow for a delayed release of these compounds.

Adrenalin seemed to influence an increased activity in the pituitary adreno-cortical mechanism of the pedigree clean animals. Evidence favoring this idea is provided by the general trend of their resistance after the hormone administration, and by the high level of cell stability which these animals showed at six hours.

Although the number of animals in each genotypic class was limited, the results obtained during this study indicated several differences which should be investigated further.

PHASE IV: CHANGES IN RED BLOOD CELL RESISTANCE AFTER THE INJECTION OF ADRENOCORTICOTROPHIC HORMONE (ACTH)

The Effects of Subcutaneous and Intramuscular ACTH Upon Red Blood Cell Resistance. The results obtained during this study are shown in Table V and Figure 7. Each animal received one subcutaneous and one intramuscular injection of adrenocorticotropic hormone (ACTH) at the beginning of the investigation. The hormone was administered

TABLE V

THE INFLUENCE OF SUBCUTANEOUS AND INTRAMUSCULAR* ACTH UPON RED BLOOD CELL RESISTANCE.**

Sample Time	Pedigree Clean			Known Carriers			Dwarfs		
	Thousands of Red Cells Per Cubic Millimeter of Blood	± Standard Deviation	Coefficient of Variation in Percent	Thousands of Red Cells Per Cubic Millimeter of Blood	± Standard Deviation	Coefficient of Variation in Percent	Thousands of Red Cells Per Cubic Millimeter of Blood	± Standard Deviation	Coefficient of Variation in Percent
Control or 0.0 Hour [2]	2,784.00	± 1,358.82	48.81	1,840.00	± 1,599.76	86.94	972.35	± 1,081.16	111.19
2.5 Hours [2]	4,041.00	± 1,572.21	38.91	2,185.00	± 1,398.26	63.99	1,516.47	± 1,366.32	90.10
5.0 Hours [2]	4,105.00	± 1,555.77	37.90	1,312.00	± 1,205.36	91.87	1,304.12	± 1,220.33	93.57
10.0 Hours [2]	5,198.00	± 1,477.58	28.43	2,089.00	± 1,534.03	73.43	1,512.00	± 1,228.97	80.85

Pedigree Clean (n = 10)
Known Carriers (n = 10)
Dwarfs (n = 17)

* Each animal received one subcutaneous and one intramuscular injection of ACTH at the beginning of the test.

** Erythrocyte resistance measured by diluting the red cells with 0.48 percent sodium chloride solution.

[2] Difference between average counts of the three genotypes was significant (P=.01).

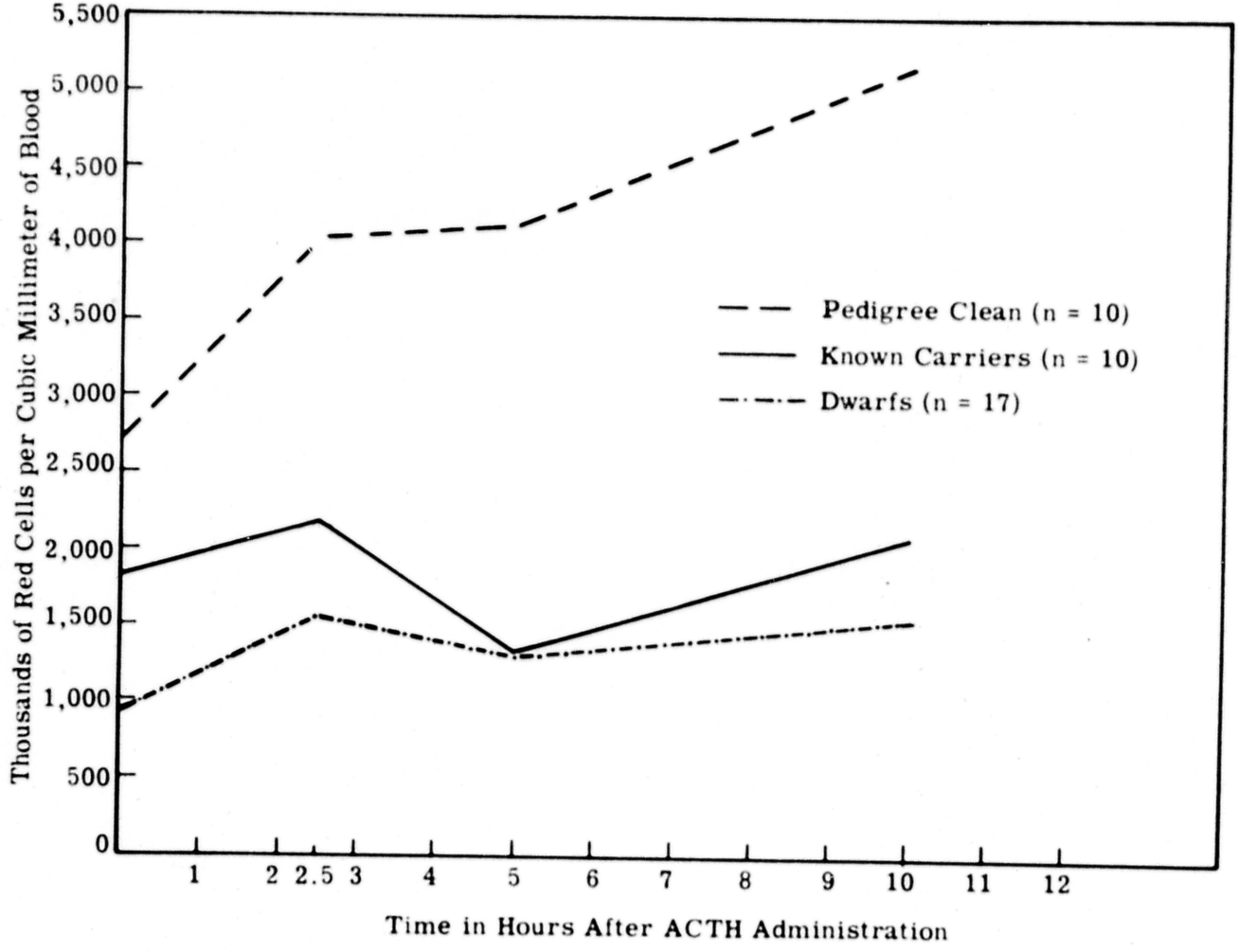

Figure 7. The Influence of Subcutaneous and Intramuscular ACTH upon Red Blood Cell Resistance.

to ten pedigree clean, ten known carriers and seventeen dwarfs at the rate of 0.25 of a unit of ACTH per pound of body weight. Erythrocyte resistance was based upon the total red cell count after the cells were diluted with a 0.48 per cent sodium chloride solution.

Average control hour erythrocyte levels were determined from blood samples collected prior to the injection of ACTH. The value obtained for the carriers was not largely different than that found during the study of initial resistance, but the dwarfs averaged higher and the pedigree clean animals slightly lower than the mean counts recorded during that phase.

The pedigree clean group demonstrated the greatest response to the subcutaneous and intramuscular injection of ACTH. Within 2.5 hours after the hormone administration, their average number of erythrocytes per cubic millimeter of blood increased slightly more than one-fold over the control level of 2,784,000. From 2.5 to 5.0 hours the count showed a gradual rise and totaled 4,105,000 cells per cubic millimeter at the end of this period.

In Figure 8 there is a graphical comparison of the average cell counts for the three genotypes prior to (0.0 hours) and five hours after the hormone injections. The average increase shown by the clean animals over this interval was not significant, but there was a highly significant difference between the average cell counts of the three genotypes at five hours.

The clean group reached a peak ten hours after the administration of ACTH and had an average erythrocyte count of 5,198,000 cells

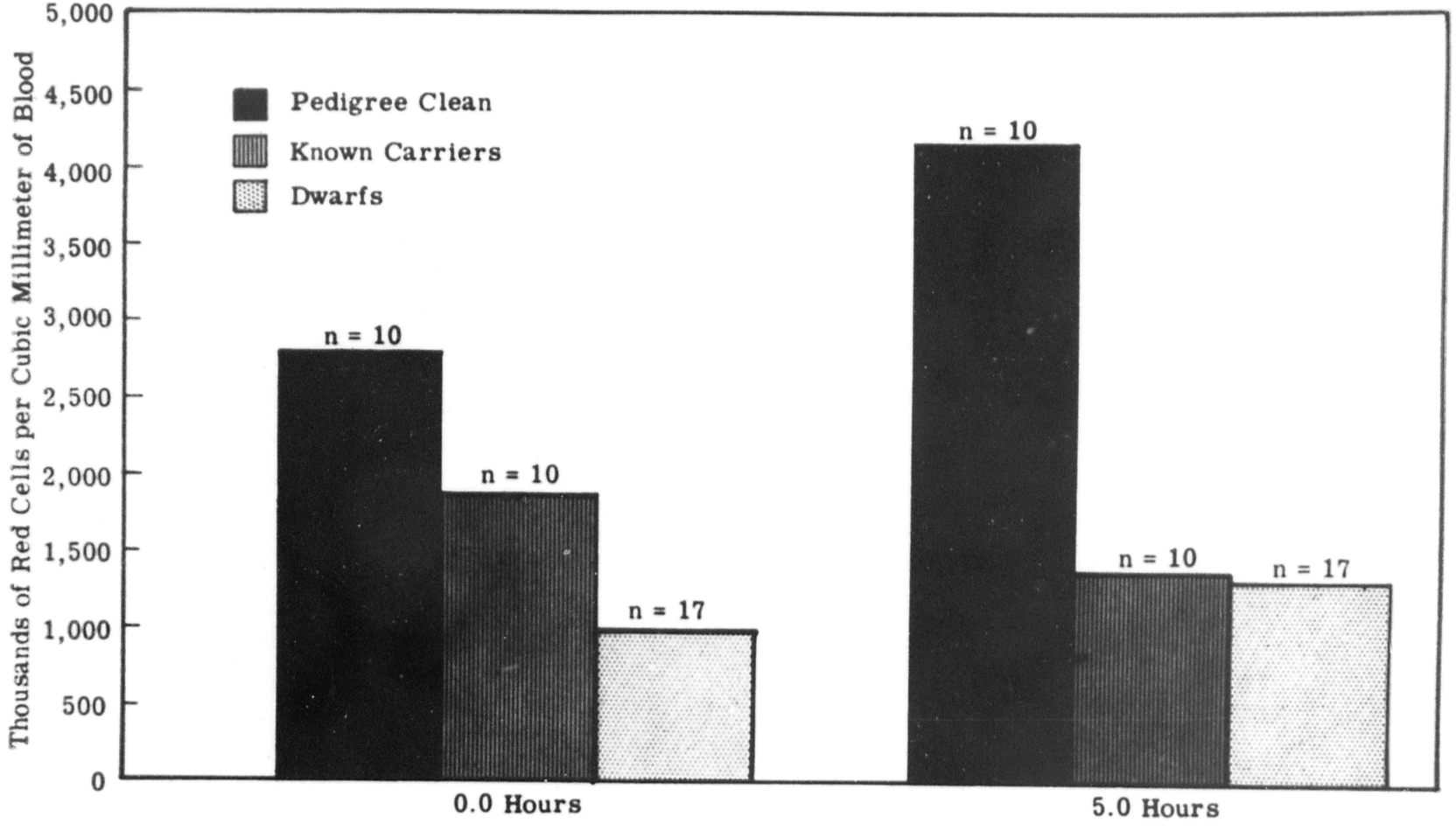

Figure 8. Changes in Red Blood Cell Resistance 5.0 Hours After the Subcutaneous and Intramuscular Injection of ACTH.

per cubic millimeter. The rise represented a one-fold increase over the five hour value and slightly more than a two-fold increase over their initial count at zero hours.

Figure 9 presents a graphical picture of the average red cell count obtained for each genotype at the end of the ten hour period. The level recorded for the clean animals at this time was significantly greater than their control value prior to the injection of ACTH, and was the highest value recorded for any genotype during the entire investigation. In addition, there was an approximate difference of 3.2 million cells per cubic millimeter between the average count of the pedigree clean animals and mean count of the other two genotypes. This was the largest difference of this type recorded during any phase of the research. There was also a highly significant difference between the average cell counts of the three groups at this hour.

The carrier and dwarf animals did not show a marked response to the ACTH administration and failed to demonstrate a significant increase in their average erythrocyte counts. They did, however, follow a similar pattern of change in regard to their red cell values. At zero hours, the carriers averaged approximately 900,000 cells per cubic millimeter more than the dwarfs. There was a slight increase in the average count of both genotypes at 2.5 hours, but this did not alter their margin of difference to any extent. By five hours after the hormone administration there was a close similarity between the mean value of each group. This resulted from a comparatively sharper decline in the carriers' level over the period from 2.5 to 5.0 hours.

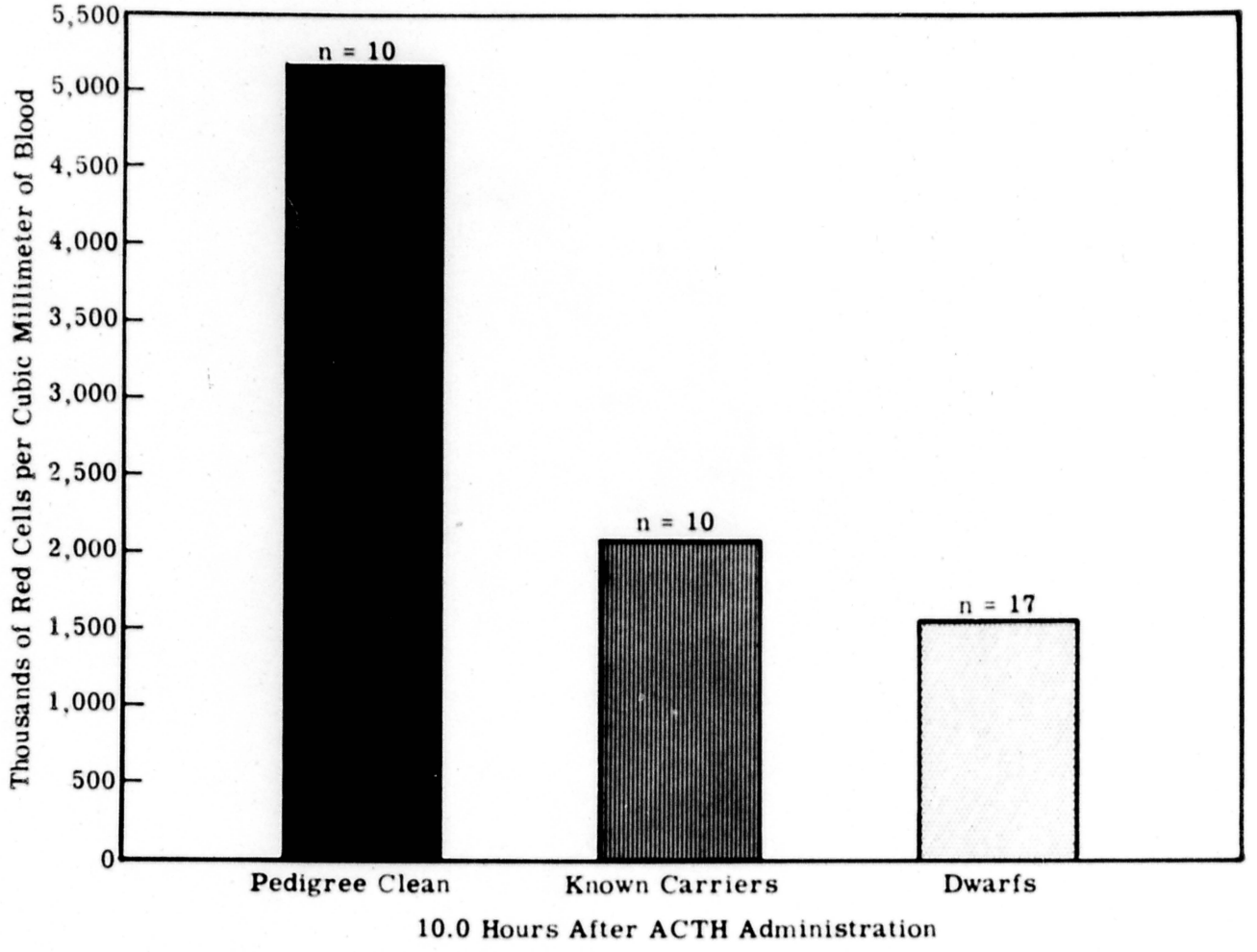

Figure 9. Genotypic Differences in Red Blood Cell Resistance 10.0 Hours After the Subcutaneous and Intramuscular Injection of ACTH.

The final sample at ten hours showed an increased average for both genotypes, but only to a level which approximated the value each attained at 2.5 hours.

The administration of adrenocorticotrophic hormone (ACTH) in a nonphysiological dosage should have induced stress and augmented the release of steroids from the adrenal cortex. It was noted in several earlier sections of this investigation that the pituitary hormone, ACTH, and the adrenal cortical hormone, cortisone, have been reported to increase red cell resistance. When the relative effectiveness of these hormones was compared ACTH was found to be the more potent substance. This may be due to the fact that cortisone is only one of several compounds found in the adrenal cortex, whereas ACTH from the pituitary may stimulate the release of several of these, causing a greater effect upon erythrocyte resistance. Although ACTH, itself, may increase red cell resistance, it is the author's belief that the important basis for its action upon erythrocyte resistance lies in its ability to increase adrenal steroid release.

The rather low level of response demonstrated by the carrier and dwarf genotypes did not favor the idea that ACTH alone increases red cell resistance, but it did indicate that these animals may be somewhat unable to maintain a balance in their pituitary and adrenal secretions when they were subjected to stress. Although the carrier and dwarf animals showed a sluggish reaction to the nonphysiological hormone administration, they illustrated transient increases in average erythrocyte resistance and did not appear to have a complete pituitary

insufficiency. However, it is thought by this investigator, that their lower levels of resistance may indicate a partial deficiency in this respect, or the failure of these substances to be released as rapidly as in normal animals.

The likelihood that some type of unbalance existed in the pituitary adreno-cortical mechanism of the carrier and dwarf animals was further magnified by the response of the pedigree clean group. Following the nonphysiological administration of ACTH, the clean animals showed a marked rise in their average red cell resistance and continued the increase throughout the ten hour period. By ten hours, they demonstrated a level of resistance that was significantly higher than the mean value recorded during the control period at zero hours. In the opinion of the author, these data indicate that the clean animals reacted to the induced stress by rapidly accelerating the secretion of pituitary and adrenal cortical hormones. It also is believed that the constant rise in erythrocyte resistance reflected the increased release of these compounds and the general pattern of stress response.

The Influence of Intravenous ACTH Upon Erythrocyte Resistance. Adrenocorticotrophic hormone (ACTH) was administered intravenously to five pedigree clean, five known carriers and six dwarfs at the rate of 0.5 of a unit of ACTH per pound of body weight. Blood samples were collected prior to and at different intervals after the ACTH injection. Erythrocyte resistance was analyzed by a total red cell count after

the cells were diluted with a 0.48 per cent sodium chloride solution. The results obtained during this section of the ACTH study are shown graphically in Figure 10 and are summarized in Table VI.

The average control hour erythrocyte count was determined for each genotype from blood samples collected prior to the nonphysiological administration of ACTH. The value obtained at this time for each genotypic group was the lowest average control count recorded for the group during the entire study of erythrocyte resistance.

At zero hours, the average number of erythrocytes per cubic millimeter of blood for the pedigree clean animals was 1,802,000. From this level their mean count showed an increase of approximately one-half-fold, and reached a peak of 2,494,000 cells per cubic millimeter at one-half hour. Within one hour after ACTH injection, the average total erythrocyte numbers had decreased slightly more than one-fold over the previous peak. The fall in average number of red cells continued over the next one hour period, and by two hours the mean count was only 890,000 cells per cubic millimeter. This was the lowest level recorded for the clean animals during any phase of the research. Following this low, the average count fluctuated upward and reached a point at six hours that was approximately equal to the one-half hour peak. Figure 11 illustrates the difference between this value and the average erythrocyte counts obtained for the known carrier and dwarf groups at that time. The difference between the average counts of the three genotypes was significant at the 5.0 per cent level.

The final blood samples were collected from the pedigree clean

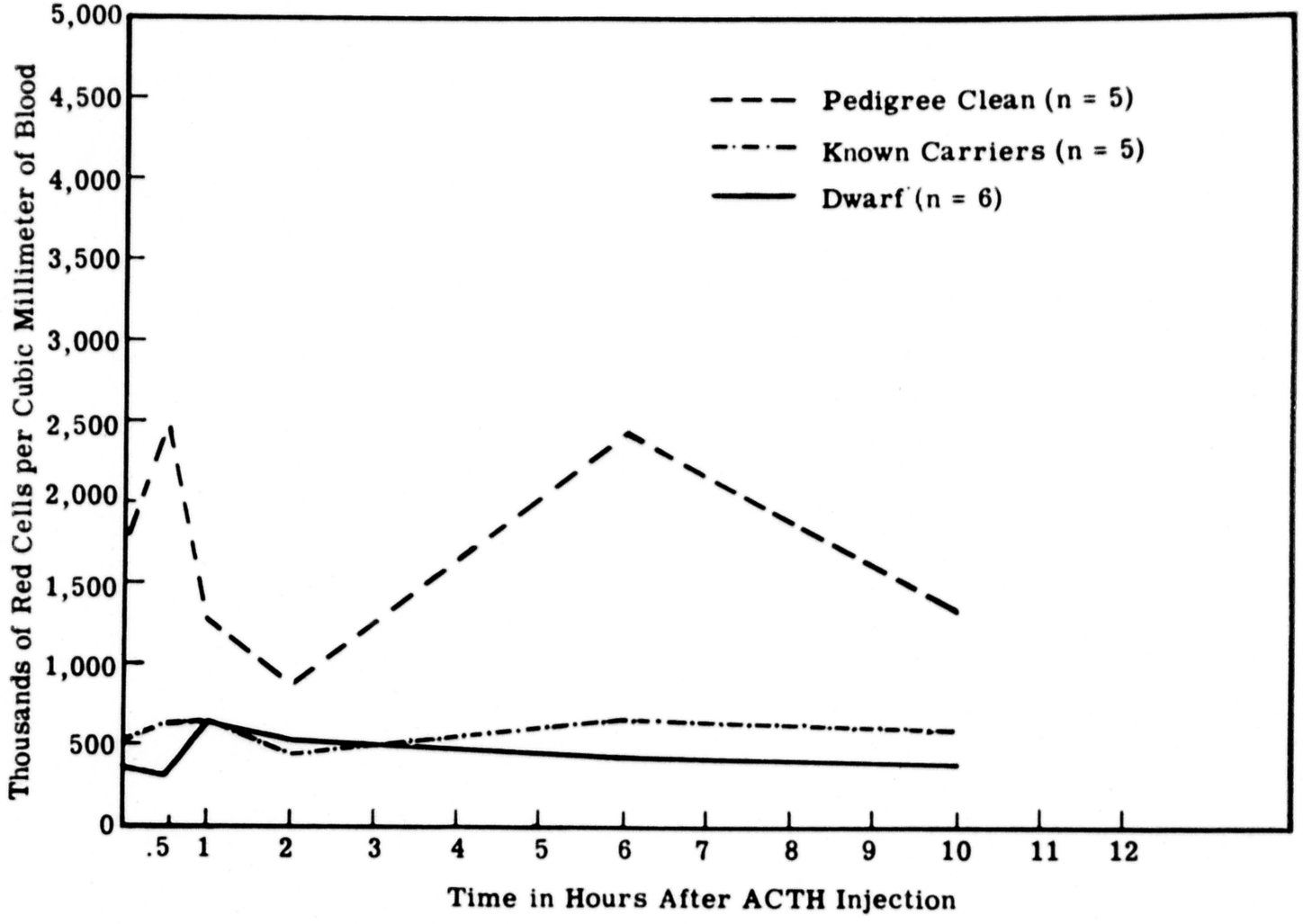

Figure 10. The Influence of Intravenous ACTH upon Red Blood Cell Resistance.

TABLE VI

THE INFLUENCE OF INTRAVENOUS ACTH UPON RED BLOOD CELL RESISTANCE.*

Sample Time	Pedigree Clean			Known Carriers			Dwarfs		
	Thousands of Red Cells Per Cubic Millimeter of Blood	±Standard Deviation	Coefficient of Variation in Percent	Thousands of Red Cells Per Cubic Millimeter of Blood	±Standard Deviation	Coefficient of Variation in Percent	Thousands of Red Cells Per Cubic Millimeter of Blood	±Standard Deviation	Coefficient of Variation in Percent
Control or 0.0 Hour [1]	1,802.00	± 1,048.91	58.21	556.00	± 470.32	84.59	393.33	± 419.57	106.67
0.5 Hour [2]	2,494.00	± 1,359.66	54.52	602.00	± 581.79	96.64	368.33	± 270.59	76.46
1.0 Hour	1,282.50	± 1,003.91	78.28	616.00	± 784.15	127.30	611.67	± 606.48	99.15
2.0 Hours	890.00	± 1,329.09	149.34	440.00	± 354.54	80.58	510.00	± 351.62	70.18
6.0 Hours [1]	2,422.00	± 1,707.37	70.49	678.00	± 525.29	77.48	420.00	± 263.97	64.23
10.0 Hours [1]	1,338.00	± 759.09	56.73	600.00	± 474.18	79.03	398.33	± 289.97	72.75

Pedigree Clean (n = 5)
Known Carriers (n = 5)
Dwarfs (n = 6)

* Erythrocyte resistance measured by diluting the red cells with 0.48 percent sodium chloride solution.

[1] Difference between average counts of the three genotypes was significant (P=.05).

[2] Difference between average counts of the three genotypes was significant (P=.01).

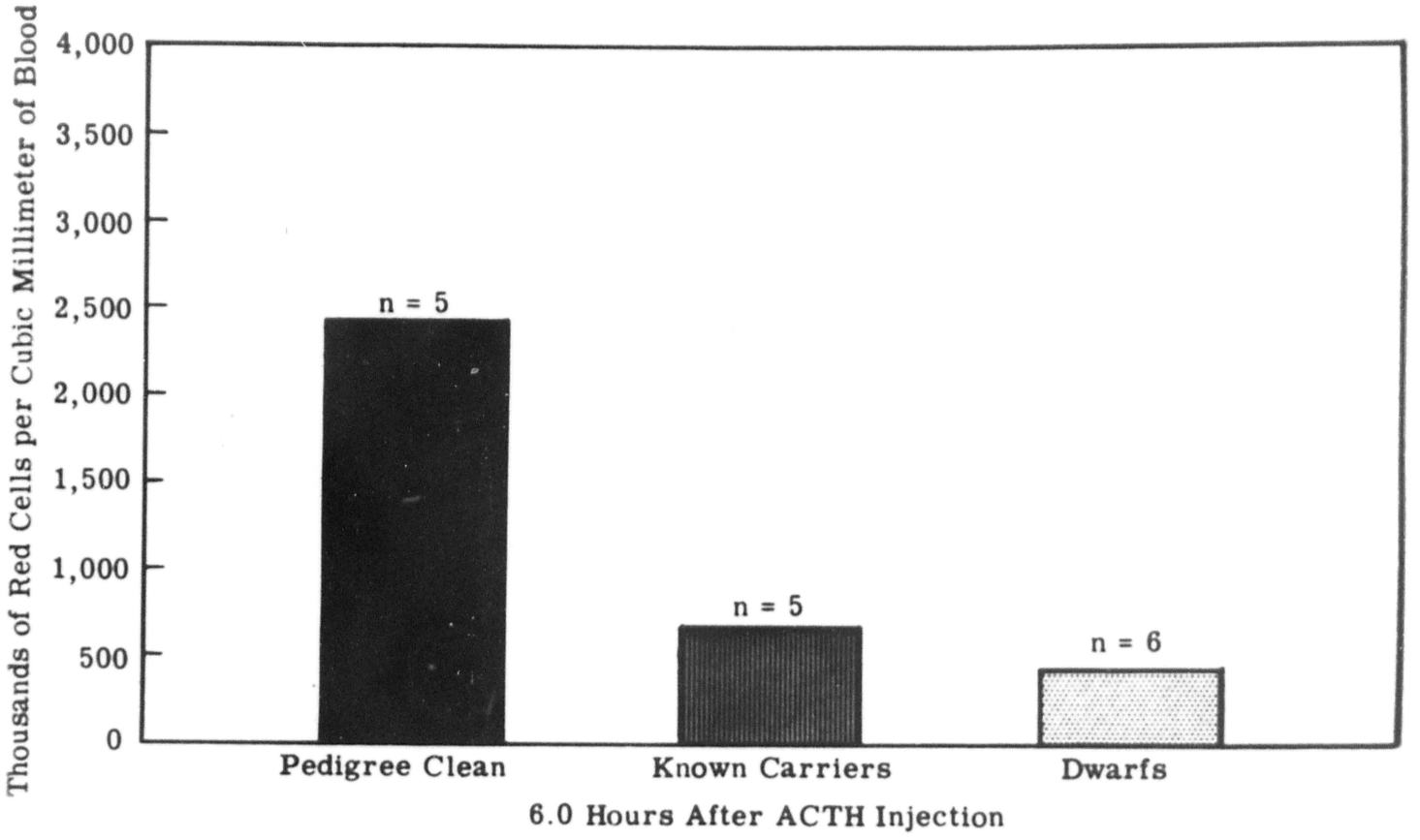

Figure 11. Genotypic Differences in Red Blood Cell Resistance 6.0 Hours After the Intravenous Injection of ACTH.

animals ten hours after the hormone administration. Their mean red cell count at that time showed a drop of about 1.0 million cells under the six hour level and equaled 1,338,000 cells per cubic millimeter.

The known carrier and dwarf animals demonstrated very little response to the intravenous injection of ACTH. There was no change of any consequence in the average erythrocyte count of either group and their mean values remained low.

The average count of the known carriers increased slightly at one-half and one hour, but dropped one-fold below the control level within two hours. From two to six hours the count increased gradually and reached a rather low peak of 678,000 cells per cubic millimeter at the end of this period. The average count showed a small drop at ten hours.

The mean red cell count for the dwarfs remained practically unchanged for the first one-half hour. By one hour it increased to a level that approximated the value attained by the known carriers at that time. This was the maximum count for the dwarfs after intravenous ACTH and was followed by a gradual decrease in the average erythrocyte number at each remaining red cell count.

The results indicated that the response to intravenous ACTH was not as marked as that which followed the subcutaneous and intramuscular injection of this hormone. The changes which occurred during the latter aspect of the ACTH study (ACTH I. V.) showed a closer resemblance to the response which took place after the intravenous injection of adrenalin (See Figure 5 and Figure 10).

Each genotype showed an increase in average number of erythrocytes per cubic millimeter of blood one-half or one hour after the intravenous administration of ACTH. It is believed by the author that such a response was not due primarily to an increased red cell resistance, but resulted essentially from splenic contraction, caused by the introduction of a foreign protein. The intensity of the reaction varied between the groups and a significant difference was observed between the average cell counts of the three genotypes one-half hour after the hormone injection.

The pedigree clean genotype showed a rather marked increase in erythrocyte resistance from two to six hours. In view of the fact that these animals were under stress from the nonphysiological hormone administration, such a rise indicated, in the opinion of the author, intense activity of the pituitary adreno-cortical mechanism, with the rapid release of ACTH and steroids from the adrenal cortex.

The response of the carrier and dwarf groups was, at best, slight. This further indicated the inability of these animals to respond rapidly to stresses induced by the nonphysiological administration of hormones.

Although nonphysiological levels of ACTH were administered in both aspects of the study, it had a more pronounced effect upon the red cell resistance of all genotypes when injected subcutaneously and intramuscularly. This suggested that a slow rate of hormone absorption was more effective for increasing red cell resistance and may be more valuable for obtaining an accurate picture of the response due to the

administration of a specific hormone. The immediate importance of this difference lies in the fact that it points to the need for more research of this type.

CHAPTER V

SUMMARY

Preliminary investigations were made to determine the effects of stress upon erythrocyte resistance in pedigree clean, known carrier and dwarf beef cattle. Stress was induced in the animals by administrating various pituitary, adrenal and pancreatic hormones in nonphysiological dosages. In the opinion of the author, the response to stress was indicated by changes in the red cell resistance.

A total of seventy-eight animals was examined in the study. Of these, there were twenty-three pedigree clean, twenty-seven known carriers and twenty-eight dwarfs. These animals represented the Hereford and Aberdeen-Angus breeds and were of both sexes.

The investigation was divided into four phases. These were:

Phase I: Initial Red Blood Cell Resistance.

Phase II: Changes in Red Blood Cell Resistance after the Injection of Insulin.

Phase III: The Influence of Adrenalin upon Red Blood Cell Resistance.

Phase IV: Changes in Red Blood Cell Resistance after the Injection of Adrenocorticotrophic Hormone (ACTH).

During each of the four phases the pedigree clean genotype demonstrated a much greater red cell resistance than either of the other two groups. Furthermore, at different times after the hormones were injected the clean animals showed marked increases in their

cellular stability which was not evident in the carrier and dwarf animals. At various times during each phase significant differences were observed between the average erythrocyte counts of the three genetic groups. These differences were most pronounced in the work with ACTH, adrenalin and forty-eight hours after a single insulin injection.

Research carried out with small laboratory animals as well as the results of this investigation, indicate that the low level of resistance shown by the carrier and dwarf animals may be due to one or more of the following factors:

1. An insufficient amount of either pituitary or adrenal hormones, or both.
2. The failure of the carrier and dwarf animals to release these substances as rapidly as normal animals.

This preliminary investigation was not designed to develop into another test for carriers of the dwarf gene; however, the knowledge acquired in the study coupled with further research may result in such a test.

BIBLIOGRAPHY

BIBLIOGRAPHY

Barcroft, J. (1925): "Recent Knowledge of the Spleen." J. Lancet, 1:319.

Barron, A. G., and Barron, E. S. G. (1937): "Mechanism of Cobalt Polycythemia; Effect of Ascorbic Acid." Proc. Soc. Exper. Biol. and Med., 35:407.

Beach, E. F., Berstein, S. S., Hummel, F. C., Williams, H. H., and Icie, G. M. (1939): "Total Sulfur, Cystine, and Methionine Content of Blood Globulins of Five Major Species." Journ. Biol. Chem., 130:115.

Bert, P. (1882): Compt. rend. Acad. Sc., 94:805. Quoted from: Grant, W. C., and Root, W. S. (1952).

Best, W. R., Limarzi, L. R., and Poncher, H. G. (1951): "Acquired Hemolytic Anemia Treated with Corticotropin." Journ. Am. Med. Association, 147:827-831.

Best, C. H., and Taylor, N. B. (1955): The Physiological Basis of Medical Practice. Ed. 6, Baltimore: The Williams and Wilkins Company, pp. 9-17, 63-92, 103-113.

Bethell, F. H., Miller, S., and Meyers, M. C. (1951):"Administration of ACTH and Cortisone in Hypersplenic Syndromes." Proc. of the Second Clinical ACTH Conference, 2:173-180.

Blount, W. P. (1939): "Normal Blood Cells in the Bovine." Vet. J., 95:222.

Bovard, K. P., Hazel, L. N., and Emmerson, M. A. (1956): "Effects of the Snorter Dwarf Gene on the Lumbar and Metacarpus in Hereford Calves at Birth." J. Animal Sci., 15:1213.

Buchanan, M. L., Bolin, F. M., Burnham, J. J., and Eveleth, D. F. (1956): "Some Skeletal Changes Associated with Dwarfism in Cattle." North Dakota Agricultural Experiment Station Bulletin, 403.

Burk, D., Hearon, J., Caroline, L., and Schade, A. J. (1946): "Reversable Complexs of Cobalt, Histidine, and Oxygen Gas." J. Biol. Chem., 165:723.

Burnett, S. H. (1917): The Clinical Pathology of the Blood of Domesticated Animals. Ed. 2, New York: Macmillan.

Canham, A. S. (1930): 16th Report, Div. Vet. Services. Pretoria: The Government Printer.

Carroll, F. D., Gregory, P. W., and Rollins, W. C. (1951): "Thyrotropic Hormone Deficiency in Homonzygous Dwarf Beef Cattle." J. Animal Sci., 10:916-921.

Coffin, D. L. (1953): Manual of Veterinary Clinical Pathology. Ed. 3, New York: Cornell Univ. Press.

Crafts, R. C. (1941): "The Effect of Endocrines on the Formed Elements of the Blood." Endocrinology, 29:596.

_____, (1946): "The Effects of Iron, Copper, and Thyroxine on the Anemia Induced by Hypophysectomy in the Adult Female Rat." Am. J. Anat., 79:267.

Craft, W. A., and Orr, H. W. (1924): "Thyroidal Influence in Cattle." J. Heredity, 15:255-256.

Crenshaw, W. W., Pipes, G. W., Ruppert, H. L. Jr., and Turner, C. W. (1957): "Indications of Normal Pituitary and Thyroid Function in Dwarf Beef Animals." Missouri Agricultural Experiment Station Bulletin, 621.

Cruz, W. O., Hahn, P. F., and Bale, W. F. (1942): "Hemoglobin Radioactive Iron Liberated by Erythrocyte Destruction (Acetylphenylhydrazine) Promptly Reutilized to Form New Hemoglobin." Am. Journ. Physiol., 135:595.

Cutler, J. T. (1934): "Studies on the Carbohydrate Metabolism of the Goat. The Blood Sugar and the Inorganic Phosphate." Journ. Biol. Chem., 106:653.

Dacie, J. V. (1950): Practical Haematology. London: Churchill.

Dallwig, H. C., Kolls, A. C., and Loevenhart, A. S. (1915): "The Mechanism Adapting the Oxygen Capacity of the Blood to the Requirements of the Tissues." Am. J. Physiol., 39:77.

Dameshek, W. (1950): "ACTH in Leukemia." Blood, 5:791.

_____, Rosenthal, M. C., and Schwartz, L. J. (1951): "The Treatment of Acquired Hemolytic Anemia with Adrenocorticotrophic Hormone (ACTH)." New England J. Med., 244:117-127.

Davis, J. E. (1941): "The Depression of Experimental Polycythemias by Various Substances in Dogs, Rabbits, and Man." J. Pharmacol. and Exper. Therap., 73:162.

_____ (1942): "The Production of Experimental Polycythemia by the Daily Administration of Epinephrine or Posterior Pituitary Solution." Am. J. Physiol., 137:669.

Dougherty, T. F., and White, A. (1944): "Influence of Hormones on Lymphoid Tissue Structure and Function. The Role of Pituitary Adrenotrophic Hormone in the Regulation of the Lymphocytes and Other Cellular Elements of the Blood." Endocrinology, 35:1-14.

Dukes, H. H. (1955): The Physiology of Domestic Animals. Ed. 7, Ithaca: Comstock Publishing Associates, pp. 18-35.

Dutcher, R. A., Jensen, C. O., and Althouse, P. M. (1951): Introduction to Agricultural Biochemistry. Ed. 1, New York: John Wiley and Sons, Inc., pp. 306-307.

Emmerson, M. A., and Hazel, L. N. (1956): "Radiographic Demonstration of Dwarf Gene Carrier Beef Animals." J. of Am. Vet. Med. Assoc., 128:381-390.

Evans, R. S., and Duane, R. T. (1949): "Acquired Hemolytic Anemia: Relation of Erythrocyte Antibody Production to Activity of the Disease: The Significance of Thrombocytopenia and Leukopenia." Blood, 4:1196.

Feldman, J. D., Rachmilewitz, M., Stein, O., and Stein, Y. (1953): "The Adrenal Cortex and Hemolysis. I. Phenylhydrazine." Blood, 8:342-348.

_____, and Rachmilewitz, M. (1954): "The Adrenal Cortex and Hemolysis, II. Immune Serum." Acta Hematol., 11:129-133.

Ferguson, L. C., Irwin, M. R., and Beach, B. A. (1941): "The Effect of Parturition on the Blood Picture of Cows in Health and During Infection with Brucella Abortus." Am. J. Vet. Res., 2:394.

_____, L. C., Irwin, M. R., and Beach, B. A. (1945): "On Variation in the Blood Cells of Healthy Cattle." J. Infect. Dis., 76:24.

Foley, C. W., Massey, J. W., and Lasley, J. F. (1956): "Variations in the Physiological Response to Stress in Dwarf and Normal Beef Cattle." J. Animal Sci., 15:1217.

Fraser, A. C. (1930): First Report, Institute of Animal Pathology, Univ. of Cambridge, 1:114. Quoted from: Holman, H. H. (1955, 1956).

Frost, R. C., and Talmage, R. V. (1951): "Fluid and Electrolyte Shifts in the Hydrated Adrenalectomized Rat." Endo. 49:606-612.

Garcia, J. F., Van Dyke, D. C., Huff, R. L., Elmlinger, P. J., and Ode, J. M. (1951): "Increase in Circulating Red Cell Volume of Normal and Hypophysectomized Rats After Treatment with ACTH." Proc. Soc. Exper. Biol. and Med., 76:707-709.

Gardner, F. (1950): "ACTH in Lukemia." Blood, 5:791.

_____, McElfresh, A. E., Harris, J. W., and Diamond, L. K. (1951): "The Effect of Adrenocorticotrophic Hormone (ACTH) in Idiopathic Acquired Hemolytic Anemias as Related to the Hemolytic Mechanisms." J. Lab. and Clin. Med., 37:444-457.

Garner, R. J., and Unsworth, K. (1953): "Seasonal Variations in the Blood Picture of Nigerian Cattle." Vet. Rec., 65:228.

Gordon, A. S., and Charipper, H. A. (1947): "Endocrine System and Hemopoiesis." Ann. New York Acad. Sci., 48:615.

Gordon, A. S., Piliero, S. J., and Landlaw, S. (1951): "The Relation of the Adrenal to Blood Formation in the Rat." Endo., 49:497.

Grant, W. C. (1951): "Measurement of Erythroid Mitotic Activity in Bone Marrow by Use of Colchicine." Proc. Soc. Exper. Biol. and Med., 77:537.

_____, and Root, W. S. (1952): "Fundamental Stimulus for Erythropoiesis." Physiol. Rev., 32:449-498.

Greatorex, J. C. (1954): "Studies on the Hematology of Calves from Birth to One Year of Age." Brit. Vet. J., 110:120.

Gregory, P. W. (1956): "Phenotypic Forms and Genetic Relationships of the Bovine Dwarf Complex." J. Animal Sci., 5:1207.

_____, Rollins, W. C., and Carroll, F. D. (1952): "Heterozygous Expression of the Dwarf Gene in Beef Cattle." The Southwestern Vet., 5:345-349.

_____, Roubicek, C. B., Carroll, F. D., Stratton, P. O., and Hilston, N. W. (1953): "Inheritance of Bovine Dwarfism and the Detection of Heterozygotes." Hilgardia, 22:407-450.

Hadley, F. B. (1949): Principles of Veterinary Science. Ed. 4, Philadelphia: W. B. Saunders Company, pp. 268-269, 275-279.

Hahn, P. F., Bale, W. F., and Bonner, J. F. Jr. (1943): "Removal of Red Cells from the Active Circulation by Sodium Pentobarbital." Am. J. Physiol., 138:415.

Hawk, P. B., Oser, B. L., and Summerson, W. H. (1954): Practical Physiological Chemistry. Ed. 13, Toronto: The Blakiston Company, pp. 467-475, 1268-1269, 1275.

Heidenrich, C. J., Lasley, J. F., and Comfort, J. E. (1955): "The Insulin Glucose Tolerance Test as Applied to Physiological Studies in Beef Cattle." J. Animal Sci., 14:1184.

Hill, J. M. and Hunter, R. B. (1951): "ACTH Therapy in Refractory Anemias." Proc. of the 2nd. Clinical ACTH Conference, 2:181-194.

Holman, H. H. (1950): Boddie's Diagnostic Methods in Veterinary Medicine. Ed. 2, Edinburgh: Oliver and Boyd, pp. 332-377.

_____ (1952): "A Negative Correlation Between Size and Number of the Erythrocytes of Cows, Sheep, Goats, and Horses." J. Path. Bact., 64:379-384.

_____ (1955): "The Blood Picture of the Cow." The Brit. Vet. J., 111:440-457.

_____ (1956): "Changes Associated with Age in the Blood Pictures of Calves and Heifers." The Brit. Vet. J., 112:91-104.

Hovermark, N. G., and Nordensen, N. G. (1950): "Hematological Changes in a Case of Rheumatoid Arthritis Treated with Adrenocorticotrophic Hormone." Acta Haemat., 4:193-200.

Hudson, G., Herdan, G., and Yoffey, J. (1952): "Effect of Repeated Injections of ACTH Upon the Bone Marrow." Brit. Med. J., 1:999-1002.

Hurtado, A., Merino, C., and Delgado, E. (1945): "Influence of Anoxemia on Hematopoietic Activity." Arch. Int. Med., 75:284.

Jakobowicz, R., Krieger, V. I., and Simmons, R. T. (1948): "Value of the Coombs Test in Detection of Isosensitization of the Newborn." Med. of Australia, 2:143.

Johnson, L. E., Harshfield, G. S. and McCone, W. (1950): "Dwarfism, an Heredity Defect in Beef Cattle." J. Heredity, 41:177-181.

Kato, K. (1937): "Iron-Cobalt Treatment of Physiologic and Nutritional Anemia in Infants." J. Pediat., 11:385.

Knisely, M. H. (1934): "Miscroscopic Observations on Circulatory Systems of Transilluminated Mammalian Spleens and Parturient Uteri." Proc. Soc. Exper. Biol. and Med., 32:212.

Kurnick, N. B. (1941): "Permeability of the Human Erythrocyte to Sodium and Potassium." J. Biol. Chem., 140-581.

Lee, M. O., and Ayres, G. B. (1936): "The Composition of Weight Lost and the Nitrogen Partition of Tissues in Rats After Hypophysectomy." Endo., 20:489.

Levin, L. (1943): "The Relation of Food Consumption, Hypophysis and Adrenal Cortex to Serum Albumin Metabolism in the Rat." Am. J. Physiol., 138:258.

Long, C. N. H. (1952): "Regulations of ACTH Secretion." Rec. Prog. Horm. Research, 7:75-105.

Lowenstein, L., Shapire, L. and Browne, J. S. L. (1951): "The Effect of Adrenocorticotrophic Hormone on the Blood and Bone Marrow of Pernicious Anemia." Proc. of the 2nd. Clinical ACTH Conf., 1:426-437.

Luisada, A. (1941): "Favism a Singular Disease Chiefly Affecting the Red Blood Cells." Medicine, 20:229.

Lush, J. L. (1930): "Duck Legged Cattle on Texas Ranches." J. Heredity, 21:85.

Manresa, M., and Orig, S. C. (1941): "Hematological Studies on Cattle; Variations of Hemoglobin and White Blood Cells in Philippine Native Breed of Cattle." Philipp. Agric., 30:375-391.

_____, Reyes, N. C., Gomez, F., Zialcita, L. P., and Falcon, P. K. (1940): "Influence of Atmospheric Temperature Upon Hemoglobin and Other Constituents of the Blood of Cattle." Emp. J. Exp. Agri., 8:97.

Marlowe, T. J., and Chambers, D. (1954): "Some Endocrine Aspects of Dwarfism in Beef Cattle." J. Animal Sci., 13:961.

Marston, H. R. (1952): "Cobalt, Copper and Molybdenum in the Nutrition of Animals and Plants." Physiol. Rev., 32:66.

Merino, C. F. (1950): "Studies on Blood Formation and Destruction in the Polycythemia of High Altitude." Blood, 5:1.

Meyer, O. O., Thewlis, E. W., and Rusch, H. P. (1940): "The Hypophysis and Hemopoiesis." Endo., 27:932.

Miller, W. T. (1934): Report of the New York State Veterinary College 1932-33. Quoted from: Holman, H. H. (1955).

Morrison, F. B. (1956): Feeds and Feeding. Ed. 22, Ithaca: The Morrison Publishing Company, pp. 107-110.

Mullick, D. N., and Pal, A. K. (1943): "A Study of the Seasonal Variation of the Blood Constituents of Normal Cattle." Ind. J. Vet. Sci., 13:146.

Neser, C. P. (1923): 9/10 Report, Div. Vet. Res. Pretoria: Govt. Print. Office.

Orten, J. M. (1935): "On the Mechanism of the Hematopoietic Action of Cobalt." Am. J. Physiol., 114:414.

Palmer, J. C., Cartwright, G. E., and Wintrobe, M. M. (1951): "The Effect of ACTH and Cortisone on the Blood of Experimental Animals." Proc. of 2nd. Clin. ACTH Conf., 1:438-453.

Petersen, W. E., et al. (1931): "Artifically Induced Hypoglycemia and Hypocalcemia in the Cow and the Relationship to Parturient Paresis in Milk Fever." J. Am. Vet. Med. Assoc., 79:217.

Price, D. A., Bogart, R., Alexander, G., and Kruger, H. (1957): "Red and White Cell Counts in Genetically Different Groups of Beef Cattle." (Abstract) J. Animal Sci., 16:1017-1122.

Price-Jones, C. (1931): "The Concentration of Hemoglobin in Normal Human Blood." J. Path. and Bact., 34:779.

Reichert, E. T., and Brown, A. P. (1909): The Crystallography of the Hemoglobins. Washington. Quoted from: Dukes, H. H. (1955).

Reznikoff, P., Foot, N. C., and Bethea, J. M. (1935): "Etiologic and Pathologic Factors in Polycythemia Vera." Am. J. M. Sc., 189:753.

Roberts, S. J. (1943): "The Effects of Various Intravenous Injections on the Horse." Am. J. Vet. Res., 4:226-239.

Roubicek, C. B., Clark, R. T., and Pahnish, O. F. (1955): "Dwarfism in Beef Cattle." A Literature Review, U.S.D.A. and Univ. of Arizona.

Rusoff, L. L., Johnston, J. E., and Branton, C. (1954): "Blood Studies of Breeding Dairy Bulls; Hematocrit, Hemoglobin, Plasma Calcium, Plasma Inorganic Phosphorus, Alkaline Phosphatase Values, Erythrocyte Count, and Leucocyte Count." J. Dairy Sci., 37:30.

_____, and Piercy, P. L. (1946): "Blood Studies of Louisiana Dairy Cows. II. Calcium, Inorganic Phosphorus, Hemoglobin Value, Erythrocyte Count, Leucocyte Count, and Percentages of Types of Leucocytes." J. Dairy Sci., 29:526.

Schneider, E. C. (1921): "Physiological Effects of Altitude." Physiol. Rev., 1:631.

Schwartz, B. M., and Stats, D. (1949): "Oxygen Saturation of Sternal Marrow Blood in Polycythemia Vera." J. Clin. Invest., 28:736.

Seligman, M. B. (1904): "Cretinism in Calves." J. Path. and Bact., 9:311-322.

Singer, K., and Motulsky, A. G. (1949): "The Developing (Coombs) Test in Spherocytic Hemolytic Anemias; Its Significance for Pathophysiology of Spherocytosis and Splenic Hemolysis." J. Lab. and Clin. Med., 34:768.

Summers. V. K., and Sheehan, J. L. (1951): "Cortisone and ACTH in Hypopituitarism." Brit. Med. J., 2:564.

Stanley, A. J., Hopps, H. C., and Shideler, A. M. (1947): "Cobalt Polycythemia. II. Relative Effects of Oral and Subcutaneous Administration of Cobaltous Chloride." Proc. Soc. Exper. Biol. and Med., 66:19.

Taylor, R. D., and Page, I. H. (1943): "Mechanism of Erythremia." Arch. Surg., 47:59.

Thorn, G. W. (1950): "Hematologic Effects of ACTH; in Discussion of ACTH in Lukemia." Blood, 5:785.

_____, Forsham, P. H., Frawley, T. F., Hill, S. R. Jr., Roche, M., Staehelin, D., and Wilson, D. L. (1950): "Medical Progress; The Clinical Usefulness of ACTH and Cortisone. New Eng. J. Med., 242:824.

Tyler, W. S., Julian, L. M., and Gregory, P. W. (1956):"Identification of the Process Responsible for the Short-headed Hereford Dwarf Based on Anatomical Studies." J. of Animal Sci., 15:1207.

Unger, L. J. (1951): "The Effects of ACTH in Acquired Hemolytic Anemia." Am. J. Clin. Path., 21:456-459.

Van Dyke, D. C., Simpson, M. E., Contopoulos, A. N., and Evans, H. M. (1957): "The Separate Existence of the Pituitary Erythropoietic Hormone." Blood, 12:539.

Vollmer, E. P., Gordon, A. S., Levenstein, I. and Charipper, H. A. (1939): "Effects of Hypophysectomy Upon the Blood Picture of the Rat." Endo., 25:970.

White, A., and Dougherty, T. F. (1945): "Effect of Prolonged Stimulation of the Adrenal Cortex and Adrenalectomy on the Numbers of Circulating Erythrocytes and Lymphocytes." Endo., 36:16-23.

Whitelaw, M. J. (1951): "A Case of Cooley's Anemia Treated with ACTH." Proc. of the 2nd. Clin. ACTH Conf., 2:195-198.

Wintrobe, M. M. (1951): Clinical Hematology. Ed. 3, London: Kimpton.

_____, Cartwright, G. E., Palmer, J. G., Kuhns, W. J., and Samuels, L. T. (1951): "Effect of Corticotropin and Cortisone on the Blood in Various Disorders in Man." Arch. Int. Med., 88:310-317.

Wogley, P. F., Shen, S. C., Gardner, F. H., and Castle, W. B. (1948): "Studies on Destruction of Red Blood Cells: The Spleen as the Source of Substance Causing Agglutination of the Red Blood Cells of Certain Patients with Acquired Hemolytic Jaundice by Antihuman Serum, and Rabbit Serum (Coomb's Serum)." J. Lab. and Clin. Med., 33:1197.

Wright, C. S., Dodd, M. C., Bouroncle, B. A., Dean, C. A., and Zollinger, R. M. (1951): "Studies of Hemagglutinins in Heredity Spherocytosis and in Acquired Hemolytic Anemia: Their Relationship to the Hypersplenic Mechanism." J. Lab. and Clin. Med., 37:165.

Young, L. E., Christian, R. M., and Izzo, M. J. (1951): "Some Newer Concepts of Congenital and Acquired Hemolytic Anemias." Med. Clin. North America, 35:571.

University Libraries
University of Missouri

Digitization Information for Theses and Dissertations project.
Scanned from microfilm; no subsequent editing done.

Local identifier

Source information

Identifier	Microfilm reel# T1958-98-T1958-109
Format	Microfilm
Content type	Text
Notes	

Capture information

Date captured	2019
Scanner manufacturer/model	ScanPro 3000 /e-ImageData
Scanning software	PowerScan 3000(TM) v5.341
Optical resolution	600 dpi
Compression	LZW
Color settings	bitonal
File types	tiff
Notes	Scanned from microfilm; no editing done.

Derivatives - Access copy

Editing software	Photoshop
Resolution	600 dpi
Color	grayscale
File types	pdf
Notes	Converted from tiff to pdf using Adobe Acrobat Pro DC.