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Dwarfism in Beef Cattle and the Influence of Dwarfism Genes on Physiological Response to Hormone-Induced Stress

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S. E. Curl, J. E. Comfort, J. F. Lasley

INTRODUCTION

The Problem of Dwarfism in Beef Cattle

Intensive research and experimentation have brought gradual advancements in control of hereditary dwarfism, one of the most misunderstood and feared problems of the beef production industry. But many purebred beef cattle breeders are still plagued by the birth of dwarfed animals. The greatest loss to the purebred breeder is through the decreased value of bloodlines in which dwarfs have been produced. Gregory and associates (1953) indicated that the percentage of heterozygous progeny available for breeding replacements (after dwarfs have been removed) may exceed 50 percent in many herds for the following reasons:

1. The frequency of the dwarf gene is high in the breeding cows of many herds.
2. Heterozygous (dwarf-gene carrier) bulls are definitely favored over homozyous (dwarf-free) bulls.

Brachycephalic Dwarfism in Beef Cattle

Several types of hereditary dwarfism have been reported in beef cattle. Many of the dwarf modifications are so rare that they will be discussed only briefly. The brachycephalic, or "snorter," type of dwarfism, first described by Johnson and associates (1950), has become frequent enough in beef cattle to create a relatively serious problem. The usual manifestation, characterized by heavy, labored breathing, is the reason for the individual's nickname, "snorter." Fransen and Andrews (1958a.) reported that the brachycephalic dwarf, small at birth, usually possesses a bulging forehead and some degree of hydrocephalus. Newborn dwarf calves are usually characterized by a protrusion of the eyes accompanied by a glassy stare, a continuous protrusion of the tip of the tongue, unusually short limbs, muscular weakness, and incoordination (Pahnish *et al.,* 1955c.). A relatively high incidence of bloat (Fransen and Andrews, 1958a.; Hafez *et al.,* 1959) and a high rate of mortality (Pahnish *et al.,* 1955b.) have also been reported among "snorter" dwarfs.

Purpose of the Investigation

Purpose of this study was to determine the response of white blood cells and blood glucose in hereditary dwarfs, dwarf-gene carriers, and dwarf-free (pedi-

gree-clean) beef cattle to injections of insulin, adrenalin, and pituitary adrenocorticotrophic hormone (ACTH). Results, it is hoped, will further the knowledge of the physiological causes of dwarfism and help develop more accurate and practical methods of detecting carriers of the dwarf gene.

REVIEW OF LITERATURE

Early Investigations of Bovine Dwarfism

Public interest was greatly aroused by the first report and description of a dwarf calf in the United States (Craft and Orr, 1924). These investigators suggested that the calf's apparent cretinous condition might have been due to an underdevelopment of the thyroid and pituitary glands. The demonstration of such endocrine abnormalities would serve to strengthen the hypothesis that this individual might have been morphologically different from the "snorter" dwarf described by Johnson *et al.* (1950). According to an earlier report (Seligmann, 1904), the first official record of bovine dwarfism was made in 1860.

Downs (1928) reported the birth of an American "Dexter monster" calf, and stated that such an individual occurred as an abortus at any time during gestation in closely inbred herds of the Dexter and Kerry breeds of cattle.

A Texas herd of "duck-legged" Hereford cattle was described by Lush (1930). Investigations revealed that the pituitary glands of these cattle were smaller than normal, but except for this endocrine abnormality and the unusually short legs of these individuals, normal Hereford characteristics were observed. Pahnish and associates (1955c) reported that the "duck-legged" condition of the grade Herefords described by Lush (1930) was traced to other than Hereford breeding and that the short legs were due to a dominant gene.

Mode of Inheritance of Dwarfism

Inheritance in Cattle

Johnson and associates (1950) were the first to present evidence that dwarfism in beef cattle is inherited. These workers reported a single autosomal recessive gene to be the mode of inheritance of the brachycephalic dwarf. These findings have been confirmed and enhanced by the more recent investigations of Gregory *et al.* (1951) and Lush and Hazel (1952). The inheritance of modifications of this type of dwarf will be discussed subsequently. The results of investigations by Arizona workers (Pahnish *et al.,* 1955a) have indicated that dwarfism in beef cattle is attributable to an autosomal recessive gene with complete penetrance. Mead and associates (1942) studied the lactation and body growth of dwarf Jersey cows. Their findings indicated that the dwarf condition might be due to a single autosomal recessive gene.

The recent reports of Julian *et al.* (1959) are in direct disagreement with the studies just discussed. These workers believe that the hypothesis of inheritance, upon which dwarfism tests are based, is in error, and have suggested that the brachycephalic dwarf is not conditioned by a single recessive gene, but that sev-

eral loci are implicated, and that dwarfism is a complex made up of several different phenotypic forms. The complex concept is in agreement with earlier genetic reports (Gregory, 1956; Gregory *et al.,* 1957). An investigation to determine the mode of dwarfism inheritance (Pahnish *et al.,* 1955c) in which 417 calves were produced by carrier parents, 74.4 percent appeared normal and 25.6 percent were dwarfs. If a single recessive gene were involved, this three-to-one ratio would have been expected.

Assuming a simple recessive gene as the mode of inheritance, High and associates (1959) stated that cattle could be of three genotypes as follows:

1. Non-carrier or "clean" individuals (homozygous for the normal allele of the dwarf gene)
2. Carriers (heterozygotes)
3. Dwarfs (homozygous for the recessive dwarf gene)

Gregory (1955) reported that more than one pair of genes is involved in the production of different morphological forms of dwarfs, and suggested a possible relationship between the "comprest" condition and the common type of dwarfism.

The results of investigations by Gregory and Carroll (1956) indicated that Hereford-Angus crossbred (F_1) dwarf hybrids are similar in all diagnostic features to the dwarfs that normally segregate in both parental breeds. On the basis of these findings, these workers postulated that both Hereford and Aberdeen Angus breeds carried the same recessive dwarf gene. Chambers *et al.* (1954) indicated that the genes responsible for dwarfism in "comprest" and conventional Hereford and Angus cattle might be allelic. The more recent findings of Burrus and Priode (1956) are in agreement with these conclusions.

Inheritance in Mice

King (1950) studied the genetic aspects of dwarfing in the house mouse and indicated that this condition was inherited as a simple recessive. Warwick and Lewis (1954) found that the frequency of a presumably recessive gene for a pygmy condition was greatly increased during five generations of selection for small size in a mouse population composed of both inbred and outbred segments.

Inheritance in the Fowl

Louisiana workers (Mayhew and Upp, 1932) studied the pedigree records of 18 dwarf chickens and concluded that the condition was likely due to a simple recessive gene. Recent investigations of sex-linked dwarfism in the fowl (Hutt, 1959) have indicated that a sex-linked recessive gene may reduce body weights of homozygous males as much as 43 percent below normal.

Dwarf Modifications in Beef Cattle

A great many differences in phenotypic expression have been observed among dwarf beef animals. The incidence of one or more of these modifications,

each with its own characteristics and mode of inheritance, has been reported in many breeds of cattle, including the three major beef breeds of the United States (Johnson *et al.,* 1950; Baker *et al.,* 1951; Gregory *et al.,* 1953).

Julian and associates (1959) have recognized at least four subgroups of dwarf types. They are:

1. The brachycephalic or short-headed dwarf, commonly referred to as the "snorter" dwarf.
2. The dolichocephalic or long-headed dwarf.
3. The "intermediate" dwarf, whose features support the concept of these researchers that dwarfism is a complex.
4. The compressed dwarf, usually varying in size from 65 to 90 percent of normal.

California workers (Hart *et al.,* 1947) reported the incidence of calves deformed at birth, apparently as a result of a maternal nutritional deficiency. These individuals were called "acorn calves," and it was concluded that their condition was not hereditary.

Stonaker and Tom (1944) investigated "compact" Shorthorn cattle. The "compact" individual exhibited a shorter head, neck, and body than "standard" Shorthorns. Pahnish and associates (1955c) stated that this condition was apparently due to a dominant gene.

Baker *et al.* (1950) described a herd of "stumpy" Shorthorns. "Stumpy" calves were distinguishable at birth by their curly-haired coats and small switches. Metabolic disturbances were found in all "stumpy" calves. This type of dwarfism was believed to be due to a simple receessive gene.

Baker *et al.* (1951) reported on a group of long-headed Angus dwarfs. From birth to two or three months of age, these individuals exhibited short, wide heads which became longer and narrower with advancing age. These investigators concluded that this condition was probably governed by a single autosomal recessive gene.

Koger and associates (1955) described dwarf Angus cattle with long, pointed noses, but phenotypically different from "long-headed" Angus dwarfs. These animals were called "ant-eater" Angus dwarfs.

Ateliotic, or "Tom Thumb" dwarfism, in which the dwarf is well proportioned but is small in all parts of the skelton, has been discussed by Winchester (1958). This investigator suggests that such a condition results from a deficiency of the growth hormone of the pituitary which influences the growth of the skeleton. Emmerson and Hazel (1956) state that such an individual occurs approximately once in 100,000 births in cattle.

Roubicek *et al.* (1955) reported the occurrence of dwarf types in many breeds, including Aberdeen Angus, African Uganda (Zebu), Ayrshire, Dexter, Guernsey, Hereford, Holstein Friesian, Jaroslav, Jersey, Oplandske (Norway), Shorthorn, Swedish Red and White, and West African Shorthorn.

A recessive type of achondroplasia, described by Punnett (1936) was referred to as the "telemark" type of dwarfism. The heterozygous individuals appeared

normal. The dwarfs, born alive after full term gestation, reportedly died of asphyxia within a few days following birth.

Mohr and Wreidt (1930) reported that a single, recessive, sub-lethal gene when in the homozygous state caused an extremely short neck, thorax, and tail in the Oplandske breed of cattle in Norway. These investigators noted a similarity between the "short-spine" condition and the "amputated" condition which is sometimes exhibited by newborn Holstein-Friesian calves.

A group of West African Shorthorns that were short in stature, humpless, and phenotypically distinct from the Zebu have been reported (Jeffreys, 1953). These animals reportedly bred true genetically.

Skeletal Characteristics of the Bovine Dwarf

The dwarf beef animal is usually distinguishable at a very early age as a result of one or more characteristic types of abnormalities of skeletal structure and development.

Hydrocephalus

Fransen and Andrews (1958b) noted that hydrocephalus of varying severity was usually present in dwarf beef cattle and suggested that this condition, marked by an excessive amount of pressure of the cerebrospinal fluid in the cranial cavity, might result from several causes. These workers added that an increased amount of cerebrospinal fluid was usually present in the bovine dwarf at necropsy.

Sykes and Moore (1942) developed a technique whereby the cerebrospinal fluid pressures of cattle could be accurately measured.

Gilman (1956)stated that congenital hydrocephalus occurs in man, cattle, swine, and various laboratory and domestic animals.

The fluid accumulation in the lateral ventricles of the brain of the dwarf calf was studied by Johnson and associates (1950). Earlier studies (Moore and Sykes, 1940) had suggested that the hydrocephalic condition resulted from a failure of the drainage mechanism of the ventricles of the brain.

Achondroplasia

As a result of investigations involving gross examination of the appendicular skeltal material from 32 phenotypically normal and 32 short-headed dwarf Herefords, Tyler and associates (1957) concluded that hypoplastic achondroplasia was the dwarfing process. These workers found that dwarfing resulted in the shortening of only the diaphyses of the bones of the appendicular skeleton, and indicated that this feature was characteristic of achondroplasia.

Julian *et al.* (1957) found that premature closure of the spheno-occipital synchrondrosis occurred in the brachycephalic dwarf, and concluded that this observation was indicative of the involvement of achondroplasia in the dwarfing processes responsible for "snorter" dwarfism.

The results of investigations by Tyler and associates (1959) indicate that a correlation exists between two intracranial projections and achondroplastic

dwarfism. These researchers observed that these projections were more prominent and frequent in the skulls of animals which manifested achondroplasia.

General Abnormalities

Many investigators, including Mohr and Wreidt (1930), Stonaker and Tom (1944), and Baker *et al.* (1951), have described various types of bovine dwarfs, with emphasis on their characteristic skeletal abnormalities.

Hutt (1934) postulated that dwarfs were not likely to occur except in the progeny of short-legged, brachycephalic individuals.

The development of several methods of detection of carriers of the gene for dwarfism has been based upon skeletal differences existing between normal, carrier, and dwarf beef cattle. Foremost among these are the x-ray method of detection (Hazel and Emmerson, 1953) and the head profile measurement (Gregory and Brown, 1952), both of which will be discussed subsequently.

The studies of Buchanan and associates (1956) indicated that certain of the skeletal abnormalities found in dwarf cattle appear together and that at least two of the syndromes may be independent.

Endocrine Aspects of Dwarfism

General Endocrinology of Dwarf Animals

Russell (1938) postulated that the hereditary dwarf was deficient in anterior pituitary and adrenocortical secretions. An earlier report (Lush, 1930) indicated that the pituitary glands of "duck-legged" cattle were smaller than normal.

Smith and MacDowell (1930) reported the cause for dwarfism in a strain of dwarf mice to be an inherited anterior pituitary deficiency. The results of a later investigation of the physiology of dwarfism in rats (Lambert and Sciuchetti, 1935) indicated that the cause of the dwarf condition was not of pituitary origin.

Bulger and Barr (1936) investigated the effects of pituitary insufficiency and administered various pituitary hormones to dwarf humans in an attempt to elucidate the specific effects of endocrine glands on chemical reactions within the body.

Fransen and Andrews (1954) have shown through the histological study of various organs that some dwarf cattle possess cystic pituitary and adrenal glands. This investigation suggests a possible abnormality of these glands in the bovine dwarf.

Marlowe and Chambers (1954) demonstrated that pituitaries of dwarf cattle revealed normal potency for thyrotrophic and adrenocorticotrophic hormones, and increased potency for gonadotrophic and growth hormones.

In a histochemical study of dwarf pituitaries. Jubb and McEntee (1955) observed an increase in the granulation and size of the acidophiles with no abnormalities in the beta or delta cells, thereby confirming the earlier reports of Fransen and Andrews (1954) and Marlowe and Chambers (1954).

Researchers at the Missouri Agricultural Experiment Station (Crenshaw *et al.,* 1957) concluded that dwarfism in beef cattle was not due to dysfunction of the pituitary-thyroid gland axis. This study failed to confirm the results of earlier investigations (Carroll *et al.,* 1951) which indicated that dwarf cattle were grossly deficient in the thyrotrophic hormone.

Since dwarfs, like hypophysectomized animals, are hypersensitive to the hypoglycemic effects of insulin, Foley and associates (1960) have recently concluded that the genes conditioning "snorter" dwarfism are in some way concerned with the anterior pituitary.

Cornelius and associates (1956) found that serum cholesterol and protein-bound iodine levels of "snorter" dwarf beef cattle were within normal limits, indicating such individuals are not primary thyroid cretins.

Andrews and Fransen (1958) observed a slight increase in rate of gain and a slight but questionable increase in height at withers of dwarf beef cattle following the administration of thyroactive iodinated protein, testosterone propionate, and diethyl stilbestrol, singly or in combination. This investigation did not provide evidence, however, that endocrine therapy can correct the dwarfism syndrome.

Foley *et al.* (1960) pointed out that dwarfs generally possess normal amounts of hormones in the endocrine glands, but added that this fact did not necessarily mean that endocrine function in the dwarf is normal.

Endocrine Regulation of Blood Sugar Levels

Insulin sensitivity. A considerable number of investigations have been concerned with elucidation of the hormonal regulation of the rise and fall of blood glucose levels within the body.

Scott and Dotti (1932) studied the lowering of blood sugar levels following insulin administration to subjects. They found that it was not in direct proportion to the amount of insulin given. These findings were confirmed by Heinbecker *et al.,* 1937. More recent studies with sheep (Reid, 1951) have shown that after a certain quantity of insulin has been administered, larger dosages of insulin do not cause a greater fall in the level of blood glucose. These findings are in agreement with results obtained by Brown and associates (1936) during a similar investigation of blood sugar levels in dairy cattle. Reid (1950) stated that an injection of one unit of insulin per kilogram of body weight is sufficient to saturate the tissue involved.

Dunn and associates (1959) have recently shown that physiologically secreted insulin, like exogenous insulin, lowers blood glucose levels almost entirely by increasing the utilization of glucose. This report is in agreement with the earlier reports of Wall *et al.* (1957), who also indicated that blood sugar levels were returned to normal by a sudden increase in the release of glucose from the liver in response to the hypoglycemia. Foley *et al.* (1960) stated that once blood sugar levels are lowered by insulin, the hormones released by the anterior pituitary and the adrenal cortex are responsible for restoring them to normal.

Engel (1953) reported that insulin exerts an influence at the onset of carbohydrate metabolism. He stated that both pituitary growth hormone and adrenal steroids have a sparing effect on glucose utilization, as reflected in glycogen accumulation.

Results obtained from the studies of Reichard *et al.* (1958), whose investigations involved the administration of C-14 labelled glucose to dogs, indicate that hepatic glucose production ceases during hyperglycemia. These results are in disagreement with those obtained during a similar isotope dilution investigation conducted by Steele and Marks (1958).

Russell (1938) found that hypophysectomized or adrenalectomized animals exhibit a greatly increased sensitivity to the hypoglycemic effect of insulin, compared to normal, intact animals.

In 1953, Mirand and Osburn reported their study of insulin sensitivity in hereditary hypopituitary dwarf mice. Following subcutaneous injections of insulin, these researchers found that dwarfs exhibited a very high degree of sensitivity to the hormone, compared to normal mice. It was noted that only 3 percent of the insulin dosage given to normal mice (two units per kilogram) was required to cause convulsive effects in the dwarfs. The recent investigations of Argyris (1959) indicate that insulin resistance in the KL strain of mice, which tolerate 200 to 500 units of insulin without going into convulsions compared to only 50 units for control (BUB) mice, is not due to an increased insulase activity. Rather, it is due to the ability of the animal to maintain a blood sugar level slightly above the compulsive level for a long period after insulin administration.

Spirtos and Halmi (1959) found that rats with electrolytic lesions in the hypothalmus displayed a greater fall in blood glucose level in response to insulin than rats bearing lesions in other parts on the brain.

Five out of ten rats bearing hypothalmic lesions showed decreased insulin sensitivity after treatment with cortisone. Eight rats with such lesions were treated with somatotrophin (STH) and all responded with improved insulin tolerance. Seventy percent of the rats with hypothalmic damage and enhanced insulin sensitivity had regressive changes in the pituitary acidophiles. These investigators concluded that increased insulin sensitivity in rats with hypothalmic damage might be due to decreased secretion of STH. The findings of these researchers are in agreement with results obtained from similar previous investigations (Ingram and Barris, 1935). A report by Spirtos and Halmi (1956) indicated that insulin sensitivity in rats was probably determined by both corticoids and STH.

Effects of adrenal hormones and pituitary ACTH

Loosli and associates (1951) observed no significant alteration in blood sugar level of mice as a result of daily administration of four milligrams of ACTH.

In a subsequent study (Mirand and Osburn, 1953), it was found that pituitary adrenocorticotrophic hormone (ACTH), adrenocortical extract (ACE), and cortisone displayed the ability to increase blood glucose levels and to reduce hypoglycemic convulsions, and thus act as anti-insulin agents. Desoxycorticosterone acetate and testosterone propionate were found to exhibit no anti-insulin action. These workers concluded that anti-insulin action may result from:

1. A decreased rate of inactivation of insulin by the blood and tissues.
2. Inadequate counter-regulatory responses (glycogenolysis and gluconeogenesis) to hypoglycemia in the liver.
3. Hypophyseal and adrenocortical deficiencies, perhaps rendering the dwarf incapable of producing anti-insulin agents, such as ACTH, under the stress conditions of fasting.

Dukes (1947) stated that the administration of adrenalin to domestic animals results in both hyperglycemia and glucosuria.

Significance of the endocrine-blood sugar relationship to the study of bovine dwarfism. The endocrine regulation of blood sugar levels has proven to be of great significance and value to investigators seeking knowledge of the physiology of dwarfism in beef cattle. Results obtained by Heidenrich *et al.* (1955) indicated that normal and dwarf cattle were significantly different in their ability to restore their blood sugar levels to normal following stress. Foley and associates (1956) reported that blood sugar levels were similar in dwarf-free and carrier cattle, but that changes due to insulin shock were different in the two genotypes. Following insulin administration, it was found that the blood sugar levels of dwarf cattle dropped to low levels more rapidly and returned to normal at a slower rate, compared with individuals free of the dwarf gene. Foley *et al.* (1960) observed highly significant differences (P less than .01) between normal and dwarf genotypes in blood sugar changes in response to varied levels of insulin dosage, and noted a significant interaction (P less than .05) between insulin dosage level and genotype.

Influence of Stress Factors on Leucocyte Kinds and Numbers in the Blood Stream

General effects of stress conditions. In 1937, Harlow and Selye defined an "alarm reaction" as a "characteristic symptom complex which appears when the general condition of an animal is seriously injured by acute, non-specific damaging influences." Today, we commonly refer to this type of reaction as "stress." Harlow and Selye (1937) found that total white cell counts increased during "alarm reactions," and concluded that this increase in numbers of white cells was primarily a result of neutrophilic leucocytosis.

Influence of adrenalin injection. Harlow and Selye (1937) reported leucocytosis with relative and often absolute lymphopenia in rats and mice injected with adrenalin or exposed to cold or surgical shock.

The results of an earlier investigation (Martin, 1932) demonstrated that there was an increased leucocyte count in the blood of humans following hypo-

dermic injections of adrenalin. Lymphocytes were reported to be the major portion of the leucocytes observed.

Following the infusion of epinephrine into the veins of human subjects, Bierman and associates (1952) observed an initial arterial leucocytosis and thrombocytosis within 30 to 60 seconds. They concluded this was primarily from the pulmonary circulation. Lucia *et al.* (1937) observed an increase in leucocytes and erythrocytes in humans following epinephrine injection. White and associates (1950) found that the changes in the leucocyte count in humans produced by subcutaneous injections of epinephrine were similar to, but not identical with, those resulting from the administration of adrenocortical hormone or adrenocorticotrophic hormone (ACTH).

Effects of adrenalectomy. During an early investigation of leucocyte changes following the removal of the adrenal glands of cats, Zwemer and Lyons (1928) found that adrenalectomy brought about a decrease in the percent of polymorphonuclear neutrophils and an increase in small lymphocytes. Corey and Britton (1932) also reported a lymphocytosis following adrenalectomy in cats, but the results of more recent investigations (Lewis, 1941; Valentine *et al.*, 1948) are in disagreement with these findings. Valentine *et al.* (1948) reported that only slight alterations from the accepted normal range of lymphocyte values occur in the adrenal-insufficient individual. Hungerford and associates (1952) observed no significant change in numbers of thoracic duct lymphocytes following administration of pituitary adrenocorticotrophic hormone (ACTH) to adrenalectomized mice. These results are in agreement with the earlier findings of Dougherty and White (1945).

Frank and Dougherty (1954) found that the lymphocytes of intact mice were smaller than those of stressed, adrenalectomized mice.

Influence of pituitary ACTH and hormones of the adrenal cortex. Dougherty and White (1944) presented evidence that the pituitary adrenotrophic hormone (ACTH) is a factor in the regulation of numbers of blood lymphocytes. Single injections of ACTH produced an absolute lymphopenia and an increase in polymorphonuclear leucocytes within a few hours in rats, mice, and rabbits. Adrenal cortical extract, adrenal cortical steroids in oil, and corticosterone produced a lymphonemia in intact and adrenalectomized animals. This effect was not observed following the administration of desoxycorticosterone. These investigators concluded that the action of the pituitary hormone on lymphoid tissue was mediated by the adrenal cortex. Subsequent research by these workers confirmed this conclusion (Dougherty and White, 1945).

In 1940, Reinhardt and Holmes studied the relationship existing between the adrenal cortex and the thymus gland. Carrey and Bryan (1935) had stated earlier that increased lymph flow was the chief agency in rapid increments of blood lymphocytes. Through the administration of ACTH to rats, Reinhardt and Li (1945) showed that stimulation of the adrenal cortex produces a rapid and persisting decline in lymphocyte numbers entering the blood stream. They

concluded that this probably resulted from a decreased outpouring of these cells from the thymus and lymph nodes.

Crafts (1941) reported that the number of circulating blood lymphocytes gradually increased following removal of the pituitary gland, which was indicative of the influence of certain hypophyseal secretions on numbers of circulating blood lymphocytes. Hungerford *et al.* (1952) observed a highly significant reduction (P less than .01) in the number of thoracic duct lymphocytes following the administration of an ACTH mixture to normal mice. These researchers found that administrations of growth hormone (beef anterior pituitary), pitressin, pitocin, adrenal cortex extract, cortisone acetate, and desoxycorticosterone glucoside did not significantly change the levels of thoracic duct lymphocytes.

A great many workers, in addition to the ones previously mentioned, have reported on the influence of pituitary adrenocorticotrophic hormone on elements of the blood. Hills *et al.* (1948) found that the administration of a single intramuscular injection of 25 milligrams of ACTH to human subjects with unimpaired adrenal function resulted in an increase of circulating neutrophils and decreased numbers of circulating lymphocytes and eosinophils. A decreased lymphocyte count in cats has been observed by British workers (Yoffey *et al.*, 1946b) following the administration of 300 units of adrenocorticotrophic hormone.

Similar results have been obtained from investigations involving rats (Yoffey and Baxter, 1946a). Dougherty and White (1943a) found that the administration of ACTH to mice resulted in leucopenia, lymphopenia, and neutrophilia. Decreased numbers of circulating lymphocytes in mice following ACTH administration have also been observed by Dougherty and White (1943b) and Kass and associates (1951). Loosli *et al.* (1951) observed an increase in the percentage of polymorphonuclear leucocytes with a corresponding decrease in lymphocytes in mice receiving 1.0 or 4.0 milligrams of ACTH daily.

Quittner and associates (1951) administered large doses of cortisone to mice and observed a decrease in circulating lymphocyte numbers.

Saunders and Adams (1950) found that a reduction in absolute numbers of circulating lymphocytes and eosinophils occurred following intravenous dosages of 25 cubic centimeters of aqueous adrenal cortex extract (ACE) to normal humans. The results of a similar study with rats (Yoffey and Baxter, 1946a) indicated a reduction in lymphocyte numbers.

Participation of the central nervous system

Porter (1953) studied the role of the central nervous system as a participator in the pituitary-adrenal response to stress stimuli. The results of investigations during the preceding year (Porter, 1952) indicated that stress-induced changes in the electrical activity of the posterior hypothalmus were correlated with a decrease in numbers of circulating eosinophils.

Relation of the influence of stress on leucocytes to the study of dwarfism. The relative significance of stress-induced leucocyte changes to the study of the physiol-

ogy of dwarfism has been substantiated (Foley *et al.,* 1956; Downs and Benson, 1959). Downs and Benson (1959) have recently demonstrated the influence of stress on numbers of circulating leucocytes in normal and dwarf mice. Lasley (1958) reported that insulin injections submitted animals to stress, facilitating the measurement of adrenal gland activity, as reflected in subsequent changes in kinds and numbers of circulating leucocytes. Different genotypes, with respect to dwarfism, exhibit different degrees of insulin sensitivity (Mirand and Osburn, 1953); they have been found to respond to insulin administration at different rates and in distinct manners (Foley *et al.,* 1956, 1960).

Physiological Differences Existing Between Dwarf, Dwarf-Carrier, and Pedigree-Clean Beef Cattle and Methods of Heterozygote Detection

As a result of intensive investigations conducted by researchers throughout the country, it has been shown that the three genotypes, with respect to dwarfism, each possess or display some characteristic feature or features which distinguish them from other members of the population. Several investigators have developed techniques by which tests for carriers of the dwarf gene, or heterozygotes, can be made. Others have merely shown that certain physiological differences do exist, and in doing so, have set the stage for further research and development of effective methods of dwarf-carrier detection.

Gregory *et al.* (1952) and Lush and Hazel (1952) reported that attempts to identify carrier animals by visual inspection had proven unsuccessful.

Determination of Genotype by Progeny Testing

Julian and associates (1959) recently stated that a high percentage of the progeny tests based upon the belief that one autosomal recessive gene conditions "snorter" dwarfism prove inaccurate after they have indicated that a bull is dwarf-free. This report adds strength to these workers' hypothesis that dwarfism is a complex, and is in agreement with previous reports. (Gregory *et al.,* 1957). Earlier reports (Lush and Hazel, 1952; United States Department of Agriculture, 1958) indicate that if a bull is mated to as many as 16 cows known to be carriers of the dwarf gene and sires all normal calves, it is almost certain that he is a non-carrier of the dwarf gene. Emmerson and Hazel (1956) have postulated that the best method of testing heifers is to breed them to dwarf bulls. Although progeny testing is expensive and time-consuming, it has been reasonably successful as a method of carrier detection.

Investigations of Skeletal Differences

Gregory and Brown (1952) developed an instrument called a profilometer. With it, they reported it was possible to distinguish between homozygous normal and heterozygous (dwarf-gene carrier) individuals by measurement from three diagnostic points on the head profile. These workers showed that the profilometer accurately reproduced the profile or contour of the bovine head.

Iowa workers (Hazel and Emmerson, 1953) found that abnormalities of the lumber vertebrae were present in the "snorter" dwarf, and developed the x-ray technique for identification of carriers of the dwarf gene. Hazel and Emmerson (1956) reported that the thoracolumbar spine of the one to ten-day old calf, when radiographed, showed evidence of longitudinal compression in the dwarf and dwarf-gene carrier, but not in the individual free of the dwarf gene. Further investigation and evaluation of radiographic techniques as methods of heterozygote detection have recently been reported by High and associates (1959). These researchers pointed out that a bull's dwarfism status could be predicted after a study of radiographs of his progeny, but they did not recommend the x-ray method as a basis for identification of individual animals with respect to genotype for dwarfism.

Through the use of an Ayer spinal fluid manometer, Dollahon *et al.* (1959b) found that cerebrospinal fluid pressures at the atlas-axis joint in dwarf-carrier cattle were significantly lower than those of homozygous normal cattle at the same joint. The difference between similar readings taken in the lumbar region was not significant, as shown in Table 1.

TABLE 1-MEAN CEREBROSPINAL FLUID PRESSURES, IN MILLIMETERS, OBTAINED FROM DWARF-CARRIER AND PEDIGREE-CLEAN COWS

Genotype of Cows	Number of Animals	Atlas-Axis Joint	Lumbar Region
Pedigree-Clean	19	279.0 ± 34.5	229.0 ± 40.6
Dwarf-Carrier	41	238.0 ± 30.0	194.0 ± 42.4

At the conclusion of a similar investigation, Fransen and Andrews (1958b) reported that the measurement of cerebrospinal fluid pressures was probably not an effective means of heterozygote detection.

Physiological Differences in Response to Stress Stimuli

Investigations conducted at the Missouri Agricultural Experiment Station have indicated that a physiological distinction exists between normal and dwarf beef cattle with regard to alteration of the blood glucose level in response to stress conditions (Heidenrich *et al.,* 1955; Foley *et al.,* 1956, 1960). However, workers at the Missouri station have not suggested blood sugar changes as a means of detecting dwarf-carrier cattle. At the conclusion of a recent investigation, Texas researchers (Deyoe *et al.,* 1959) also indicated that these physiological differences could not be considered as a basis for carrier detection until verified by further experimentation.

Lasley (1958) reported that the dwarf-carrier detection test developed at the Missouri station was based on the ability of dwarf-free and dwarf-carrier cattle to respond to insulin injections. The test measured changes in the total cell counts, including cell fragments, and changes in the proportions of kinds of white blood cells at one and two hours following insulin injections. This investigator stated that carriers were usually slower to respond to insulin than pedigree-clean in-

dividuals. The recent investigations of Downs and Benson (1959) have enhanced the study of leucocyte response to non-specific stress conditions.

Measurement of Differences in Spermatazoa Constituents

In an investigation of certain cytochemical and cytological aspects of bovine dwarfism, Leuchtenberger and associates (1956) found that the desoxyribose-nucleic acid (DNA) content of individual spermatogenic cells was subject to a considerably greater variation in dwarf bulls than in normal animals. In one dwarf and two suspected carriers of dwarfism, these researchers found the mean amount of DNA per spermatogenic cell nucleus to be markedly deficient, compared with corresponding cells of normal bulls. Chromosomal analysis showed that the variation in DNA values in the dwarfs tested was not due to alteration in chromosome number.

Measurement of Differences in Blood Constituents

Fransen and Andrews (1954) reported that the formed elements of the blood were similar in dwarf and normal cattle. More recent investigations of the blood of "snorter" dwarfs (Cornelius *et al.,* 1956) revealed that serum proteins, calcium, magnesium, phosphorus, protein-bound iodine, and serum cholesterol were all within normal limits. In this study, which involved 38 Hereford and Angus dwarfs ranging in age from six days to 14 months, hematological values appeared normal except for the differential count, where the differences between normal and dwarf lymphocytes and neutrophils were found to be highly significant with a random probability of less than .001 in both cases. It was pointed out that, since total white cell counts do not vary from the normal, differences in the differential counts were not relative changes.

Fransen and Andrews (1958a) showed that the blood plasma cholesterol levels of dwarf beef cattle were significantly lower than those of normal beef cattle. They found that dwarf bulls possessed higher cholesterol levels than dwarf heifers (Table 2). These workers stated that seasonal differences in blood plasma cholesterol levels of dwarf cattle were not significant.

Results of recent investigations of Dollahon and associates (1959a) indicated that the erythrocytes obtained from dwarf-carrier animals were larger than those obtained from non-carrier animals, as indicated by a significantly higher (P less than .05) mean corpuscular volume in the dwarf-carrier group. The RNA content of the plasma protein was significantly higher (P less than .05) in the dwarf-carrier group, and the DNA content of the plasma protein was higher in the dwarf-carrier group. This difference was highly significant (P less than .01). Although each of these measurements exhibited a significant or highly significant difference, these investigators stated that the feasibility of using one or more of them as methods of heterozygote detection was doubtful.

TABLE 2-BLOOD PLASMA CHOLESTEROL LEVELS OF DWARF AND PEDIGREE-CLEAN BEEF CATTLE

		Number of Animals	Number of Determinations	Plasma Cholesterol Levels Average (mgs./100 mls.)	Plasma Cholesterol Levels Range (mgs./100 mls.)
Normal	Steers	18	26	105.3 ± 3.1	102.8 - 107.8
	Females	24	45	100.2 ± 2.7	96.7 - 103.7
Dwarf	Less than 180 days old	33	87	83.0 ± 5.2	76.2 - 90.8
	More than 180 days old	20	88	79.9 ± 4.6	66.8 - 94.7
	Bulls	22	81	89.4 ± 5.4*	74.4 -105.3
	Heifers	24	94	73.5 ± 4.5	64.0 - 84.5
All Normals		42	71	102.5 ± 2.0**	99.4 -105.5
All Dwarfs		46	175	81.1 ± 3.6	68.9 - 94.4

*Significantly higher than dwarf heifers (Probability of chance occurrence less than .05)

**Significantly higher than all dwarf animals (Probability of chance occurence less than .01)

MATERIALS AND METHODS

Experimental Animals

In this study, non-physiological dosages of insulin, adrenalin, and pituitary adrenocorticotrophic hormone (ACTH) were administered to each of 22 Hereford beef animals. These included 5 dwarfs, 7 pedigree-clean cows, and 10 mature cows which were known to be carriers of the dwarf gene. Two of the five dwarfs were bulls. Four of the dwarfs were considered mature, while the other was about a year old.

Data from the dwarf and dwarf-carrier animals were collected from the University of Missouri experimental herd maintained near Columbia. Data from the pedigree-clean animals were obtained from the herd of a cooperative breeder, located approximately 100 miles from Columbia. All data were obtained during the summer months. The animals used in the study were maintained on pasture consisting of bluegrass and annual grasses native to the area. The pastures were in good condition.

Procedures in the Field

The hormones were administered to each of the 22 experimental animals. A 10 cubic centimeter syringe and a 16 gauge hypodermic needle were used for the hormone injections.

Regular zinc insulin was injected into the jugular vein of the neck of each animal at a dosage level of 0.8 unit of insulin per kilogram of body weight.

Four blood samples were collected from the jugular vein of each animal at different time intervals before and after insulin injection. Using a 16 gauge hypodermic needle, the blood samples were collected into tubes containing two potassium oxalate tablets which provided an anti-coagulant effect. The first blood sample was collected immediately before insulin injection. One hour after administration of the hormone, a second blood sample was collected. A third sample was collected two hours after insulin administration, and eight hours after injection, a fourth blood sample was collected.

The following measurements were made on each blood sample: blood glucose content, total white cell count (in 0.7 percent hydrochloric acid solution), and differential white cell count.

Adrenalin was administered to each of the experimental animals intravenously, at the level of 0.4 cubic centimeter of adrenalin (diluted 1 to 10,000) per hundred pounds of body weight. The techniques used in the collection of the blood samples were the same as those used during the insulin tolerance test, except that, in this case, five blood samples were collected and at different time intervals than during the insulin test.

Blood samples were collected from each individual immediately before adrenalin administration, and at 0.5 hours (because of the rapid effect of adrenalin), 1 hours, 2 hours, and 4 hours following injection of the hormone.

The determinations that were made on blood samples during the insulin tolerance test were also made on the samples collected before and after adrenalin administration.

In a test to determine the tolerance of the experimental animals to nonphysiological dosages of pituitary adrenocorticotrophic hormone (ACTH), 80 Upjohn units of ACTH per animal (regardless of body weight) were administered interamuscularly. The techniques used in blood collection were the same as those used in the hormone tolerance tests cited previously.

Four blood samples were collected in the following manner. The first blood sample was collected just prior to ACTH injection, and succeeding samples were taken at 1 hour, 4 hours, and 8 hours following hormone administration.

The same measurements that were involved in the hormone tolerance tests, with the exception of the blood glucose determination, were made on each blood sample collected before and after ACTH administration. (After testing several samples of blood collected at varying intervals following ACTH administration, it was concluded that the influence of ACTH on the level of blood sugar was not great enough to be of particular significance to this study.)

Blood samples were collected at different time intervals because of the difference in the rapidity of optimal influence of each hormone on the various blood elements.

After the collection of blood from each animal, samples were prepared immediately for blood sugar determinations by the modified Folin-Wu method. Thirty-five milliliters of distilled water and five milliliters of well-mixed oxalate blood were mixed in an Erlenmeyer flask by shaking until complete hemolysis

was observed. Five milliliters of a 10 percent sodium tungstate solution were then added to the flask and mixed with the other ingredients. The flask was shaken continuously while five milliliters of 0.667 normal sulfuric acid were added. The remainder of the procedure for glucose determination was undertaken in the laboratory.

Within 15 minutes following the collection of each blood sample, dry slides (blood smear) were made in duplicate in the following manner. A precleaned glass slide was placed on a flat surface; a small drop of blood was placed on the slide. The edge of the second slide was placed on the first slide at an angle of approximately 30 degrees and was slowly drawn in contact with the drop of blood. The second slide, used as a spreader, was then pushed slowly across the first slide. This resulted in a thin and evenly-distributed film of blood. The blood smear was allowed to dry before it was stained.

Procedures in the Laboratory

The protein-free solution was filtered through Whatman Number 41 filter paper for the blood glucose determination. At this point, 1 cubic centimeter of the filtrate and 1 cubic centimeter of distilled water were pipetted into a graduated Folin-Wu blood sugar tube; each tube was heated in boiling water for 6 minutes following the addition of 2 cubic centimeters of alkaline copper tartrate solution.

The blood sugar tubes were transferred to a running water bath upon completion of the six-minute heating period, and were cooled for 10 minutes. At the end of the cooling period, 2 cubic centimeters of phosphomolybdic acid were pipetted into each tube and allowed to equilibrate with its contents for 2 minutes. Each tube was then filled to the 25 cubic centimeters mark with distilled water. After the contents of each tube had been adequately mixed, it was compared with a distilled water standard, or "blank," in the colorimeter, and the blood sugar content was determined.

In making the total white blood cell count, the first step of the procedure was dilution of the blood in a 0.7 percent solution of hydrochloric acid. Such a solution was prepared by adding 9.6 cubic centimeters of 37 percent hydrochloric acid to 490.4 cubic centimeters of distilled water.

A white cell pipette was used to make the dilution. The stem was marked in portions of 0.0 and 1.0, with the mixing chamber of bulb-like portion marked from 1.0 to 11.0. The blood was drawn to the 0.5 mark on the stem, and the mixture of blood and diluting fluid was drawn to the 11.0 mark. This resulted in a dilution of one part of blood in 20 parts of diluting fluid.

The blood and diluting fiuid were mixed by shaking the pipette for 2 minutes. The diluent fluid in the stem of the pipette was discarded after the solution was well mixed, since it did not play a part in the dilution of the blood.

Charging of the counting chamber was accomplished by allowing a portion of the solution in the pipette stem to move by capillary action under the cover

glass of the counting chamber. If the chamber was over-filled or flooded, it was wiped clean and recharged.

The white blood cells were counted under the low power objective (100 x magnification) on the red blood cell side of the hemocytometer. The cells counted were in the four corner groups of four sets of 16 squares each.

The formula for determining the number of white blood cells per cubic millimeter of undiluted blood is:

$$\frac{\text{cells counted x 20 (dilution) x 10 (depth)}}{\text{number of square millimeters counted (4)}}$$

Immediately after washing, the counting chamber was cleaned with water and acetone and dried with a soft lint-free cloth. Distilled water was aspirated through the pipette with a suction apparatus until the pipette was entirely free of blood. A small amount of acetone was then aspirated through the pipette for drying purposes. The bead in the bulb-like portion of the pipette moved freely upon agitation when the pipette had been cleaned and dried properly.

Modified Wright's stain was used to stain the blood smears that had been prepared in the field for white cell differential counts. Given below is the procedure used to make 1 liter of modified Wright's stain:

1. To 30.0 milliliters of glycerin, add 2.0 grams of powdered Wright's stain and 0.3 grams of powdered Giemsa's stain. Grind these ingredients to a light paste in a mortar.
2. In 970 milliliters of methyl alcohol, dissolve 1.6 grams of monobasic potassium phosphate and 3.2 grams of dibasic sodium phosphate. (buffer solution).
3. Add the buffer solution to the stain-glycerine paste and agitate the combination for 20 minutes on a shaker machine.
4. Filter the stain after allowing it to stand for 24 hours. The stain is then ready for use.

After 10 drops of modified Wright's stain had been placed on a slide, the stain was immediately diluted with 20 drops of distilled water. Optimal results were obtained when the stain was allowed to remain on the slide for 15 seconds. At the end of the staining time, the stain was washed from the slides by flooding them with distilled water for 15 seconds. The slides were than placed on a drying rack to dry.

The differential white cell counts were made under the high power objective (430 x magnification) and each type of white cell observed was recorded as the slide was surveyed. Two hundred white cells were counted on each of the two slides for a total count of 400 cells on each blood sample.

RESULTS AND DISCUSSION

Animals are known to possess many homeostatic mechanisms which keep various elements in the blood stream in proper balance. An attempt was made

to determine if there was a flaw in the homeostatic mechanisms of dwarf and dwarf-gene carrier cattle.

Three hormones were selected as the stressing agents:

(1) *Insulin.* Insulin shock, induced by the administration of nonphysiological dosages of the pancreatic hormone, is believed to stimulate an increased secretion of ACTH by the hypophysis. The pituitary hormone, in turn, stimulates the adrenal cortex, causing it to release glucocorticoids. These substances act as deaminization agents and break down amino acids to form a carbohydrate residue which goes to the liver and subsequently enters the blood stream as blood glucose.

(2) *Adrenalin.* When an animal is subjected to stress conditions, the hormone, epinephrine, or adrenalin, is released by the medulla of the adrenal gland. This hormone is often called the emergency hormone because it is believed to cause a shifting of blood to the skeletal muscles to enable the body to cope with emergencies, and is thought to mediate an increased secretion of ACTH from the anterior lobe of the pituitary gland.

(3) *ACTH.* This hormone was administered to investigate the possibility of any defects in secretion of ACTH by the anterior lobe of the hypophysis.

Lasley (1958) reported that changes in kinds and numbers of circulating leucocytes are attributable to increased activity of the adrenal gland in response to stress stimuli.

As noted previously, 22 experimental animals, including 5 dwarfs, 7 pedigree-clean cows, and 10 dwarf-carrier cows were used in this investigation. Since interactions could not be determined by standard analysis of variance procedures when using unequal subclass numbers, the data obtained were divided into two groups, referred to in the analyses of variance as Sample I and Sample II.

Sample I was composed of data obtained from 5 dwarfs, 5 pedigree-clean cows selected at random, and 5 dwarf-carrier cows chosen in the same manner. For Sample II, data from the 5 dwarfs were repeated in the calculations, and data from the 5 remaining dwarf-carrier cows were used. Since only two pedigree-clean cows remained, three of the individuals used in Sample I were selected at random and repeated in Sample II, thus providing five individual responses for each genotype in each of the two samples.

Dividing the data into two groups, made it more suitable for standard analysis of variance procedures. If values indicating significant differences were observed in one sample and not in the other, differences were regarded as non-conclusive. Such differences will be referred to in the discussion of the analysis of variance as non-significant differences.

Significant differences between cattle of the three genotypes before and after hormone injection are illustrated in Figures 1 through 10, and means and standard deviations for each of the 23 problems analyzed are given in Tables 3 through 25. The corresponding analyses of variance, which were used to determine the significance or non-significance of differences observed in the study,

will be placed in the appendix portion of the manuscript and presented in Tables 3A through 25A.

Influence of Insulin on Various Blood Components in Dwarf, Dwarf-Carrier, and Pedigree-Clean Beef Cattle

Percentages of Lymphocytes

Table 3 shows percentages of lymphocytes in the blood of dwarf, dwarf-carrier, and pedigree-clean beef cattle before and after the administration of in-

TABLE 3-MEANS AND STANDARD DEVIATIONS FOR PERCENTAGES OF LYMPHOCYTES IN THE BLOOD OF DWARF, DWARF-CARRIER, AND PEDIGREE CLEAN BEEF CATTLE AT DIFFERENT INTERVALS FOLLOWING INTRAVENOUS INJECTIONS OF 0.8 UNITS OF INSULIN PER KILOGRAM OF BODY WEIGHT

Time of	Dwarf Mean ± Standard Deviation	Dwarf-Carrier Mean ± Standard Deviation	Pedigree-Clean Mean ± Standard Deviation
0.0 hour	63.76 ± 6.25	56.12 ± 8.38	51.70 ± 8.95
1.0 hour	58.24 ± 6.30	54.61 ±10.39	49.87 ± 9.36
2.0 hours	58.42 ± 9.74	52.00 ±10.65	45.33 ± 8.71
8.0 hours	61.44 ± 6.86	46.57 ±11.67	46.82 ±12.90

sulin. The percentage of lymphocytes before insulin injection was highest in the blood of the dwarf cattle, with a mean of 63.76 percent. The blood of the dwarf-carrier cattle was intermediate in this respect, averaging 56.12 percent lymphocytes, while the blood of the pedigree-clean animals averaged 51.70 percent.

Interestingly, a gradual and persisting decline in the percentage of lymphocytes occurred in the blood of the dwarf-carrier cattle throughout the period of blood sample collection following the administration of insulin. In both pedigree-clean and dwarf cattle, the percentage of lymphocytes ceased to decline and began to increase prior to the eighth hour after insulin shock. The physiological differences are illustrated in Figure 1.

Decreased proportions of circulating lymphocytes in response to stress stimuli have also been observed in rats (Harlow and Selye, 1937) and in mice (Harlow and Selye, 1937; Dougherty and White, 1943a; Loosli *et al.,* 1951).

As shown in Table 3A, highly significant differences (P less than .01) were found among cattle of the three genotypes with regard to percentage of circulating lymphocytes before and after insulin injection. The genotype x time interval interaction and the analysis of variance between hours, were not significant. The non-significant genotype x time interval interaction indicates that at the different time intervals, the reaction of genotype to insulin injection was not significantly different from that of the other genotypes.

Percentages of Neutrophils

Cattle of the three genotypes did not differ significantly in percentage of circulating neutrophils before and after insulin injection (Table 4A). Neither

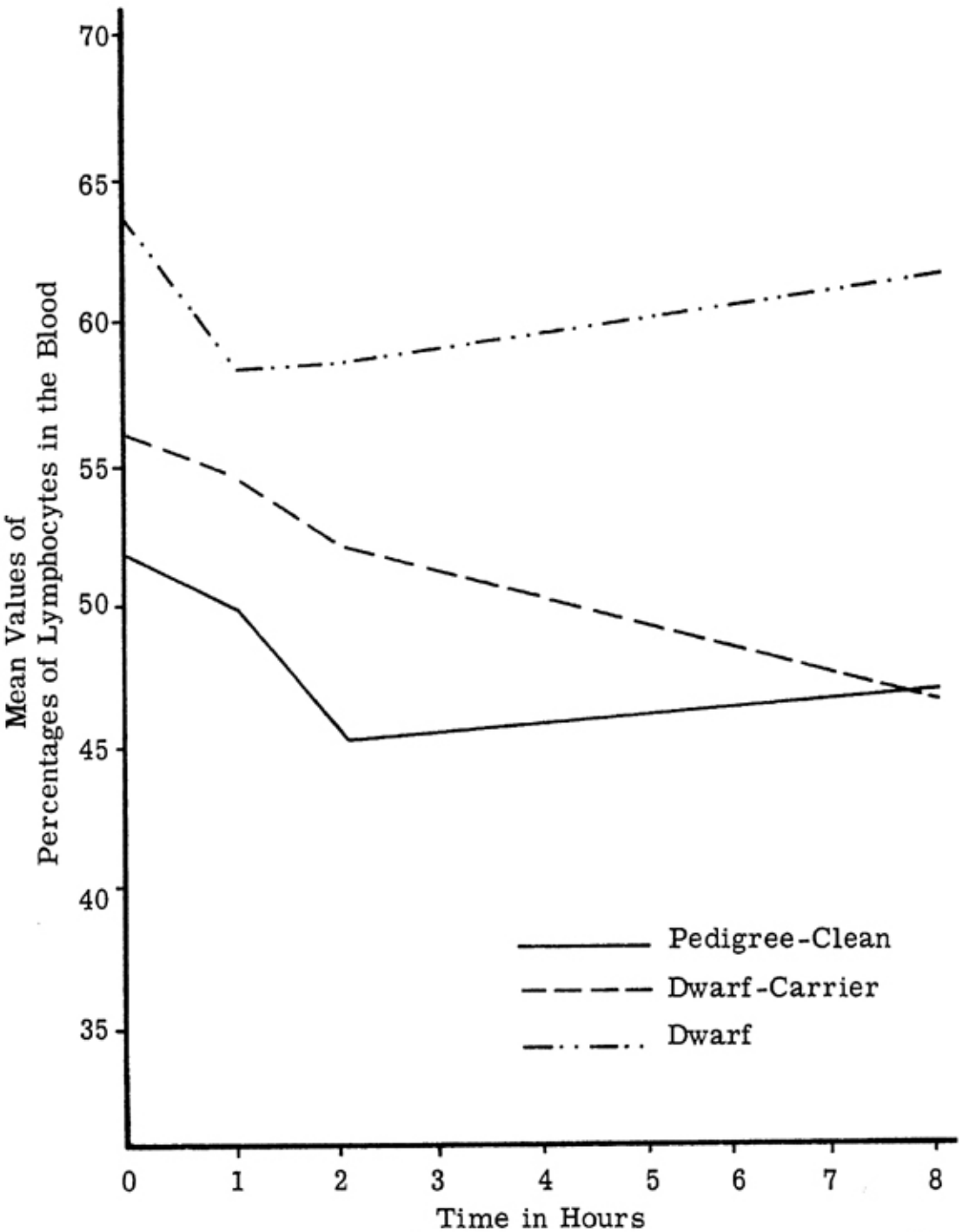

Fig. 1 - Influence of insulin injection on percentages of circulating lymphocytes.

did they differ significantly in the genotype x time interval interaction or the between-hours analysis.

Peripheral neutropenia occurred (Table 4) in the dwarf cattle prior to the collection of the fourth blood sample at eight hours following the injection of insulin. Neutrophilia persisted, however, throughout the test period in the pedi-

gree-clean and dwarf-carrier cattle, which responded similarly with regard to neutrophil changes following insulin injection.

Before administration of the hormone, neutrophils were found in highest percentages in the blood of the dwarf-carrier cattle, as shown in Table 4. Percentages of neutrophils were intermediate in the dwarfs and lowest in the pedigree-clean cattle. As has been noted previously, however, genotype differences were not significant.

TABLE 4-MEANS AND STANDARD DEVIATIONS FOR PERCENTAGES OF NEUTROPHILS IN THE BLOOD OF DWARF, DWARF-CARRIER, AND PEDIGREE-CLEAN BEEF CATTLE AT DIFFERENT INTERVALS FOLLOWING INTRAVENOUS INJECTIONS OF 0.8 UNIT OF INSULIN PER KILOGRAM OF BODY WEIGHT

Time of Sample	Dwarf Mean ± Standard Deviation	Dwarf-Carrier Mean ± Standard Deviation	Pedigree-Clean Mean ± Standard Deviation
0.0 hour	31.02 ± 4.89	34.78 ± 10.13	29.94 ± 8.13
1.0 hour	36.82 ± 6.31	36.46 ± 12.99	34.11 ± 10.00
2.0 hours	39.60 ± 6.25	40.76 ± 16.97	39.49 ± 9.94
8.0 hours	37.70 ± 6.83	48.14 ± 13.89	44.74 ± 13.24

Percentages of Eosinophils

As presented in Table 5A, differences between genotypes in eosinophil percentage before and after insulin injection were found to be very highly significant (P less than .005). Non-significant differences were observed between hours of sampling and in the genotype x time interval interaction.

Before insulin injection and throughout the sampling period, pedigree-clean cattle were highest, dwarf-carrier cattle intermediate, and dwarf cattle lowest in percentage of eosinophils (Table 5). Declining percentages of eosinophils were

TABLE 5-MEANS AND STANDARD DEVIATIONS FOR PERCENTAGES OF EOSINOPHILS IN THE BLOOD OF DWARF, DWARF-CARRIER, AND PEDIGREE-CLEAN BEEF CATTLE AT DIFFERENT INTERVALS FOLLOWING INTRAVENOUS INJECTIONS OF 0.8 UNIT OF INSULIN PER KILOGRAM OF BODY WEIGHT

Time of Sample	Dwarf Mean ± Standard Deviation	Dwarf-Carrier Mean ± Standard Deviation	Pedigree-Clean Mean ± Standard Deviation
0.0 hour	5.22 ± 7.17	9.09 ± 7.33	18.36 ± 6.88
1.0 hour	4.94 ± 2.45	9.13 ± 6.89	16.02 ± 4.54
2.0 hours	2.78 ± 3.45	7.99 ± 7.03	15.18 ± 4.10
8.0 hours	0.86 ± 0.31	5.27 ± 4.66	8.44 ± 7.01

observed in all genotypes during the test period. Eosinophil percentage in the dwarf-carrier cattle, however, remained fairly constant and did not begin a decline until after collection of the second blood sample at one hour following insulin injection. These genotype differences are illustrated in Figure 2.

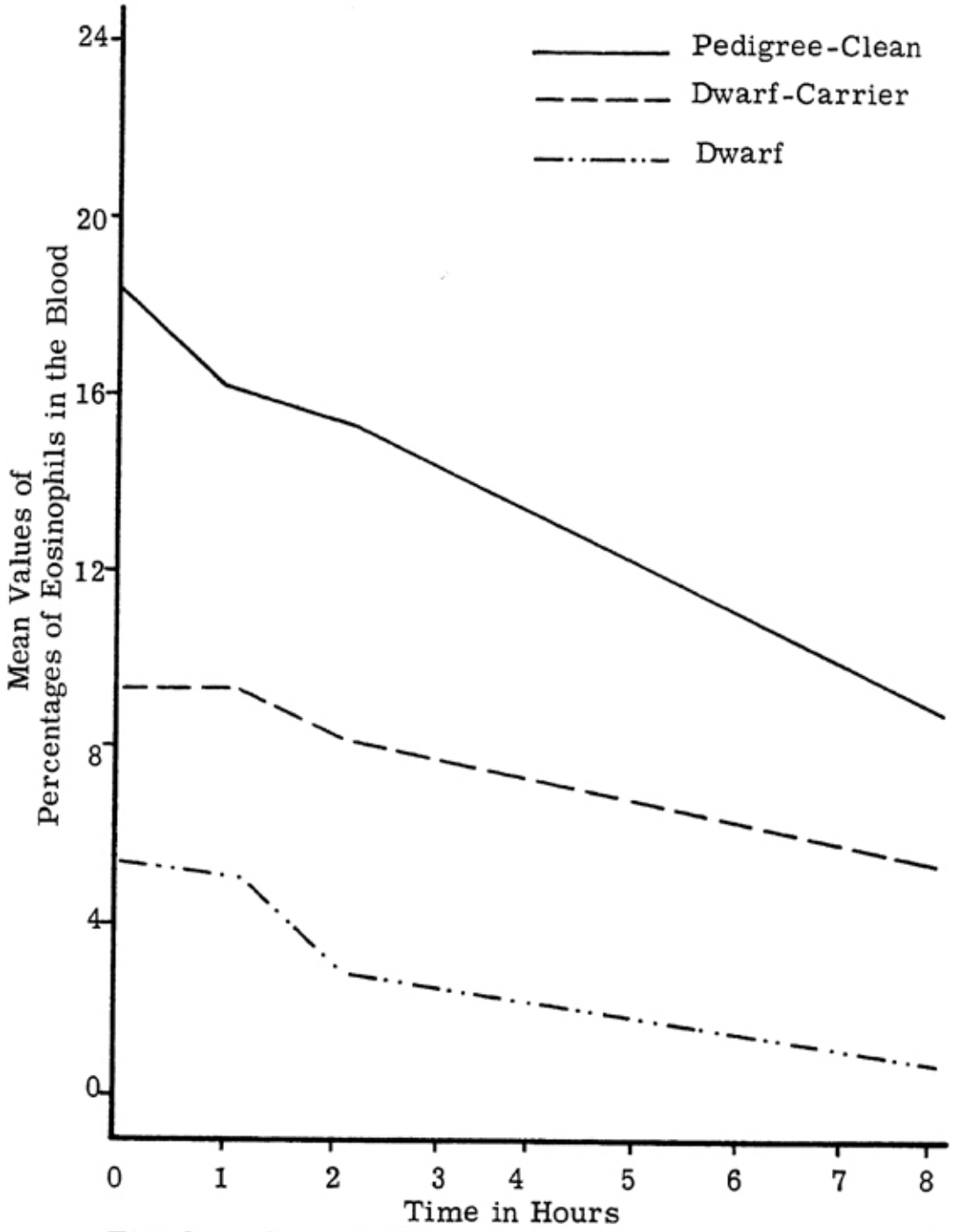

Fig. 2 - Influence of insulin injection on percentages of circulating eosinophils.

Numbers of Lymphocytes

No significant differences were observed between genotypes or between hours of sampling, with regard to numbers of circulating lymphocytes before and after insulin injection (Table 6A). The genotype x time interval interaction also was non-significant.

Throughout the test period, the blood of the dwarf cattle contained greater numbers of lymphocytes than that of the pedigree-clean and dwarf-carrier cattle

(Table 6). This measurement fluctuated to a greater extent in the dwarf animals than in the pedigree-clean or dwarf-carrier cattle. The greater physiological alteration within the dwarf animals as a result of stress indicates a possible abnormality within the dwarfed individual in the homeostatic mechanisms whose function is to counteract unfavorable conditions.

TABLE 6-MEANS AND STANDARD DEVIATIONS FOR NUMBERS OF LYMPHOCYTES PER 1/50 CUBIC MILLIMETER OF BLOOD IN DWARF, DWARF-CARRIER, AND PEDIGREE-CLEAN BEEF CATTLE AT DIFFERENT INTERVALS FOLLOWING INTRAVENOUS INJECTIONS OF 0.8 UNIT OF INSULIN PER KILOGRAM OF BODY WEIGHT

Time of Sample	Dwarf Mean ± Standard Deviation	Dwarf-Carrier Mean ± Standard Deviation	Pedigree-Clean Mean ± Standard Deviation
0.0 hour	108.70 ± 20.09	87.53 ± 21.90	79.60 ± 18.73
1.0 hour	92.06 ± 21.90	83.07 ± 22.08	82.71 ± 23.45
2.0 hours	92.20 ± 24.21	88.00 ± 21.26	84.16 ± 29.07
8.0 hours	133.34 ± 17.72	82.38 ± 16.00	88.15 ± 16.32

During the first two hours after administration of insulin, the blood of the dwarf-carrier cattle contained slightly greater numbers of lymphocytes than that of the pedigree-clean animals.

Table 6 shows insulin shock exerted considerable lymphocytotic effect in the dwarf cattle between two and eight hours following insulin injection. A gradual increase in numbers of lymphocytes occurred for the duration of the test period in the pedigree-clean cattle. The other genotypes were not consistent in alterations of lymphocyte numbers in response to the stress stimuli.

Numbers of Neutrophils

Differences in numbers of circulating neutrophils between hours of blood sample collection were highly significant (P less than .01). See Table 7A. Neither the hours x genotype interaction nor the analysis of variance between genotypes was significant.

Neutrophilic leucocytosis occurred in each genotype for the duration of the eight-hour test period following insulin injection (Table 7). This response

TABLE 7-MEANS AND STANDARD DEVIATIONS FOR NUMBERS OF NEUTROPHILS PER 1/50 CUBIC MILLIMETER OF BLOOD IN DWARF, DWARF, CARRIER, AND PEDIGREE-CLEAN BEEF CATTLE AT DIFFERENT INTERVALS FOLLOWING INTRAVENOUS INJECTIONS OF 0.8 UNIT OF INSULIN PER KILOGRAM OF BODY WEIGHT

Time of Sample	Dwarf Mean ± Standard Deviation	Dwarf-Carrier Mean ± Standard Deviation	Pedigree-Clean Mean ± Standard Deviation
0.0 hour	52.34 ± 17.89	53.00 ± 13.74	47.41 ± 12.54
1.0 hour	57.82 ± 15.28	56.69 ± 19.48	54.99 ± 13.94
2.0 hours	63.56 ± 17.58	68.61 ± 27.97	71.10 ± 18.77
8.0 hours	84.50 ± 29.72	81.38 ± 35.33	88.97 ± 37.60

accounted for the highly significant differences between hours of sample collection. The most rapid increase in numbers of neutrophils entering the blood stream occurred in the pedigree-clean individuals.

The submission of humans (Hills *et al.*, 1948) and mice (Dougherty and White, 1943a) to stress conditions has also been shown to result in increased numbers of circulating neutrophils. Harlow and Selye (1937) indicated that increasing neutrophil numbers in the circulation was the primary cause of the increase in numbers of total leucocytes resulting from "alarm reactions."

Numbers of Eosinophils

Differences between the three genotypes for the dwarf gene were very highly significant (P less than .005) with regard to numbers of circulating eosinophils before and after insulin injection (Table 8A). Figure 3 illustrates this physiological distinction between genotypes.

Blood of the dwarf-carrier cattle was intermediate in numbers of eosinophils and blood of pedigree-clean cattle contained the greatest numbers of eosinophils. Table 8 and Figure 3 show the numbers for these two groups remained fairly constant for the first two hours following the injection of insulin. Be-

TABLE 8-MEANS AND STANDARD DEVIATIONS FOR NUMBERS OF EOSINOPHILS PER 1/50 CUBIC MILLIMETER OF BLOOD IN DWARF, DWARF-CARRIER, AND PEDIGREE-CLEAN BEEF CATTLE AT DIFFERENT INTERVALS FOLLOWING INTRAVENOUS INJECTIONS OF 0.8 UNIT OF INSULIN PER KILOGRAM OF BODY WEIGHT

Time of Sample	Dwarf Mean ± Standard Deviation	Dwarf-Carrier Mean ± Standard Deviation	Pedigree-Clean Mean ± Standard Deviation
0.0 hour	10.18 ± 15.01	13.84 ± 11.33	29.35 ± 13.96
1.0 hour	7.98 ± 4.91	13.23 ± 10.01	26.67 ± 9.51
2.0 hours	5.04 ± 6.70	13.69 ± 13.07	27.88 ± 9.40
8.0 hours	1.96 ± 0.84	9.30 ± 8.02	16.54 ± 14.01

tween the collection of the third and fourth blood samples, eosinophil numbers decreased in the blood of the cattle of each experimental group. Hills *et al.* (1948) indicated that the induction of stress resulted in a decline in numbers of circulating eosinophils in humans.

Other differences in numbers of eosinophils (Table 8A) were not significant.

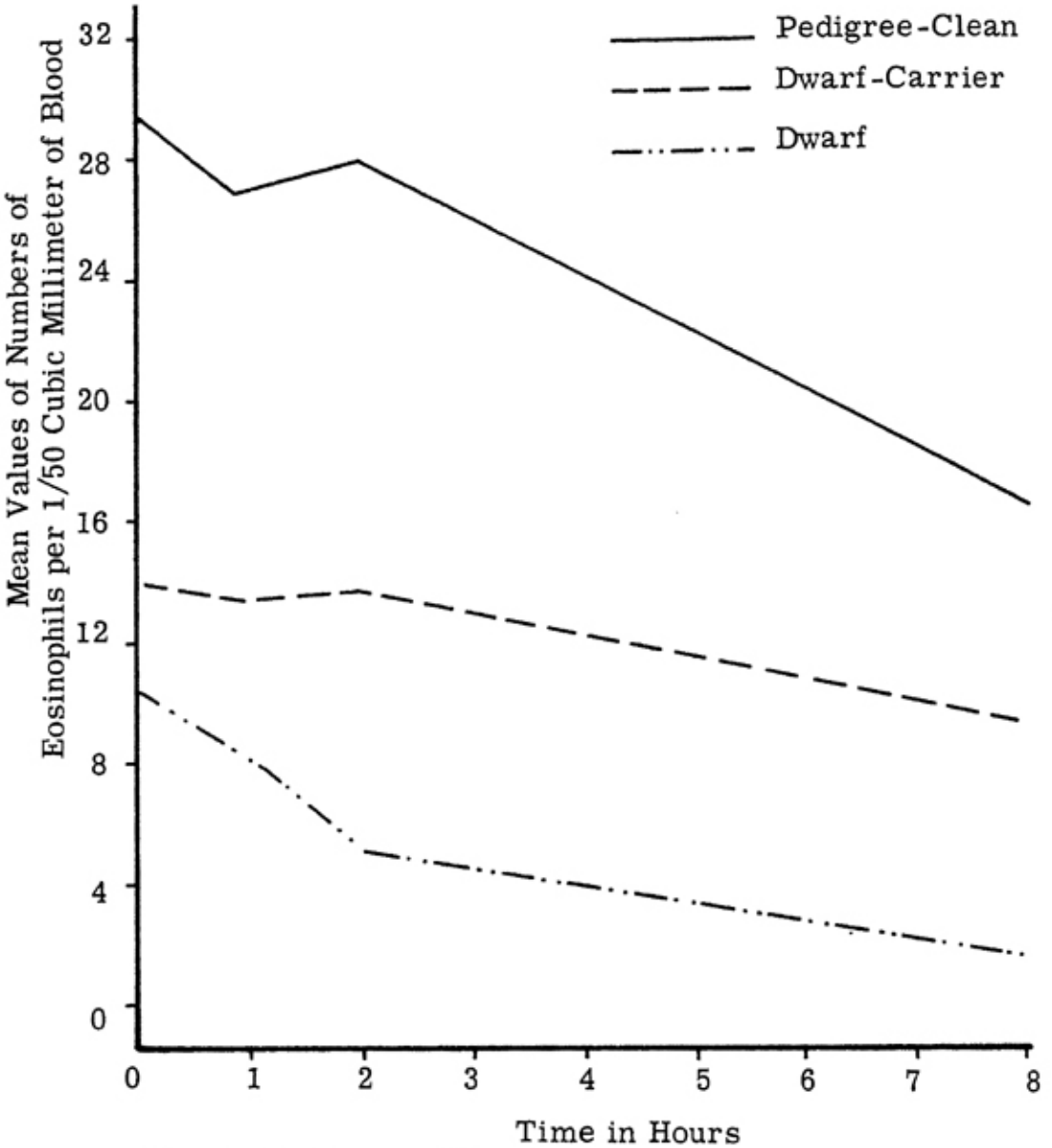

Fig. 3 - Influence of insulin injection on numbers of circulating eosinophils.

Levels of Blood Glucose

Very highly significant differences (P less than .005) existed between genotypes and between hours of blood sampling (Table 9A), with regard to levels of blood glucose before and after insulin injection. The genotype x time interval interaction was not significant. The differences in blood sugar changes between genotypes and between hours are illustrated in Figure 4.

Pedigree-clean cattle were consistently higher in their blood sugar level than dwarf and dwarf-carried cattle.

A rapid decrease in blood sugar level during the first hour following insulin injection occurred in each of the three genotypes.

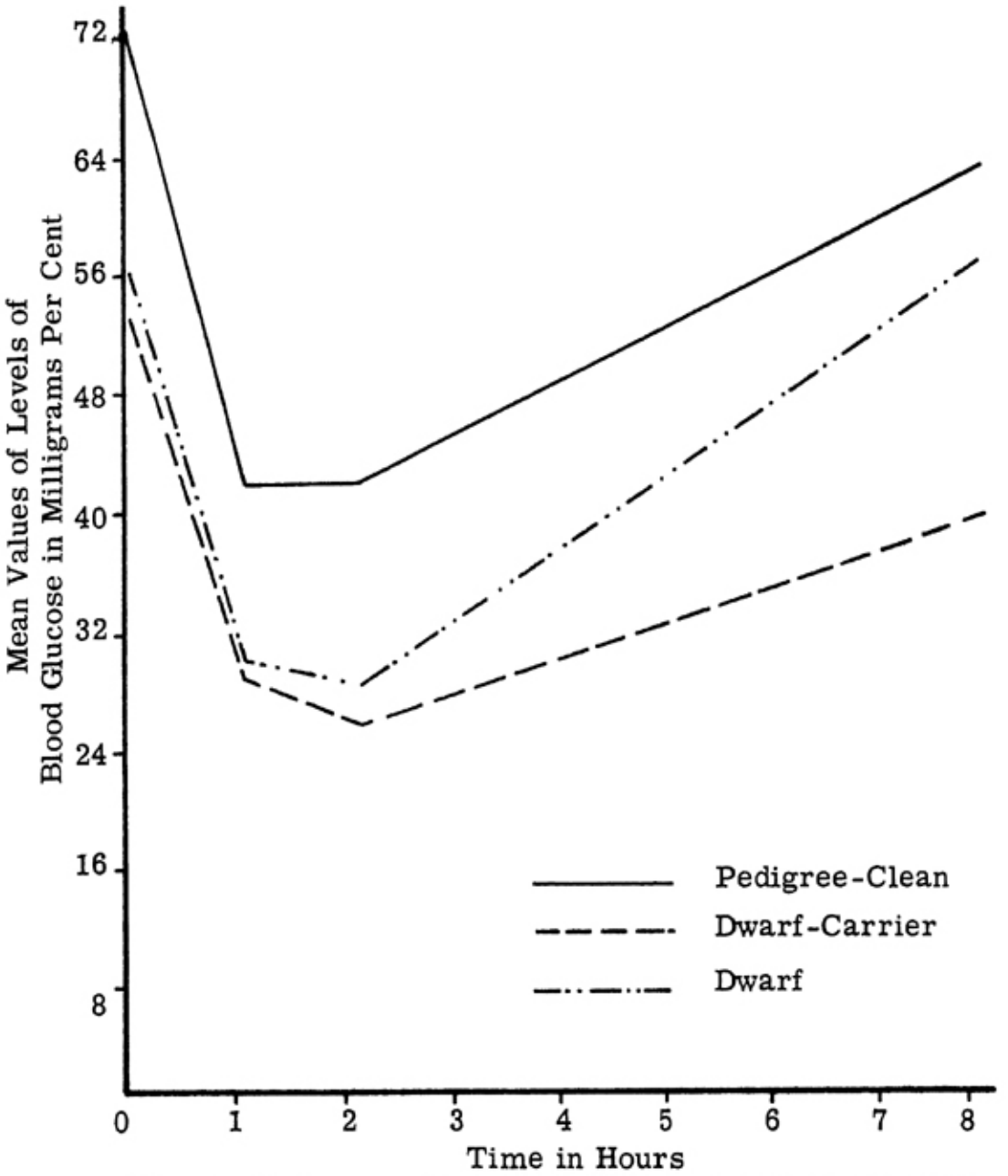

Fig. 4 - Influence of insulin injection on levels of blood glucose.

A previous investigation conducted at the Missouri station (Foley *et al.*, 1956) indicated that the blood sugar levels of dwarf cattle, in response to insulin injection, dropped more rapidly and returned to normal slower than those of pedigree-clean cattle. Results of this study, however, fail to confirm these findings. As shown in Table 9 and Figure 4, the blood sugar level had returned to normal eight hours after insulin injection in the dwarf cattle, but was still sub-normal in the pedigree-clean cattle.

Several factors must be considered in discussing this disagreement of results. In the earlier investigation, two insulin injections were administered 48 hours apart and two different methods of blood sugar analysis were employed.

TABLE 9-MEANS AND STANDARD DEVIATIONS FOR LEVELS OF BLOOD GLUCOSE IN MILLIGRAMS PER CENT IN THE BLOOD OF DWARF, DWARF-CARRIER, AND PEDIGREE-CLEAN BEEF CATTLE AT DIFFERENT INTERVALS FOLLOWING INTRAVENOUS INJECTIONS OF 0.8 UNIT OF INSULIN PER KILOGRAM OF BODY WEIGHT

Time of Sample	Dwarf Mean ± Standard Deviation	Dwarf-Carrier Mean ± Standard Deviation	Pedigree-Clean Mean ± Standard Deviation
0.0 hour	56.53 ± 3.58	53.24 ± 16.86	71.79 ± 11.16
1.0 hour	29.93 ± 2.91	28.74 ± 17.88	41.37 ± 9.83
2.0 hours	28.16 ± 6.46	25.65 ± 11.59	41.47 ± 11.22
8.0 hours	56.73 ± 6.21	39.27 ± 23.21	63.08 ± 18.75

Results of the current study were based upon one insulin injection and one method of blood sugar analysis. The number of dwarfs used in each of the experiments is also of significance. Twelve dwarfs were injected in the earlier experiment and only five in this one.

Environment is also known to exert considerable effect on blood sugar level. In this study, the dwarfs were tested during the summer months. They were fat and were possibly in better condition than the dwarfs used in the 1956 study. Foley *et al.* (1960) reviewed literature suggesting that many factors, including age, lactation, plane of nutrition, and estrus, might cause variation in the blood sugar level.

Blood sugar changes in response to insulin injection cannot be suggested as a means of detecting carriers of the dwarf gene until verified by further experimentation. Since environment is known to influence blood sugar levels, a study to determine the factors of environment and the magnitude of their influence in this respect would seem to be of considerable significance and interest to the animal physiologist.

Total Numbers of White Cells

The differences observed in total numbers of white cells between hours of blood sample collection were very highly significant (P less than .005), as shown in Table 10A. The genotype x time interval interaction and genotype differences before and after insulin administration were not significant.

In cattle of each genotype, a decline in numbers of white blood cells occurred during the first hour after injection of the hormone (Table 10), although leucocyte numbers in the pedigree-clean cattle were relatively stable. By the second hour following insulin injection, white blood cell numbers had begun to increase in each of the genotypes. Increasing numbers of white cells entered the blood stream in each genotype throughout the remainder of the test period. The most rapid increase occurred in the dwarf animals, indicating that the physiological mechanism which maintains leucocyte numbers within the normal range may be inadequate in some dwarfs. Other factors, such as differences in insulin sensitivity, may also be involved. The reason for the initial decline is not fully

TABLE 10-MEANS AND STANDARD DEVIATIONS FOR TOTAL NUMBERS OF WHITE CELLS PER 1/50 CUBIC MILLIMETER OF BLOOD IN DWARF, DWARF-CARRIER, AND PEDIGREE-CLEAN BEEF CATTLE AT DIFFERENT INTERVALS FOLLOWING INTRAVENOUS INJECTIONS OF 0.8 UNIT OF INSULIN PER KILOGRAM OF BODY WEIGHT

Time of Sample	Dwarf Mean ± Standard Deviation	Dwarf-Carrier Mean ± Standard Deviation	Pedigree-Clean Mean ± Standard Deviation
0.0 hour	171.20 ± 31.93	150.84 ± 18.43	159.70 ± 19.21
1.0 hour	157.80 ± 31.74	146.84 ± 20.52	159.50 ± 16.28
2.0 hours	160.80 ± 35.47	169.76 ± 24.90	183.10 ± 31.57
8.0 hours	219.80 ± 41.80	181.07 ± 22.02	196.30 ± 36.65

understood, and possibly warrants further investigation. Genotype differences with regard to the initial response to insulin shock (Table 10) are of considerable interest from the physiological standpoint.

Leucocytosis in response to stress stimuli has also been found in humans (Martin, 1932; Lucia *et al.,* 1937; Bierman *et al.,* 1952), in rats (Harlow and Selye, 1937), and in mice (Harlow and Selye, 1937).

Influence of Adrenalin on Various Blood Components in Dwarf, Dwarf-Carrier, and Pedigree-Clean Beef Cattle

Percentages of Lymphocytes

Differences between cattle of the three genotypes with regard to percentage of lymphocytes before and after adrenalin injection were very highly significant (P less than .005), as shown in Table 11A. The genotype x time interval interaction and the differences between hours of sampling were not significant. Genotype differences are illustrated in Figure 5.

The blood of the dwarf cattle prior to injection again contained a higher percentage of lymphocytes than that of either of the other genotypes. Blood of the dwarf-carrier cattle was intermediate in this respect (Table 11; Figure 5).

TABLE 11-MEANS AND STANDARD DEVIATIONS FOR PERCENTAGES OF LYMPHOCYTES IN THE BLOOD OF DWARF, DWARF-CARRIER, AND PEDIGREE-CLEAN BEEF CATTLE AT DIFFERENT INTERVALS FOLLOWING INTRAVENOUS INJECTIONS OF 0.4 CUBIC CENTIMETER OF ADRENALIN (DILUTED 1 TO 10,000) PER HUNDRED POUNDS OF BODY WEIGHT

Time of Sample	Dwarf Mean ± Standard Deviation	Dwarf-Carrier Mean ± Standard Deviation	Pedigree-Clean Mean ± Standard Deviation
0.0 hour	61.54 ± 6.18	53.80 ± 5.84	50.70 ± 8.27
0.5 hour	61.26 ± 7.98	53.87 ± 6.80	47.87 ± 8.67
1.0 hour	59.28 ± 7.86	51.26 ± 7.69	47.72 ± 10.49
2.0 hours	60.16 ± 6.31	54.78 ± 6.03	43.53 ± 9.54
4.0 hours	63.98 ± 6.14	50.00 ± 8.44	55.13 ± 19.81

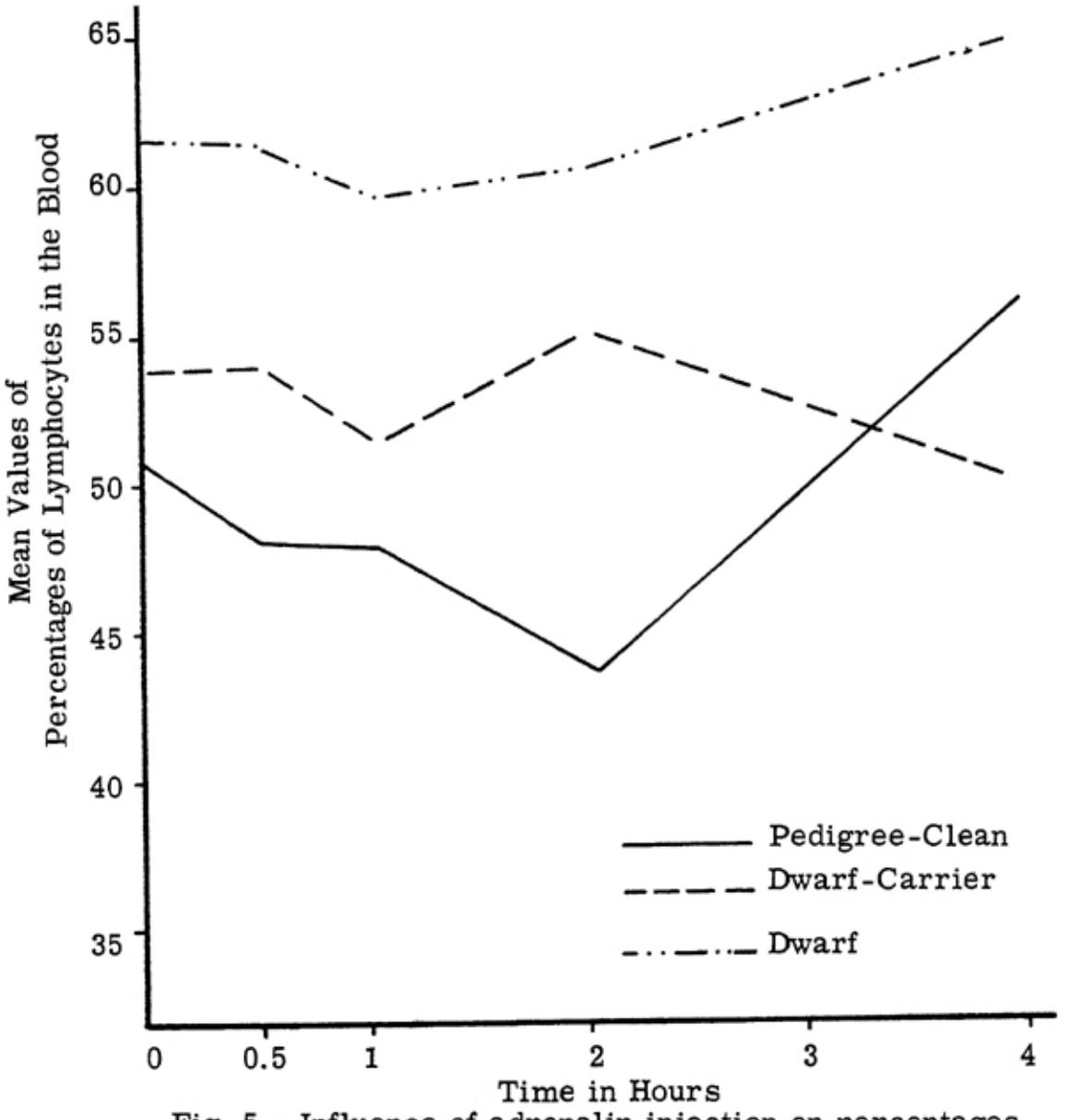

Fig. 5 - Influence of adrenalin injection on percentages of circulating lymphocytes.

Because of a rapid increase in lymphocytes entering the blood stream between the second and fourth hours following adrenalin administration (Table 14; Figure 7), accompanied by peripheral neutropenia (Table 15) and decreased numbers of eosinophils (Table 16; Figure 8), an increased percentage of lymphocytes was present at the conclusion of the test period in the blood of the pedigree-clean cattle. During this period, the percentage of lymphocytes decreased in the dwarf-carrier cattle and increased in the dwarf cattle.

The highly significant differences between cattle of the three genotypes in percentage of circulating lymphocytes in response to adrenalin injection (Table 11A) and to insulin injection (Table 3A) are of considerable interest. They indicate possible differences in the homeostatic mechanisms of pedigree-clean,

dwarf, and dwarf-carrier cattle which maintain a proper balance of blood constituents in the presence of stressful conditions.

In the pedigree-clean cattle, the percentage of lymphocytes declined during the first two hours following adrenalin injection. Following the administration of adrenalin to rats and mice, Harlow and Selye (1937) also found that the hormone caused lymphopenia.

Percentages of Neutrophils

Dwarf, dwarf-carrier, and pedigree-clean cattle did not differ significantly in percentage of nuetrophils before and after adrenalin injection (Table 12A). Neither the differences between hours of sampling nor the genotype x time interval interaction was significant. Therefore, the differences between genotypes in percentage of lymphocytes before and after adrenalin injection (Table 11A) seem to have a substantial influence upon the *percentage of eosinophils,* (Table 13A) rather than upon the *proportion of both neutrophils and eosinophils* in the circulation.

Throughout the test period, alterations in the percentages of neutrophils were relatively small, especially in the dwarf cattle, as presented in Table 12.

TABLE 12-MEANS AND STANDARD DEVIATIONS FOR PERCENTAGES OF NEUTROPHILS IN THE BLOOD OF DWARF, DWARF-CARRIER, AND PEDIGREE-CLEAN BEEF CATTLE AT DIFFERENT INTERVALS FOLLOWING INTRAVENOUS INJECTIONS OF 0.4 CUBIC CENTIMETER OF ADRENALIN (DILUTED 1 TO 10,000) PER HUNDRED POUNDS OF BODY WEIGHT

Time of Sample	Dwarf Mean ± Standard Deviation	Dwarf-Carrier Mean ± Standard Deviation	Pedigree-Clean Mean ± Standard Deviation
0.0 hour	32.58 ± 5.40	28.29 ± 4.52	29.38 ± 10.53
0.5 hour	32.44 ± 9.15	28.27 ± 6.53	31.33 ± 10.49
1.0 hour	32.54 ± 9.35	32.52 ± 6.54	33.05 ± 10.01
2.0 hours	33.90 ± 8.04	30.74 ± 5.21	38.98 ± 9.79
4.0 hours	28.98 ± 7.05	35.84 ± 9.44	32.99 ± 14.98

A gradual increase was noted in the percentage of neutrophils in the blood of pedigree-clean cattle during the first two hours following adrenalin injection. Following the removal of the adrenal glands of cats, Zwemer and Lyons (1928) observed a decrease in percentages of polymorphonuclear neutrophils. The results of this early study of the effects of adrenalectomy are also indicative of the role of epinephrine in stimulating increased proportions of neutrophils.

Percentages of Eosinophils

Table 13A and Figure 6 show that differences between cattle of the three genotypes, with regard to eosinophil percentage before and after adrenalin injection, were very highly significant (P less than .005). The genotype x time interval interaction and differences between hours of blood sampling were not significant in this respect.

Prior to the injection of adrenalin, the blood of the dwarf cattle contained a very low percentage of eosinophils, in comparison with the other genotypes (Table 13; Figure 6). This percentage remained at relatively low levels throughout the test period and accounts for the immense differences between genotypes.

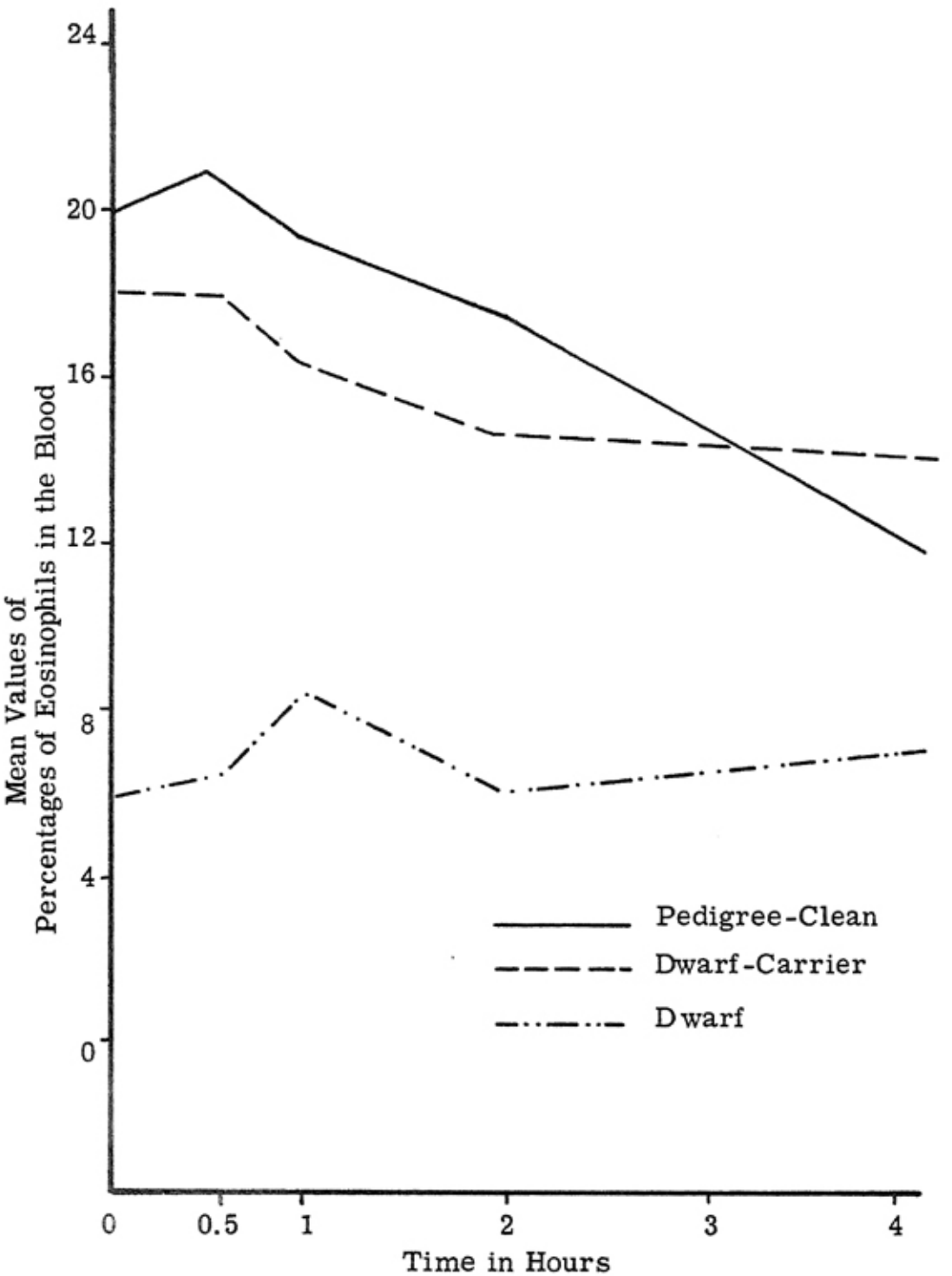

Fig. 6 - Influence of adrenalin injection on percentages of circulating eosinophils.

Prior to the administration of adrenalin, the blood of the dwarf cattle contained higher percentages of lymphocytes (Table 11; Figure 5) and neutrophils (Table 12) than that of the dwarf-carrier and pedigree-clean cattle. This was not true of the neutrophils, however, at the beginning of the insulin tolerance test (Table 4).

TABLE 13-MEANS AND STANDARD DEVIATIONS FOR PERCENTAGES OF EOSINOPHILS IN THE BLOOD OF DWARF, DWARF-CARRIER, AND PEDIGREE-CLEAN BEEF CATTLE AT DIFFERENT INTERVALS FOLLOWING INTRAVENOUS INJECTIONS OF 0.4 CUBIC CENTIMETER OF ADRENALIN (DILUTED 1 TO 10,000) PER HUNDRED POUNDS OF BODY WEIGHT

Time of Sample	Dwarf Mean ± Standard Deviation	Dwarf-Carrier Mean ± Standard Deviation	Pedigree-Clean Mean ± Standard Deviation
0.0 hour	5.88 ± 3.15	17.90 ± 6.13	19.92 ± 8.89
0.5 hour	6.30 ± 5.33	17.84 ± 6.68	20.80 ± 9.06
1.0 hour	8.18 ± 4.23	16.20 ± 4.71	19.23 ± 9.30
2.0 hours	5.94 ± 2.43	14.46 ± 3.55	17.49 ± 5.49
4.0 hours	7.04 ± 3.04	14.15 ± 3.16	11.88 ± 8.15

Numbers of Lymphocytes

The differences between genotypes, with regard to numbers of circulating lymphocytes before and after adrenalin injection, were significant at the .005 level of probability (Table 14A; and Figure 7). Non-significant differences were found between hours of sampling, and in the genotype x time interval interaction.

Throughout the test period, the blood of the dwarf cattle contained much greater numbers of lymphocytes than that of either of the other genotypes (Table 14, Figure 7). The differences between dwarf-carrier and pedigree-clean cattle were relatively small in this respect during the first hour following adrenalin injection. Between the second and fourth hours after injection, considerable lymphocytosis occurred in the blood of the pedigree-clean and dwarf cattle.

TABLE 14-MEANS AND STANDARD DEVIATIONS FOR NUMBERS OF LYMPHOCYTES PER 1/50 CUBIC MILLIMETER OF BLOOD IN DWARF, DWARF-CARRIER, AND PEDIGREE-CLEAN BEEF CATTLE AT DIFFERENT INTERVALS FOLLOWING INTRAVENOUS INJECTIONS OF 0.4 CUBIC CENTIMETER OF ADRENALIN (DILUTED 1 TO 10,000) PER HUNDRED POUNDS OF BODY WEIGHT

Time of Sample	Dwarf Mean ± Standard Deviation	Dwarf-Carrier Mean ± Standard Deviation	Pedigree-Clean Mean ± Standard Deviation
0.0 hour	102.24 ± 25.26	80.28 ± 17.64	80.88 ± 15.35
0.5 hour	102.56 ± 28.79	78.85 ± 17.87	79.02 ± 10.58
1.0 hour	106.10 ± 23.49	78.07 ± 13.33	78.98 ± 8.30
2.0 hours	101.04 ± 25.45	87.78 ± 15.04	81.09 ± 20.78
4.0 hours	121.58 ± 28.34	85.35 ± 15.35	105.69 ± 31.81

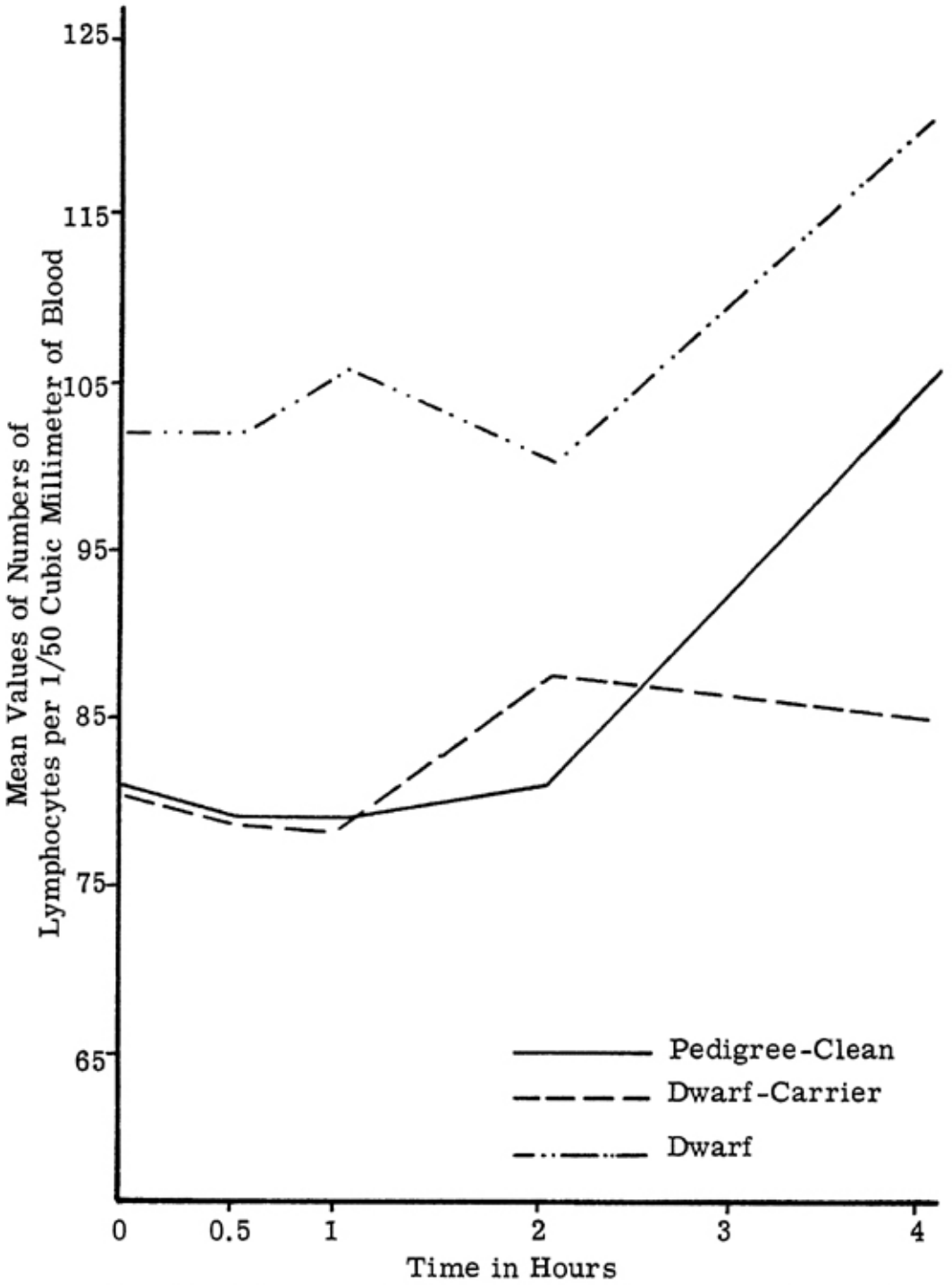

Fig. 7 - Influence of adrenalin injection on numbers of circulating lymphocytes.

Lymphocyte numbers decreased in the blood of the dwarf-carrier cattle during this period. This relationship between pedigree-clean and dwarf-carrier cattle in response to adrenalin injection could be of considerable importance if verified by further experimentation. It might provide a rapid and inexpensive means of heterozygote detection.

The removal of the adrenal glands has been reported to exert a lymphocytotic effect in cats (Zwemer and Lyons, 1928; Corey and Britton, 1932). The results of more recent investigations (Lewis, 1941; Valentine *et al.,* 1948), however, are in disagreement with these findings.

Numbers of Neutrophils

Non-significant differences were found between genotypes and between hours of blood sample collection, with regard to numbers of circulating neutrophils before and after adrenalin injection (Table 15A). The genotype x time interval interaction was also non-significant. A gradual increase in neutrophil numbers occurred in the blood of the pedigree-clean cattle during the first two hours following adrenalin injection (Table 15). Harlow and Selye (1937) reported that the increase in numbers of white blood cells during "alarm reactions" was primarily a result of neutrophilic leucocytosis.

TABLE 15-MEANS AND STANDARD DEVIATIONS FOR NUMBERS OF NEUTROPHILS PER 1/50 CUBIC MILLIMETER OF BLOOD IN DWARF, DWARF-CARRIER, AND PEDIGREE-CLEAN BEEF CATTLE AT DIFFERENT INTERVALS FOLLOWING INTRAVENOUS INJECTIONS OF 0.4 CUBIC CENTIMETER OF ADRENALIN (DILUTED 1 TO 10,000) PER HUNDRED POUNDS OF BODY WEIGHT

Time of Sample	Dwarf Mean ± Standard Deviation	Dwarf-Carrier Mean ± Standard Deviation	Pedigree-Clean Mean ± Standard Deviation
0.0 hour	53.48 ± 12.67	41.71 ± 9.26	47.46 ± 17.81
0.5 hour	53.14 ± 17.73	40.92 ± 9.85	53.06 ± 19.64
1.0 hour	62.32 ± 29.67	49.92 ± 12.43	57.45 ± 22.43
2.0 hours	57.64 ± 21.41	50.14 ± 15.37	73.94 ± 24.22
4.0 hours	58.86 ± 28.95	62.78 ± 24.33	65.96 ± 31.71

The greatest numbers of neutrophils were found in the blood of the dwarf cattle prior to and during the first hour following adrenalin injection. Pedigree-clean cattle were intermediate in this respect. It is of interest to note that the dwarf and pedigree-clean cattle reached their peak in neutrophil numbers at one and two hours, respectively, while numbers of neutrophils were greatest in the blood of the dwarf-carrier cattle in the sample collected at the close of the four-hour test period.

Numbers of Eosinophils

As shown in Table 16A, the differences between cattle of the three genotypes with regard to numbers of eosinophils before and after the administration of adrenalin highly significant (P less than .005). Genotype differences are illustrated in Figure 8. Non-significant differences were found between hours of sampling. The genotype x time interval interaction also was not significant.

The blood of the dwarf cattle contained the fewest eosinophils throughout the sampling period; the dwarf-carrier cattle were intermediate in this respect (Table 16; Figure 8). Note that eosinophil numbers in the blood stream of the dwarf-carrier cattle seemed to gradually decline for the first two hours follow-

ing administration of adrenalin, while the pedigree-clean and dwarf cattle reached a peak in eosinophil numbers at 30 minutes and one hour, respectively, following adrenalin injection (Figure 8).

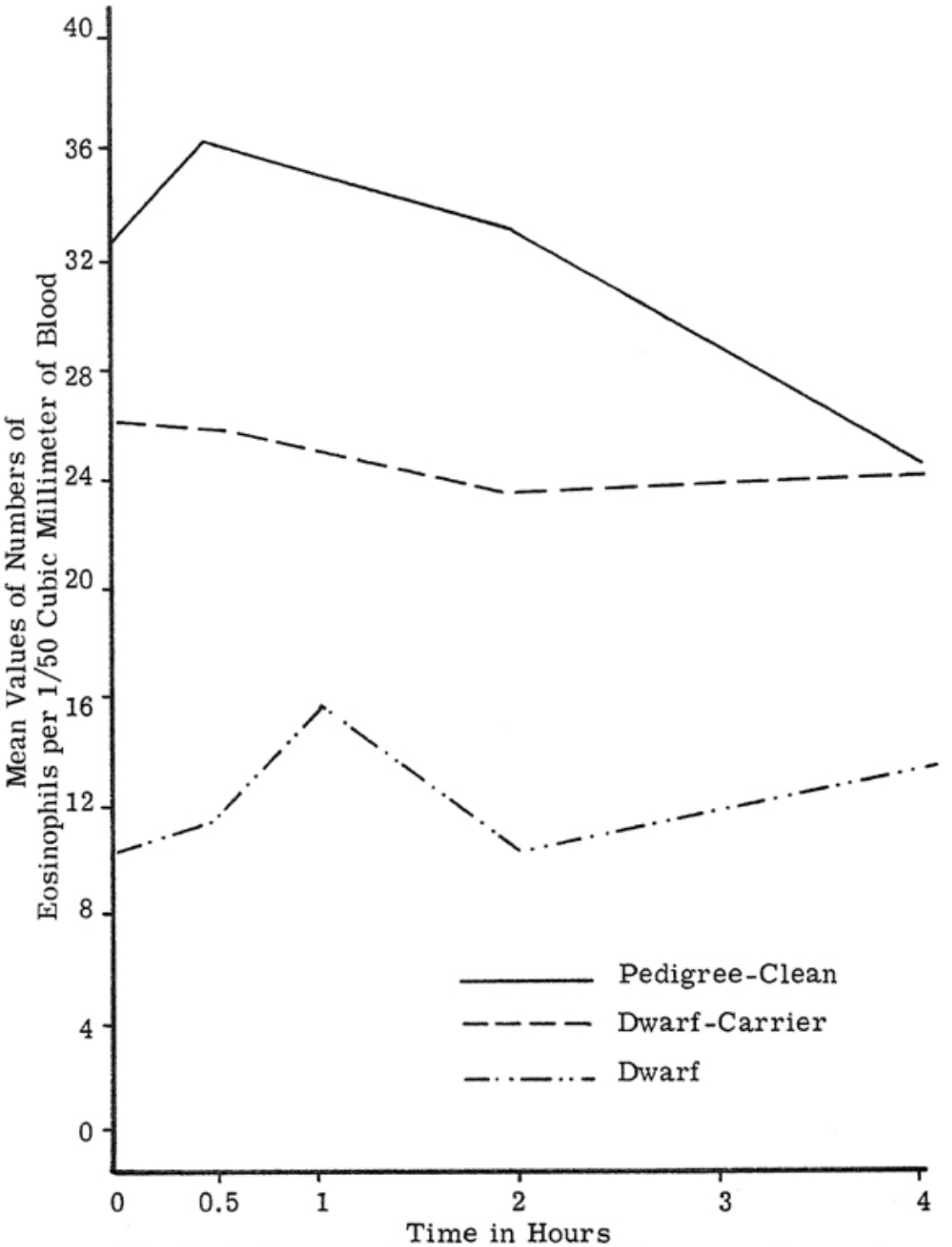

Fig. 8 - Influence of adrenalin injection on numbers of circulating eosinophils.

TABLE 16-MEANS AND STANDARD DEVIATIONS FOR NUMBERS OF EOSINOPHILS PER 1/50 CUBIC MILLIMETER OF BLOOD IN DWARF, DWARF-CARRIER, AND PEDIGREE-CLEAN BEEF CATTLE AT DIFFERENT INTERVALS FOLLOWING INTRAVENOUS INJECTIONS OF 0.4 CUBIC CENTIMETER OF ADRENALIN (DILUTED 1 TO 10,000) PER HUNDRED POUNDS OF BODY WEIGHT

Time of Sample	Dwarf Mean ± Standard Deviation	Dwarf-Carrier Mean ± Standard Deviation	Pedigree-Clean Mean ± Standard Deviation
0.0 hour	10.30 ± 5.96	26.00 ± 7.65	32.60 ± 16.60
0.5 hour	11.50 ± 9.48	25.78 ± 10.02	36.25 ± 19.97
1.0 hour	15.60 ± 9.67	24.92 ± 7.83	35.07 ± 23.16
2.0 hours	10.22 ± 4.77	23.42 ± 6.51	33.07 ± 14.97
4.0 hours	13.30 ± 6.76	24.07 ± 5.21	24.48 ± 20.14

Levels of Blood Glucose

Figure 9 and Table 17A show that differences in blood sugar levels of the three genotypes before and after adrenalin injection were highly significant (P less than .005). The genotype x time interval interaction and the analysis of variance between hours were non-significant.

Means and standard deviations for blood glucose levels of each genotype are presented in Table 17. The initial blood sample collected from pedigree-clean

TABLE 17-MEANS AND STANDARD DEVIATIONS FOR LEVELS OF BLOOD GLUCOSE IN MILLIGRAMS PER CENT IN THE BLOOD OF DWARF, DWARF-CARRIER, AND PEDIGREE-CLEAN BEEF CATTLE AT DIFFERENT INTERVALS FOLLOWING INTRAVENOUS INJECTIONS OF 0.4 CUBIC CENTIMETER OF ADRENALIN (DILUTED 1 TO 10,000) PER HUNDRED POUNDS OF BODY WEIGHT

Time of Sample	Dwarf Mean ± Standard Deviation	Dwarf-Carrier Mean ± Standard Deviation	Pedigree-Clean Mean ± Standard Deviation
0.0 hour	41.04 ± 2.60	51.69 ± 7.32	76.68 ± 12.57
0.5 hour	48.48 ± 2.67	58.11 ± 3.34	78.84 ± 10.79
1.0 hour	48.14 ± 4.53	60.44 ± 8.97	80.80 ± 12.07
2.0 hours	48.74 ± 4.18	55.95 ± 11.19	78.55 ± 10.33
4.0 hours	45.08 ± 2.50	54.43 ± 9.61	75.61 ± 17.38

cattle before adrenalin injection contained an average of 76.68 milligrams of glucose per 100 milliliters of blood, compared with 51.69 milligrams percent for the dwarf-carrier cattle and 41.04 milligrams percent for the dwarf cattle. These genotype differences account for the magnitude of the calculated F ratios in the analysis of variance (Table 17A).

Dukes (1947) stated that the administration of adrenalin to domestic animals resulted in hyperglycemia. Results of this investigation demonstrate such an effect in cattle of each genotype during the first 30 minutes following injection of the hormone (Table 17; Figure 9).

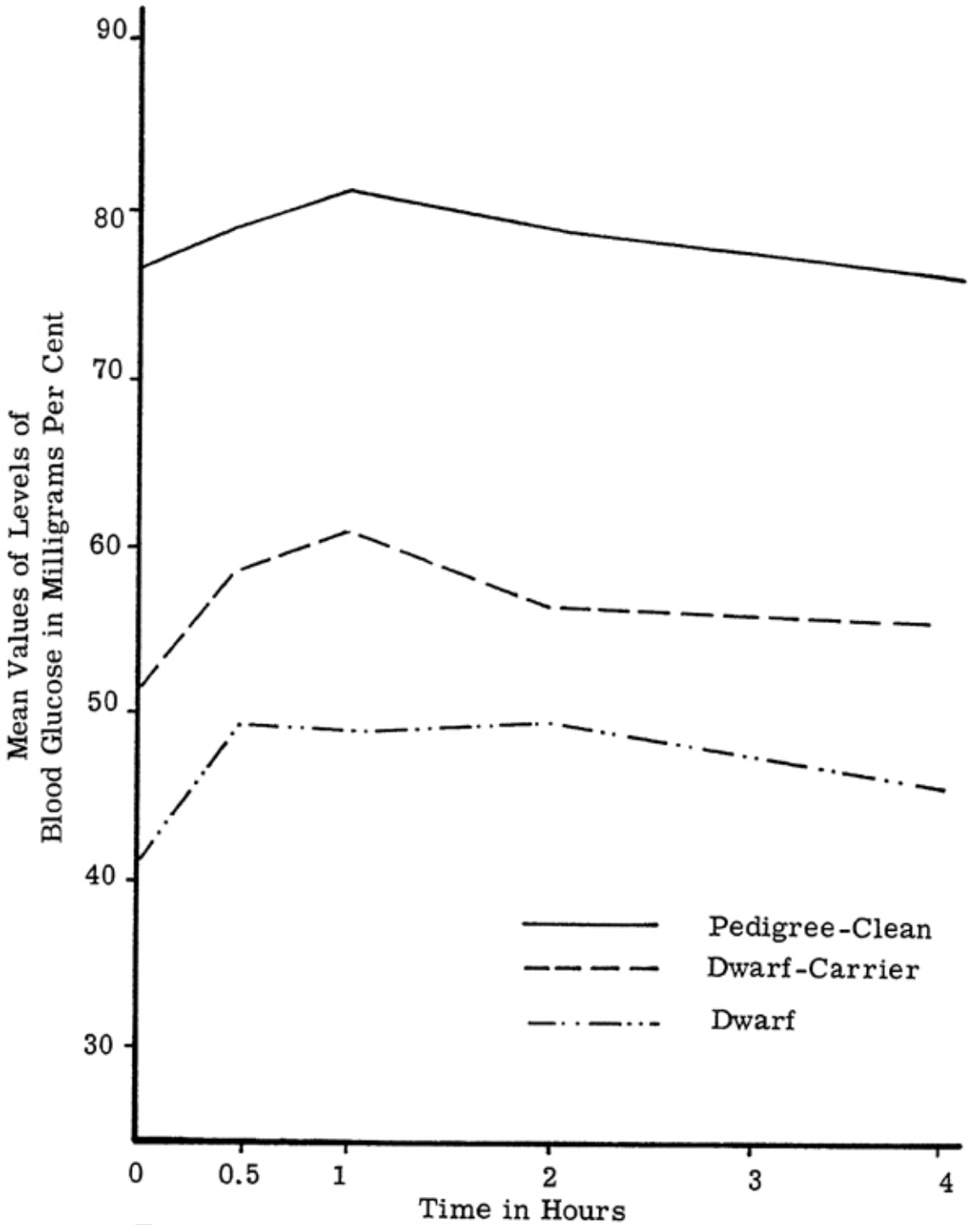

Fig. 9 - Influence of adrenalin injection on levels of blood glucose.

At the end of the four-hour test period following adrenalin injection, only the pedigree-clean cattle had reduced their blood sugar levels to normal. Following the administration of insulin, Heidenrich and associates (1955) reported that normal and dwarf cattle were significantly different in their ability to restore their blood sugar levels to normal following stress.

In another investigation involving the injection of insulin, Foley *et al.* (1956) found that the blood sugar levels of dwarf cattle dropped to low levels more rapidly and returned to normal at a slower rate, as compared with pedigree-clean cattle. The influence exerted upon blood sugar levels of these two genotypes by adrenalin is also of considerable interest from a physiological standpoint. Following the injection of adrenalin, which increases the level of blood sugar whereas insulin decreases it, the blood sugar levels of the dwarf cattle rose to their peak more rapidly and return to normal at a slower rate, compared with pedigree-clean cattle. Therefore, the blood glucose levels of dwarf cattle are evidently affected more by stressful conditions, whether they exert a hypoglycemic or hyperglycemic influence, than are those of pedigree-clean individuals. Likely, a flaw exists in the dwarf's homeostatic mechanism for facilitating restoration of normal blood glucose levels following stress.

Total Numbers of White Cells

Differences between genotypes and between hours of blood sample collection were not significant in total numbers of white blood cells before and after adrenalin injection (Table 18A). The genotype x time interval interaction was not significant.

Following the injection of adrenalin, a rapid increase in numbers of white cells occurred in the blood of the pedigree-clean cattle (Table 18). An almost

TABLE 18-MEANS AND STANDARD DEVIATIONS FOR TOTAL NUMBERS OF WHITE CELLS PER 1/50 CUBIC MILLIMETER OF BLOOD IN DWARF, DWARF-CARRIER, AND PEDIGREE-CLEAN BEEF CATTLE AT DIFFERENT INTERVALS FOLLOWING INTRAVENOUS INJECTIONS OF 0.4 CUBIC CENTIMETER OF ADRENALIN (DILUTED 1 TO 10,000) PER HUNDRED POUNDS OF BODY WEIGHT

Time of Sample	Dwarf Mean ± Standard Deviation	Dwarf-Carrier Mean ± Standard Deviation	Pedigree-Clean Mean ± Standard Deviation
0.0 hour	166.20 ± 36.18	147.92 ± 23.16	160.30 ± 25.18
0.5 hour	158.80 ± 36.79	145.07 ± 22.74	168.40 ± 26.24
1.0 hour	180.80 ± 50.44	152.71 ± 16.91	170.30 ± 34.36
2.0 hours	163.60 ± 36.92	161.14 ± 28.27	175.40 ± 37.58
4.0 hours	190.80 ± 55.20	172.00 ± 27.24	199.00 ± 30.19

equally rapid decrease took place in the dwarf cattle. A slight decrease in numbers of circulating leucocytes occurred in the heterozygotes. Although genotype differences were not regarded as significant, it should be pointed out that these differences were significant (P less than .05) in Sample I (Table 18A). Since genotype differences were not significant at the .05 level of probability in Sample II, however, significance must be regarded as non-conclusive. The irregularity of white cell numbers in the blood of the dwarf cattle following adrenalin injection is of interest in its contrast to the more gradual and persisting changes occurring within the circulation of the normal-appearing individuals. Such is in-

dicative of the relative inability of the dwarf animal to cope with, or counteract, sudden environmental changes. This occurred in a somewhat similar manner following the administration of insulin (Table 10).

Leucocytosis in response to adrenalin injection, which occurred within the first 30 minutes following injection in the pedigree-clean cattle, but not in the dwarf and dwarf-carrier animals, has also been reported in humans (Martin, 1932; Lucia *et al.,* 1937; Bierman *et al.,* 1952) and in rats and mice (Harlow and Selye, 1937). White and associates (1950) stated that the changes in the leucocyte count in humans as a result of adrenalin injection were similar to, but not identical with, those produced by the injection of another stressing agent used in this study, the pituitary adrenocorticotrophic hormone (ACTH), which will be discussed subsequently. As noted previously, adrenalin, which is secreted by the medulla of the adrenal gland in response to stress, is believed to mediate an increased secretion of this hormone of the hypophysis.

Influence of Pituitary Adrenocorticotrophic Hormone on Various Blood Components in Dwarf, Dwarf-Carrier, and Pedigree-Clean Beef Cattle

Percentages of Lymphocytes

Genotype differences in lymphocyte percentage before and after intramuscular injection of pituitary adrenocorticotrophic hormone (ACTH) were not significant (Table 19A). Neither was the genotype x time interval interaction, but differences between hours of sampling were highly significant (P less than .005).

ACTH did not exert a pronounced influence on percentages of lymphocytes during the first hour subsequent to injection of the hormone (Table 19).

TABLE 19-MEANS AND STANDARD DEVIATIONS FOR PERCENTAGES OF LYMPHOCYTES IN THE BLOOD OF DWARF, DWARF-CARRIER, AND PEDIGREE-CLEAN BEEF CATTLE AT DIFFERENT INTERVALS FOLLOWING INTRAMUSCULAR INJECTIONS OF 80 UP JOHN UNITS OF ACTH PER ANIMAL

Time of Sample	Dwarf Mean ± Standard Deviation	Dwarf-Carrier Mean ± Standard Deviation	Pedigree-Clean Mean ± Standard Deviation
0.0 hour	56.56 ± 8.85	60.59 ± 7.32	55.63 ± 10.13
1.0 hour	56.54 ± 8.06	59.10 ± 7.76	57.92 ± 10.71
4.0 hours	41.20 ± 8.52	41.91 ± 6.18	39.73 ± 8.70
8.0 hours	51.74 ± 5.90	36.16 ± 5.41	34.53 ± 8.84

By the fourth hour following hormone administration, however, a substantial decrease in the percentage of lymphocytes had occurred in the cattle of each genotype.

It is of physiological interest to note that lymphocyte percentages were at their lowest levels in the pedigree-clean and dwarf-carrier cattle at eight hours following ACTH injection, while by this time, lymphocyte percentage in the

dwarf cattle had risen to heights only slightly less than those found in the initial blood sample. This is a further indication of insufficiency in the adrenal-pituitary homeostatic mechanisms of the dwarf animal, and suggests a possible flaw in its adrenal cortex. Extensive research with small animals, including the investigations of Dougherty and White (1943a) and Loosli *et al.* (1951), has shown that ACTH, which stimulates the adrenal cortex to release glucocorticoids, causes a decrease in the percentage of circulating lymphocytes. The glucocorticoids are believed to be the factor directly responsible for this decrease in lymphocyte percentages. On the basis of this investigation, therefore, the glucocorticoid supply in dwarf cattle appears sufficient to cause decreased percentages of blood lymphocytes only for a limited period following the release of ACTH by the hypophysis in response to stress.

Percentages of Neutrophils

Non-significant differences between genotypes were found with regard to the percentage of neutrophils in the circulation before and after ACTH injection. The genotype x time interval interaction was also non-significant (Table 20A), but differences between hours of sample collection were highly significant (P less than .005).

Table 20 shows that proportions of neutrophils increased throughout the test period following ACTH injection in the pedigree-clean and dwarf-carrier cattle, but reached their peak in the dwarf cattle in the sample collected at four

TABLE 20-MEANS AND STANDARD DEVIATIONS FOR PERCENTAGES OF NEUTROPHILS IN THE BLOOD OF DWARF, DWARF-CARRIER, AND PEDIGREE-CLEAN BEEF CATTLE AT DIFFERENT INTERVALS FOLLOWING INTRAMUSCULAR INJECTIONS OF 80 UPJOHN UNITS OF ACTH PER ANIMAL

Time of Sample	Dwarf Mean ± Standard Deviation	Dwarf-Carrier Mean ± Standard Deviation	Pedigree-Clean Mean ± Standard Deviation
0.0 hour	25.14 ± 7.50	23.07 ± 7.39	27.93 ± 7.81
1.0 hour	28.26 ± 4.97	27.01 ± 8.52	29.77 ± 8.93
4.0 hours	49.48 ± 11.29	50.66 ± 8.44	53.39 ± 7.97
8.0 hours	43.80 ± 5.80	60.26 ± 6.99	61.50 ± 8.45

hours following administration of the hormone. The decrease in neutrophil percentage in the dwarf cattle between the fourth and eighth hours following ACTH injection corresponds with the dwarfs' increasing percentage of lymphocytes (Table 19) during this period.

Throughout the period of sampling, a higher percentage of neutrophils was found in the blood of the pedigree-clean cattle than in that of the other genotypes.

Percentages of Eosinophils

Differences between hours of sampling in percentage of eosinophils before and after ACTH injection were highly significant (P less than .005, Table 21A). ACTH injection also caused pronounced differences between hours of sampling in percentages of lymphocytes (Table 19A) and in percentages of neutrophils (Table 20A). The genotype x time interval interaction and the differences between genotypes were not significant.

The blood of the dwarf cattle contained a higher percentage of eosinophils than that of the other genotypes prior to ACTH injection and throughout the sampling period (Table 21). A declining percentage of eosinophils occurred in cattle of each genotype throughout the test period following hormone administration.

TABLE 21-MEANS AND STANDARD DEVIATIONS FOR PERCENTAGES OF EOSINOPHILS IN THE BLOOD OF DWARF, DWARF-CARRIER, AND PEDIGREE-CLEAN BEEF CATTLE AT DIFFERENT INTERVALS FOLLOWING INTRAMUSCULAR INJECTIONS OF 80 UPJOHN UNITS OF ACTH PER ANIMAL

Time of Sample	Dwarf Mean ± Standard Deviation	Dwarf-Carrier Mean ± Standard Deviation	Pedigree-Clean Mean ± Standard Deviation
0.0 hour	18.30 ± 5.47	16.33 ± 5.06	16.43 ± 3.93
1.0 hour	15.20 ± 5.00	13.88 ± 4.45	12.37 ± 4.81
4.0 hours	9.32 ± 4.98	7.41 ± 3.69	6.87 ± 1.88
8.0 hours	4.46 ± 2.09	3.56 ± 3.14	3.95 ± 2.10

Numbers of Lymphocytes

Highly significant (P less than .005) differences were found between genotypes before and after injection of ACTH in the cattle tested in Sample II (Table 22A) in numbers of circulating lymphocytes. However, since these genotype differences were not great enough in the cattle in Sample I to be significant at the .05 level of probability, genotype differences must be considered non-significant.

Throughout the test period, numbers of circulating lymphocytes were greater in the dwarf cattle than in either of the other genotypes (Table 22). Between four and eight hours following ACTH injection, immense differences occurred between the dwarf cattle and the normal-appearing cattle in numbers of circulating lymphocytes. During this period, lymphocyte numbers decreased in the pedigree-clean and dwarf-carrier cattle, while they increased considerably in the dwarf cattle. This would indicate that a deficiency may exist in the physiological mechanism in dwarf cattle which restores lymphocyte numbers to normal following stress.

That the pituitary adrenocorticotrophic hormone is a factor in the regulation of numbers of circulating lymphocytes has been well substantiated (Doug-

TABLE 22-MEANS AND STANDARD DEVIATIONS FOR NUMBERS OF LYMPHOCYTES PER 1/50 CUBIC MILLIMETER OF BLOOD IN DWARF, DWARF-CARRIER, AND PEDIGREE-CLEAN BEEF CATTLE AT DIFFERENT INTERVALS FOLLOWING INTRAMUSCULAR INJECTIONS OF 80 UPJOHN UNITS OF ACTH PER ANIMAL

Time of Sample	Dwarf Mean ± Standard Deviation	Dwarf-Carrier Mean ± Standard Deviation	Pedigree-Clean Mean ± Standard Deviation
0.0 hour	99.30 ± 16.97	87.56 ± 19.21	93.90 ± 24.70
1.0 hour	115.94 ± 26.97	96.59 ± 19.86	96.50 ± 24.17
4.0 hours	110.92 ± 20.08	102.90 ± 22.15	92.20 ± 14.92
8.0 hours	132.02 ± 28.53	88.22 ± 10.46	86.20 ± 18.78

herty and White, 1943b, 1944, 1945; Reinhardt and Li, 1945; Yoffey *et al.*, 1946a, 1946b; Hills *et al.*, 1948; Kass *et al.*, 1951).

The administration of ACTH, which stimulates secretion of adrenal glucocorticoids, has been shown to result in decreased numbers of circulating lymphocytes in humans (Hills *et al.*, 1948), in cats (Yoffey *et al.*, 1946b), in rats (Reinhardt and Li, 1945; Yoffey and Baxter, 1946a), and in mice (Dougherty and White, 1943b; Kass *et al.*, 1951). Crafts (1941) reported that hypophysectomy, which removes all endogenous ACTH secretion, results in increased numbers of circulating lymphocytes.

Numbers of Neutrophils

The genotype x time interval interaction and genotype differences in neutrophil numbers before and after ACTH injection were not significant (Table 23A). Differences between hours of sampling were significant at the .005 level. Although the genotype x time interval interaction was regarded as non-significant, note that a significant interaction (P less than .05) was found in Sample II.

ACTH was exerted only slight neutrophilic effects in the pedigree-clean cattle, in comparison with those in the other genotypes, during the first hour following injection (Table 23). Between one and four hours subsequent to ACTH administration, neutrophil numbers increased rapidly in the circulation of animals of each genotype. The dwarf cattle seemed to reach their peak in

TABLE 23-MEANS AND STANDARD DEVIATIONS FOR NUMBERS OF NEUTROPHILS PER 1/50 CUBIC MILLIMETER OF BLOOD IN DWARF, DWARF-CARRIER AND PEDIGREE-CLEAN BEEF CATTLE AT DIFFERENT INTERVALS FOLLOWING INTRAMUSCULAR INJECTIONS OF 80 UPJOHN UNITS OF ACTH PER ANIMAL

Time of Sample	Dwarf Mean ± Standard Deviation	Dwarf-Carrier Mean ± Standard Deviation	Pedigree-Clean Mean ± Standard Deviation
0.0 hour	45.18 ± 17.61	34.40 ± 15.84	47.40 ± 15.77
1.0 hour	57.78 ± 13.74	44.67 ± 18.77	49.30 ± 15.64
4.0 hours	136.44 ± 43.44	122.95 ± 24.62	127.50 ± 32.74
8.0 hours	110.72 ± 21.38	149.11 ± 28.21	157.90 ± 40.34

neutrophil numbers around the middle of the eight-hour test period. Numbers of circulating neutrophils continued to increase during the last half of the sampling period in the pedigree-clean and dwarf-carrier cattle.

Increased numbers of neutrophils following ACTH injection have also been reported in humans (Hills *et al.,* 1948) and in mice (Dougherty and White, 1943a).

Numbers of Eosinophils

Significant differences (P less than .05) were found between genotypes in eosinophil numbers before and after ACTH injection (Table 24A). Differences between hours of sampling were highly significant (P less than .005). The genotype x time interval interaction was not significant. Figure 10 illustrates the differences between genotypes and between hours of sample collection.

Table 24 and Figure 10 show eosinophil numbers to be higher throughout the test period in the blood of the dwarf animals than in that of the other genotypes. Figure 10 shows that eosinophil numbers dropped more rapidly following ACTH injection in the pedigree-clean cattle than in the other genotypes. Peripheral neutropenia occurred somewhat similarly throughout the sampling period in the dwarf and dwarf-carrier cattle.

TABLE 24-MEANS AND STANDARD DEVIATIONS FOR NUMBERS OF EOSINOPHILS PER 1/50 CUBIC MILLIMETER OF BLOOD IN DWARF, DWARF-CARRIER, AND PEDIGREE-CLEAN BEEF CATTLE AT DIFFERENT INTERVALS FOLLOWING INTRAMUSCULAR INJECTIONS OF 80 UPJOHN UNITS OF ACTH PER ANIMAL

Time of Sample	Dwarf Mean ± Standard Deviation	Dwarf-Carrier Mean ± Standard Deviation	Pedigree-Clean Mean ± Standard Deviation
0.0 hour	32.90 ± 13.18	24.47 ± 11.21	27.60 ± 7.69
1.0 hour	30.70 ± 10.01	23.19 ± 9.19	20.70 ± 8.19
4.0 hours	25.08 ± 13.51	18.82 ± 12.18	16.40 ± 4.88
8.0 hours	10.86 ± 4.45	8.74 ± 7.83	10.20 ± 5.80

Decreased numbers of eosinophils have also been found in humans (Hills *et al.,* 1948) following intramuscular injection of adrenocorticotrophic hormone.

Total Numbers of White Cells

Table 25A shows that differences between hours of sampling, with regard to total numbers of white blood cells before and after ACTH injection, were highly significant (P less than .005). It will be recalled that in Sample II of the analysis of variance of lymphocyte numbers (Table 22A), genotype differences before and after the administration of ACTH were highly significant (P less than .005), while in Sample I, these differences were non-significant. This difference between samples is reflected in the analysis of total numbers of white cells (Table 25A). Because of the disagreement between samples, this determination is regarded as non-conclusive and warrants further study. The genotype x time interval interaction was not significant.

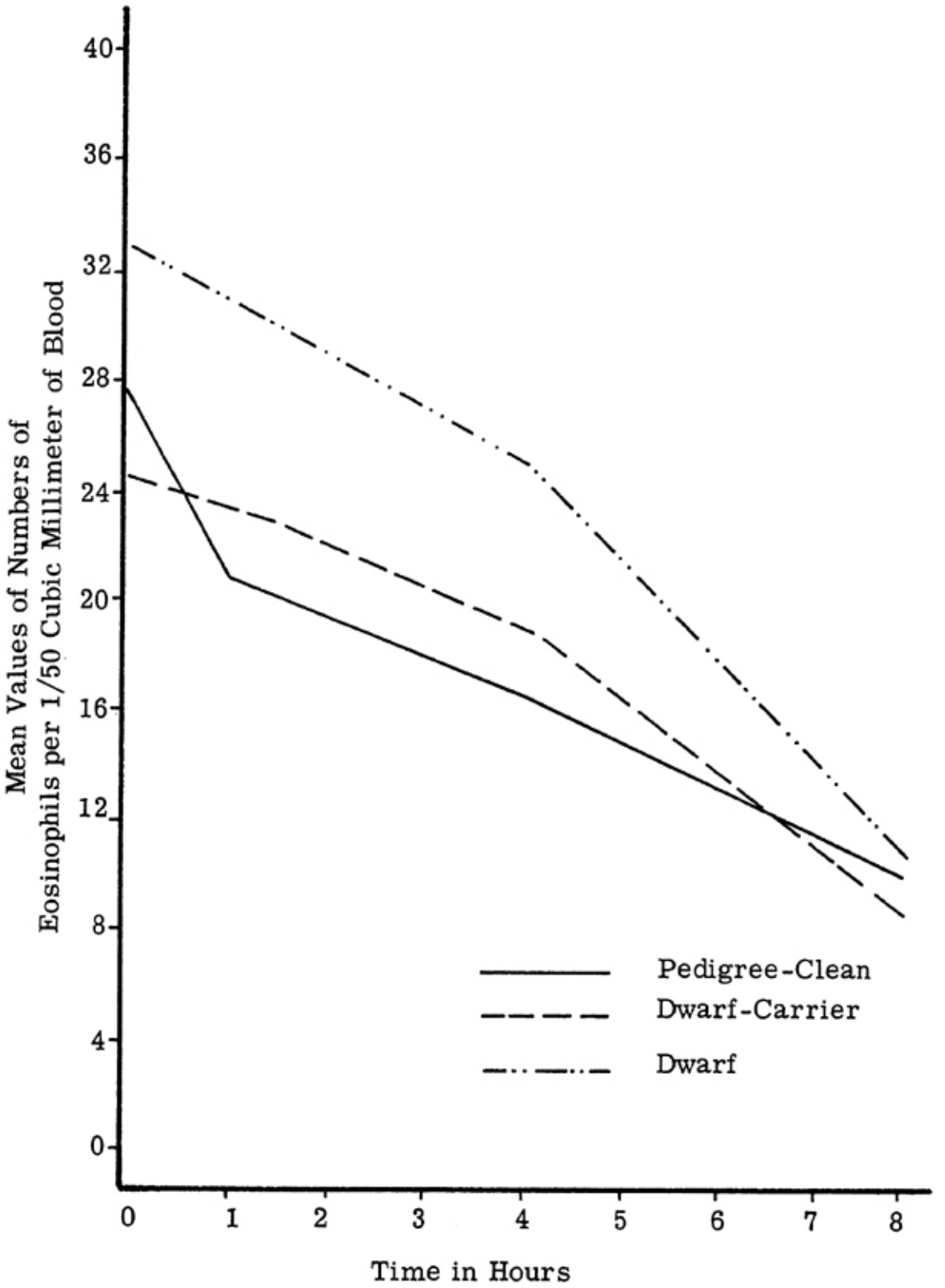

Fig. 10 - Influence of adrenocorticotrophic hormone injection on numbers of circulating eosinophils.

The response of each genotype to the stress stimuli initiated by ACTH injection is of considerable interest from a physiological standpoint. Table 25 shows white cell numbers in the dwarf and dwarf-carrier cattle rose rapidly during the first four hours following ACTH injection, while in the pedigree-clean cattle, a decline in leucocyte numbers occurred during the first hour subsequent to injection of the hormone. Between the first and fourth hours of the test period, white cell numbers increased at a rapid rate in the pedigree-clean individuals.

TABLE 25-MEANS AND STANDARD DEVIATIONS FOR TOTAL NUMBERS OF WHITE CELLS PER 1/50 CUBIC MILLIMETER OF BLOOD IN DWARF, DWARF-CARRIER, AND PEDIGREE-CLEAN BEEF CATTLE AT DIFFERENT INTERVALS FOLLOWING INTRAMUSCULAR INJECTIONS OF 80 UPJOHN UNITS OF ACTH PER ANIMAL

Time of Sample	Dwarf Mean ± Standard Deviation	Dwarf-Carrier Mean ± Standard Deviation	Pedigree-Clean Mean ± Standard Deviation
0.0 hour	177.40 ± 28.64	146.46 ± 37.15	168.80 ± 30.44
1.0 hour	204.40 ± 30.08	164.46 ± 33.99	166.10 ± 25.07
4.0 hours	272.40 ± 38.20	245.84 ± 42.82	235.90 ± 33.95
8.0 hours	253.60 ± 41.69	246.07 ± 27.10	254.40 ± 43.05

White *et al.* (1950) indicated that leucocyte numbers in humans were altered similarly by injections of adrenalin and ACTH. Results of this investigation indicate that numbers of circulating leucocytes are affected to a greater extent during a four-hour time period by ACTH injection (Table 25) than by the administration of adrenalin (Table 11), although the immediate influence of adrenalin on numbers of white blood cells was shown to be greater than that of ACTH.

SUMMARY

Previous investigations at the Missouri Agricultural Experiment Station indicated that dwarf, dwarf-carrier, and pedigree-clean beef cattle possibly differed significantly in their ability to maintain normal body function in the presence of a stressful environment. Therefore, a study was made to determine the physiological response of the three genotypes for the dwarf gene to hormone-induced stress. More accurate and practical methods of identifying heterozygous individuals, or carriers of the dwarf gene were objectives of the study.

Each of 22 Hereford beef animals used in the investigation, including 5 dwarfs, 7 pedigree-clean cows, and 10 dwarf-carrier cows, was injected with non-physiological dosages of insulin, adrenalin, and pituitary adrenocorticotrophic hormone (ACTH). Samples of blood were collected from each experimental animal immediately before the injection of each hormone, and at various intervals following hormone administration.

In the laboratory, measurements were made on various blood components in each sample of blood collected. The total number of leucocytes per 1/50

cubic millimeter of blood was determined in each sample. Differential white blood cell counts were made to determine the average percentages of circulating lymphocytes, neutrophils, and eosinophils in the cattle of each genotype at various intervals before and after hormone administration.

To give a more accurate evaluation of alterations within the blood stream, the actual numbers of lymphocytes, neutrophils, and eosinophils present were calculated from the total number of white blood cells in the circulation and the percentages of each of the major types of leucocytes present.

Levels of blood glucose before and after hormone injection were determined in each genotype by the modified Folin-Wu method of blood sugar analysis,

Before and after the injection of insulin, dwarf, dwarf-carrier, and pedigree-clean cattle differed significantly in percentages of lymphocytes (P less than .01), percentages of eosinophils (P less than .005), and numbers of eosinophils (P less than .005) in the blood, and also in levels of blood glucose (P less than .005). Significant differences (P less than .005) occurred between genotypes in percentages and numbers of lymphocytes and percentages and numbers of eosinophils in the circulation, and in levels of blood glucose before and after the injection of adrenalin. Genotypes differed significantly (P less than .05) in numbers of eosinophils before and after the injection of ACTH.

Significant differences in numbers of circulating neutrophils (P less than .01), levels of blood glucose (P less than .005), and total numbers of white cells (P less than .005) occurred between hours of blood sample collection before and after the administration of insulin. Differences between hours of sampling before and after adrenalin injection were not significant. Prior to and following the injection of ACTH, percentages of lymphocytes, percentages of neutrophils, percentages of eosinophils, numbers of neutrophils, numbers of eosinophils, and total numbers of white cells in the blood were significantly different (P less than .005) between hours of blood sample collection.

No significant genotype x time interval interactions occurred following hormone treatments in this investigation. This means that individuals of the three genotypes did not differ significantly in their response to the hormones administered. However, significant differences did occur between animals of the three genotypes in the average levels of several blood components before and after hormone injection.

Although genotype differences were found in several characteristics, none of these, singly or in combination, were of sufficient magnitude to positively identify individuals as to genotype for the dwarf gene. Certain of the genotype differences, such as those found in numbers and percentages of eosinophils, do appear promising enough to suggest that more detailed research, as to their physiological causes, be conducted.

There were indications, as have been noted in previous studies at the Missouri Agricultural Experiment Station, that the adrenal-pituitary axis does not function normally in dwarf cattle. The evidence obtained does not conclusively indicate whether this is a direct or indirect effect of the gene for dwarfism.

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APPENDIX

TABLE 3A-ANALYSIS OF VARIANCE FOR PERCENTAGES OF LYMPHOCYTES IN THE BLOOD OF DWARF, DWARF-CARRIER, AND PEDIGREE-CLEAN BEEF CATTLE FOLLOWING INTRAVENOUS INJECTIONS OF 0.8 UNIT OF INSULIN PER KILOGRAM OF BODY WEIGHT

Source	Degrees of Freedom	Mean Square		F Ratio	
		Sample I	Sample II	Sample I	Sample II
Between Hours	3	95.18	35.62	1.31	0.42
Between Genotypes	2	375.90	1085.62	5.16**	12.83***
Hours x Genotypes	6	31.66	29.87	0.43	0.35
Within	48	72.84	84.64		
Total	59				

**Probability of chance occurrence less than .01.
***Probability of chance occurrence less than .005.

TABLE 4A-ANALYSIS OF VARIANCE FOR PERCENTAGES OF NEUTROPHILS IN THE BLOOD OF DWARF, DWARF-CARRIER, AND PEDIGREE-CLEAN BEEF CATTLE FOLLOWING INTRAVENOUS INJECTIONS OF 0.8 UNIT OF INSULIN PER KILOGRAM OF BODY WEIGHT

Source	Degrees of Freedom	Mean Square		F Ratio	
		Sample I	Sample II	Sample I	Sample II
Between Hours	3	272.48	253.33	3.06*	1.60
Between Genotypes	2	215.25	18.59	2.42	0.12
Hours x Genotypes	6	32.11	42.93	0.36	0.27
Within	48	89.00	158.35		
Total	59				

*Probability of chance occurrence less than .05.

TABLE 5A-ANALYSIS OF VARIANCE FOR PERCENTAGES OF EOSINOPHILS IN THE BLOOD OF DWARF, DWARF-CARRIER, AND PEDIGREE-CLEAN BEEF CATTLE FOLLOWING INTRAVENOUS INJECTIONS OF 0.8 UNIT OF INSULIN PER KILOGRAM OF BODY WEIGHT

Source	Degrees of Freedom	Mean Square		F Ratio	
		Sample I	Sample II	Sample I	Sample II
Between Hours	3	50.79	113.20	2.20	2.65
Between Genotypes	2	548.67	944.65	23.72***	22.11***
Hours x Genotypes	6	2.13	9.80	0.09	0.23
Within	48	23.13	42.72		
Total	59				

***Probability of chance occurrence less than .005.

TABLE 6A-ANALYSIS OF VARIANCE FOR NUMBERS OF LYMPHOCYTES IN THE BLOOD OF DWARF, DWARF-CARRIER, AND PEDIGREE-CLEAN BEEF CATTLE FOLLOWING INTRAVENOUS INJECTIONS OF 0.8 UNIT OF INSULIN PER KILOGRAM OF BODY WEIGHT

Source	Degrees of Freedom	Mean Square		F Ratio	
		Sample I	Sample II	Sample I	Sample II
Between Hours	3	291.62	873.81	0.74	1.12
Between Genotypes	2	1479.63	2109.65	3.74*	2.71
Hours x Genotypes	6	498.18	177.94	1.26	0.23
Within	48	395.21	777.28		
Total	59				

*Probability of chance occurrence less than .05.

TABLE 7A-ANALYSIS OF VARIANCE FOR NUMBERS OF NEUTROPHILS IN THE BLOOD OF DWARF, DWARF-CARRIER, AND PEDIGREE-CLEAN BEEF CATTLE FOLLOWING INTRAVENOUS INJECTIONS OF 0.8 UNIT OF INSULIN PER KILOGRAM OF BODY WEIGHT

Source	Degrees of Freedom	Mean Square		F Ratio	
		Sample I	Sample II	Sample I	Sample II
Between Hours	3	2837.31	2551.25	8.16***	4.85**
Between Genotypes	2	357.96	190.46	1.03	0.36
Hours x Genotypes	6	76.56	62.70	0.22	0.12
Within	48	347.77	525.77		
Total	59				

**Probability of chance occurrence less than .01.
***Probability of chance occurrence less than .005.

TABLE 8A - ANALYSIS OF VARIANCE FOR NUMBERS OF EOSINOPHILS IN THE BLOOD OF DWARF, DWARF-CARRIER, AND PEDIGREE-CLEAN BEEF CATTLE FOLLOWING INTRAVENOUS INJECTIONS OF 0.8 UNIT OF INSULIN PER KILOGRAM OF BODY WEIGHT

Source	Degrees of Freedom	Mean Square		F Ratio	
		Sample I	Sample II	Sample I	Sample II
Between Hours	3	106.16	219.42	1.23	1.38
Between Genotypes	2	1857.92	3021.29	21.51***	18.94***
Hours x Genotypes	6	9.04	23.68	0.10	0.15
Within	48	86.36	159.53		
Total	59				

***Probability of chance occurrence less than .005.

TABLE 9A - ANALYSIS OF VARIANCE FOR LEVELS OF BLOOD GLUCOSE IN THE BLOOD OF DWARF, DWARF-CARRIER, AND PEDIGREE-CLEAN BEEF CATTLE FOLLOWING INTRAVENOUS INJECTIONS OF 0.8 UNIT OF INSULIN PER KILOGRAM OF BODY WEIGHT

Source	Degrees of Freedom	Mean Square		F Ratio	
		Sample I	Sample II	Sample I	Sample II
Between Hours	3	1986.85	2903.09	13.27***	27.39***
Between Genotypes	2	1157.05	2895.76	7.73***	27.32***
Hours x Genotypes	6	41.70	167.66	0.28	1.58
Within	48	149.74	105.98		
Total	59				

***Probability of chance occurrence less than .005.

TABLE 10A - ANALYSIS OF VARIANCE FOR TOTAL NUMBERS OF WHITE CELLS IN THE BLOOD OF DWARF, DWARF-CARRIER, AND PEDIGREE-CLEAN BEEF CATTLE FOLLOWING INTRAVENOUS INJECTIONS OF 0.8 UNIT OF INSULIN PER KILOGRAM OF BODY WEIGHT

Source	Degrees of Freedom	Mean Square		F Ratio	
		Sample I	Sample II	Sample I	Sample II
Between Hours	3	4278.51	4693.66	6.47***	6.00***
Between Genotypes	2	563.89	2850.02	0.85	3.64*
Hours x Genotypes	6	413.55	909.46	0.63	1.16
Within	48	661.15	782.18		
Total	59				

*Probability of chance occurrence less than .05.
***Probability of chance occurrence less than .005.

TABLE 11A-ANALYSIS OF VARIANCE FOR PERCENTAGES OF LYMPHOCYTES IN THE BLOOD OF DWARF, DWARF-CARRIER, AND PEDIGREE-CLEAN BEEF CATTLE FOLLOWING INTRAVENOUS INJECTIONS OF 0.4 CUBIC CENTIMETER OF ADRENALIN (DILUTED 1 TO 10,000) PER HUNDRED POUNDS OF BODY WEIGHT

Source	Degrees of Freedom	Mean Square		F Ratio	
		Sample I	Sample II	Sample I	Sample II
Between Hours	4	52.06	106.28	0.62	1.43
Between Genotypes	2	740.01	682.38	8.79***	9.17***
Hours x Genotypes	8	52.08	132.44	0.62	1.78
Within	60	84.22	74.42		
Total	74				

***Probability of chance occurrence less than .005.

TABLE 12A-ANALYSIS OF VARIANCE FOR PERCENTAGES OF NEUTROPHILS IN THE BLOOD OF DWARF, DWARF-CARRIER, AND PEDIGREE-CLEAN BEEF CATTLE FOLLOWING INTRAVENOUS INJECTIONS OF 0.4 CUBIC CENTIMETER OF ADRENALIN (DILUTED 1 TO 10,000) PER HUNDRED POUNDS OF BODY WEIGHT

Source	Degrees of Freedom	Mean Square		F Ratio	
		Sample I	Sample II	Sample I	Sample II
Between Hours	4	55.53	37.18	0.78	0.77
Between Genotypes	2	37.47	82.19	0.53	1.71
Hours x Genotypes	8	36.31	62.95	0.51	1.31
Within	60	70.97	48.09		
Total	74				

TABLE 13A-ANALYSIS OF VARIANCE FOR PERCENTAGES OF EOSINOPHILS IN THE BLOOD OF DWARF, DWARF-CARRIER, AND PEDIGREE-CLEAN BEEF CATTLE FOLLOWING INTRAVENOUS INJECTIONS OF 0.4 CUBIC CENTIMETER OF ADRENALIN (DILUTED 1 TO 10,000) PER HUNDRED POUNDS OF BODY WEIGHT

Source	Degrees of Freedom	Mean Square		F Ratio	
		Sample I	Sample II	Sample I	Sample II
Between Hours	4	28.49	67.24	1.36	1.97
Between Genotypes	2	979.12	1173.88	46.85***	34.43***
Hours x Genotypes	8	20.09	38.84	0.96	1.14
Within	60	20.90	34.09		
Total	74				

***Probability of chance occurrence less than .005.

TABLE 14A-ANALYSIS OF VARIANCE FOR NUMBERS OF LYMPHOCYTES IN THE BLOOD OF DWARF, DWARF-CARRIER, AND PEDIGREE-CLEAN BEEF CATTLE FOLLOWING INTRAVENOUS INJECTIONS OF 0.4 CUBIC CENTIMETER OF ADRENALIN (DILUTED 1 TO 10,000) PER HUNDRED POUNDS OF BODY WEIGHT

Source	Degrees of Freedom	Mean Square		F Ratio	
		Sample I	Sample II	Sample I	Sample II
Between Hours	4	616.68	1482.21	1.29	3.58
Between Genotypes	2	3247.87	3986.24	6.81***	9.64***
Hours x Genotypes	8	126.36	493.09	0.27	1.19
Within	60	476.66	413.61		
Total	74				

*Probability of chance occurrence less than .05.

***Probability of chance occurrence less than .005.

TABLE 15A - ANALYSIS OF VARIANCE FOR NUMBERS OF NEUTROPHILS IN THE BLOOD OF DWARF, DWARF-CARRIER, AND PEDIGREE-CLEAN BEEF CATTLE FOLLOWING INTRAVENOUS INJECTIONS OF 0.4 CUBIC CENTIMETER OF ADRENALIN (DILUTED 1 TO 10,000) PER HUNDRED POUNDS OF BODY WEIGHT

Source	Degrees of Freedom	Mean Square		F Ratio	
		Sample I	Sample II	Sample I	Sample II
Between Hours	4	421.35	455.48	1.15	1.14
Between Genotypes	2	1089.56	437.88	2.96	1.09
Hours x Genotypes	8	155.89	229.85	0.42	0.57
Within	60	367.52	399.99		
Total	74				

TABLE 16A - ANALYSIS OF VARIANCE FOR NUMBERS OF EOSINOPHILS IN THE BLOOD OF DWARF, DWARF-CARRIER, AND PEDIGREE-CLEAN BEEF CATTLE FOLLOWING INTRAVENOUS INJECTIONS OF 0.4 CUBIC CENTIMETER OF ADRENALIN (DILUTED 1 TO 10,000) PER HUNDRED POUNDS OF BODY WEIGHT

Source	Degrees of Freedom	Mean Square		F Ratio	
		Sample I	Sample II	Sample I	Sample II
Between Hours	4	50.01	83.79	0.41	0.50
Between Genotypes	2	3312.23	3525.73	26.86***	20.94***
Hours x Genotypes	8	49.59	93.50	0.40	0.56
Within	60	123.31	168.36		
Total	74				

***Probability of chance occurrence less than .005.

TABLE 17A-ANALYSIS OF VARIANCE FOR LEVELS OF BLOOD GLUCOSE IN THE BLOOD OF DWARF, DWARF-CARRIER, AND PEDIGREE-CLEAN BEEF CATTLE FOLLOWING INTRAVENOUS INJECTIONS OF 0.4 CUBIC CENTIMETER OF ADRENALIN (DILUTED 1 TO 10,000) PER HUNDRED POUNDS OF BODY WEIGHT

Source	Degrees of Freedom	Mean Square		F Ratio	
		Sample I	Sample II	Sample I	Sample II
Between Hours	4	108.83	145.19	1.56	1.95
Between Genotypes	2	5269.13	8199.32	75.73***	110.25***
Hours x Genotypes	8	18.74	11.95	0.27	0.16
Within	60	69.58	74.37		
Total	74				

***Probability of chance occurrence less than .005.

TABLE 18A-ANALYSIS OF VARIANCE FOR TOTAL NUMBERS OF WHITE CELLS IN THE BLOOD OF DWARF, DWARF-CARRIER, AND PEDIGREE-CLEAN BEEF CATTLE FOLLOWING INTRAVENOUS INJECTIONS OF 0.4 CUBIC CENTIMETER OF ADRENALIN (DILUTED 1 TO 10,000) PER HUNDRED POUNDS OF BODY WEIGHT

Source	Degrees of Freedom	Mean Square		F Ratio	
		Sample I	Sample II	Sample I	Sample II
Between Hours	4	1456.02	2148.45	1.22	1.85
Between Genotypes	2	4400.04	2402.76	3.67*	2.07
Hours x Genotypes	8	134.14	381.09	0.11	0.33
Within	60	1197.33	1163.01		
Total	74				

*Probability of chance occurrence less than .05.

TABLE 19A-ANALYSIS OF VARIANCE FOR PERCENTAGES OF LYMPHOCYTES IN THE BLOOD OF DWARF, DWARF-CARRIER, AND PEDIGREE-CLEAN BEEF CATTLE FOLLOWING INTRAMUSCULAR INJECTIONS OF 80 UPJOHN UNITS OF ACTH PER ANIMAL

Source	Degrees of Freedom	Mean Square		F Ratio	
		Sample I	Sample II	Sample I	Sample II
Between Hours	3	1545.55	1651.08	20.04***	22.12***
Between Genotypes	2	18.58	46.12	0.24	0.62
Hours x Genotypes	6	142.01	175.67	1.84	2.35*
Within	48	77.14	74.64		
Total	59				

*Probability of chance occurrence less than .05.
***Probability of chance occurrence less than .005.

TABLE 20A-ANALYSIS OF VARIANCE FOR PERCENTAGES OF NEUTROPHILS IN THE BLOOD OF DWARF, DWARF-CARRIER, AND PEDIGREE-CLEAN BEEF CATTLE FOLLOWING INTRAMUSCULAR INJECTIONS OF 80 UPJOHN UNITS OF ACTH PER ANIMAL

Source	Degrees of Freedom	Mean Square		F Ratio	
		Sample I	Sample II	Sample I	Sample II
Between Hours	3	3637.44	3962.48	55.53***	63.73***
Between Genotypes	2	12.38	119.66	0.19	1.92
Hours x Genotypes	6	118.60	166.09	1.81	2.67*
Within	48	65.50	62.18		
Total	59				

*Probability of chance occurrence less than .05.
***Probability of chance occurrence less than .005.

TABLE 21A-ANALYSIS OF VARIANCE FOR PERCENTAGES OF EOSINOPHILS IN THE BLOOD OF DWARF, DWARF-CARRIER, AND PEDIGREE-CLEAN BEEF CATTLE FOLLOWING INTRAMUSCULAR INJECTIONS OF 80 UPJOHN UNITS OF ACTH PER ANIMAL

Source	Degrees of Freedom	Mean Square		F Ratio	
		Sample I	Sample II	Sample I	Sample II
Between Hours	3	491.87	536.58	29.61***	26.06***
Between Genotypes	2	40.76	22.25	2.45	1.08
Hours x Genotypes	6	1.41	8.70	0.08	0.42
Within	48	16.61	20.59		
Total	59				

***Probability of chance occurrence less than .005.

TABLE 22A-ANALYSIS OF VARIANCE FOR NUMBERS OF LYMPHOCYTES IN THE BLOOD OF DWARF, DWARF-CARRIER, AND PEDIGREE-CLEAN BEEF CATTLE FOLLOWING INTRAMUSCULAR INJECTIONS OF 80 UPJOHN UNITS OF ACTH PER ANIMAL

Source	Degrees of Freedom	Mean Square		F Ratio	
		Sample I	Sample II	Sample I	Sample II
Between Hours	3	208.78	609.13	0.46	1.95
Between Genotypes	2	1153.42	5022.45	2.55	16.08***
Hours x Genotypes	6	766.78	378.01	1.70	1.21
Within	48	451.61	312.31		
Total	59				

***Probability of chance occurrence less than .005.

TABLE 23A - ANALYSIS OF VARIANCE FOR NUMBERS OF NEUTROPHILS IN THE BLOOD OF DWARF, DWARF-CARRIER, AND PEDIGREE-CLEAN BEEF CATTLE FOLLOWING INTRAMUSCULAR INJECTIONS OF 80 UPJOHN UNITS OF ACTH PER ANIMAL

Source	Degrees of Freedom	Mean Square		F Ratio	
		Sample I	Sample II	Sample I	Sample II
Between Hours	3	34605.35	41112.02	46.83***	64.84***
Between Genotypes	2	93.02	44.17	0.13	0.07
Hours x Genotypes	6	864.92	1626.83	1.17	2.57*
Within	48	738.90	634.10		
Total	59				

*Probability of chance occurrence less than .05.
***Probability of chance occurrence less than .005.

TABLE 24A - ANALYSIS OF VARIANCE FOR NUMBERS OF EOSINOPHILS IN THE BLOOD OF DWARF, DWARF-CARRIER, AND PEDIGREE-CLEAN BEEF CATTLE FOLLOWING INTRAMUSCULAR INJECTIONS OF 80 UPJOHN UNITS OF ACTH PER ANIMAL

Source	Degrees of Freedom	Mean Square		F Ratio	
		Sample I	Sample II	Sample I	Sample II
Between Hours	3	992.04	812.89	9.29***	8.99***
Between Genotypes	2	379.16	472.05	3.55*	5.22**
Hours x Genotypes	6	30.37	61.54	0.28	0.68
Within	48	106.73	90.40		
Total	59				

*Probability of chance occurrence less than .05.
**Probability of chance occurrence less than .01.
***Probability of chance occurrence less than .005.

TABLE 25A-ANALYSIS OF VARIANCE FOR TOTAL NUMBERS OF WHITE CELLS IN THE BLOOD OF DWARF, DWARF-CARRIER, AND PEDIGREE-CLEAN BEEF CATTLE FOLLOWING INTRAMUSCULAR INJECTIONS OF 80 UPJOHN UNITS OF ACTH PER ANIMAL

Source	Degrees of Freedom	Mean Square		F Ratio	
		Sample I	Sample II	Sample I	Sample II
Between Hours	3	25521.47	37530.99	19.01***	47.79***
Between Genotypes	2	3065.12	9529.87	2.28	12.13***
Hours x Genotypes	6	839.65	1325.04	0.63	1.69
Within	48	1342.72	785.33		
Total	59				

***Probability of chance occurrence less than .005.