"SNORTER" DWARFISM IN THE BOVINE. CERTAIN CYTOLOGICAL AND HISTOLOGICAL COMPARISONS OF THREE GENOTYPES IN HEREFORD CATTLE

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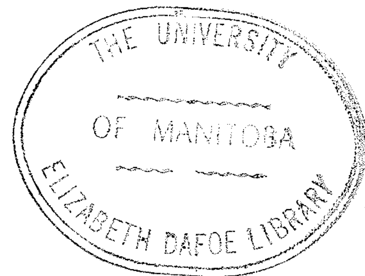

by

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ABSTRACT

"SNORTER" DWARFISM IN THE BOVINE.

CERTAIN CYTOLOGICAL AND HISTOLOGICAL COMPARISONS OF THREE GENOTYPES IN HEREFORD CATTLE

BY

B. RATHNA KUMAR BENJAMIN

An investigation was undertaken to determine karyotypic and histological differences among three genotypes for 'snorter' dwarfism. Each genotype was represented by four Hereford females.

Bovine leucocyte cultures prepared by a modification of the techniques employed for humans was used to conduct chromosomal studies.

No karyotypic abnormalities were detected in animals belonging to the 3 genotypes. All animals had a diploid number of 60 chromosomes. The 58 autosomes were acrocentric and the sex chromosomes were submetacentric. No differences were observed in the karyotypes of normal, carrier and dwarf animals which would indicate that karyotyping is not a useful technique to distinguish between the 3 genotypes for snorter dwarfism.

Biopsied skin samples were used for the histological study. The following skin characteristics were studied: skin

thickness, number of sweat glands per square centimeter of skin, the volume of sweat glands, and the depth at which these glands were located from the skin surface. Skin thickness was measured using tuberculin callipers. The sweat gland dimensions were measured from thick sections, using an eye piece micrometer and their depth from the skin surface was measured on the same sections. The number of sweat glands per square centimeter of skin was estimated after making the necessary correction for shrinkage due to histological processing. Of the 4 characteristics studied only skin thickness differed significantly among the 3 genotypes. The skin was thickest in normal animals and thinnest in dwarfs. The carriers showed intermediate values. No significant differences were noted among the 3 genotypes for the remaining 3 skin characteristics.

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INTRODUCTION

The prosperity of the meat animal industry depends essentially upon a high reproductive rate and rapid efficient growth. Factors which impede the reproduction or growth, whether genetic or nutritional, cannot be ignored by the livestock breeder nor the researcher constantly trying to improve the efficiency with which meat is produced. One such condition confronting the cattle breeder and researcher is the 'snorter' form of dwarfism.

The 'snorter' dwarf animal exhibits symptoms of laboured breathing from which the name 'snorter' dwarf is derived. The snoring sound is similar to that exhibited by cattle suffering from 'snoring disease' caused by Schistosoma nasilis (a fluke) of the tropics. Except for this snoring there is no similarity whatsoever between the two maladies. Dwarf animals are pot-bellied and show a distinct tendency to bloat. Mal occlusion of jaws is seen because of prognathism (over shot mandible) and the forehead is broader and shorter than that of normal animals which leads to the term brachycephalic dwarfs. Median prominance of the forehead is another common symptom encountered in dwarfs. The animals are short and thick because of retarded growth and in general they are less viable. Those heifers which live and reproduce often exhibit dystocia. Dwarf bulls have difficulty in ser-

ving because of their short stature. Dwarf symptoms become more pronounced as age advances. The phenotypic expression of 'snorter' dwarfs was described in considerable detail by Johnson et al. (1950), Lindley (1951), Gregory et al. (1951) and Carroll et al. (1951). The condition was first described in Hereford cattle by Johnson et al. (1951) who pointed out that these dwarfs were different from those described by earlier investigators (Mead et al. 1942; Lush, 1930). Gregory et al. (1951) also noted this difference and further added that these dwarfs differed from 'stumpy' Shorthorns described by Baker et al. (1950). The 'stumpy Shorthorns can be recognized at an early age by their curly coat and short tail. Moreover, they live and reproduce. The reproductive behaviour and performance of 'snorter' dwarfs in Herefords were described by Pahnish et al. (1955b).

'Snorter' dwarfism is known to be conditioned by a single autosomal recessive gene (Johnson et al. 1950; Gregory et al. 1951 and Lush and Hazel, 1952). Lush and Hazel (1952) explained that the 'snorter' dwarf gene segregates as a simple Mendelian recessive. Dwarfism is manifested by animals having the recessive gene in the homozygous condition only. Animals possessing the gene in the heterozygous condition or homozygous dominant condition were apparently normal. Pahnish et al. (1955a) obtained a phenotypic normal and dwarf ratio of 3:1 by mating heterozygous animals. Dwarf matings

resulted in dwarf progeny only. It was shown that the dwarf conditioning gene has complete penetrance (Gregory et al. 1951 and Pahnish et al. 1955a).

Isolated instances of dwarfism among cattle have been known for centuries. The earliest reference to a dwarf breed of cattle was made by Darwin who observed the breed known as Niata cattle in South America during his voyage. An elaborate description was given by Darwin in his posthumous publication (1899). These animals were characterized by short and broad forehead and upturned nostrils. Their mandibles were longer than the corresponding maxillae. This breed was known to breed true, that is, Niata matings invariably produced offspring with Niata features. He further postulated that these animals would be the first to perish in the event of drought and consequent drying up of pastures. He reasoned that these animals cannot browse on twigs or reeds since their lips do not meet for the purposes of prehension. The Hereford 'snorter' dwarfs in many respects resembled Niata cattle. Jeffreys (1953) reported about a strain of dwarf cattle existing in Nigeria. He refuted the claim that these cattle were a stunted variety of Shorthorns resulting from adverse environmental conditions. He explained that these dwarfs remained as dwarfs even when provided an ideal environment. According to him, the dwarfism in this breed was of genetic origin and not a simple environmental

response. Zeuner (1963) reported a general decrease in the body size of animals as a result of domestication. He enumerated the various effects of domestication on wild animals. Bohlken (1964) examined the skulls of cattle and found that domestication has had a broadening and shortening effect on the shape of the skull.

The high incidence of dwarfism in beef breeds posed a serious threat to the livestock industry from 1950 to 1960 and is still a minor problem. The loss may be explained in two ways. Firstly, the dwarfs themselves are a loss to a commercial breeder because of their uneconomical growth and poor viability. Secondly, a seed-stock farmer is unable to sell as foundation stock animals carrying or suspected of carrying dwarfism. The alarming increase of dwarfs in the three major beef breeds naturally caused concern among cattle breeders and research workers of North America. Hereditary dwarfism was not a serious problem among dairy herds since the dwarf body type was never considered ideal by dairymen and hence not propagated by selection (Bone, 1963). It was first a problem among pure bred herds. The problem was so threatening that a Californian rancher in 1953 imported 2 Hereford bulls and 9 cows from England as a measure to counteract dwarfism - this was considered to be the biggest importation of the breed since 1817. It is interesting to note that no dwarfism was reported among Herefords

from Herefordshire, England, the habitat of the breed. Briggs (1958) recorded that pioneer Hereford breeders sacrificed extreme size to achieve symmetry and refinement.

It was realized that effective genetic control of dwarfism depends upon identification of heterozygous normal or 'carrier' animals and homozygous normal or 'clean' animals at an early age (Gregory et al. 1953). Several tests have been evolved in an attempt to identify dwarfs, carriers and normal animals at an early age. It is not difficult to identify dwarfs particularly at a later age but so far there is no single method of positively differentiating heterozygous normal animals from homozygous normal animals. Many parameters such as anatomical, physiological and haematological-have been tried with the aim of separating different genotypes for snorter dwarfism but no single test has been absolutely successful.

REVIEW OF LITERATURE

A. Etiology of the Dwarf syndrome

Different forms of dwarfism have been observed from time to time. Craft and Orr (1924) reported a case of a dwarf calf in the Hereford breed and they attributed the abnormality to thyroid and pituitary deficiencies. Lush (1930) observed short-leggedness in Hereford herds in Texas. According to him the syndrome was conditioned by a single dominant gene inherited in a Mendelian manner. Brandt (1941), Gregory *et al*. (1942) and Mead *et al*. (1942) observed cases of achondroplasia in dairy cattle. They attributed the condition to recessive genes. Crew (1923, 1924) described the pathology of achondroplastic Dexter bull-dog calves and indicated that the syndrome was caused by a dominant gene. Mead *et al*. (1946) observed a dominant mutation in dairy cattle which reduced their size. Gaztambide (1949) found dwarfism in Peurto Rican herds and advocated the use of their germ plasm in genetic experiments. The conformation of these animals was beefy. Van Marle and Lombart (1964) reported a low incidence of dwarfs in the Afrikaner breed. One of these dwarfs resembled the Hereford dwarfs described by Gregory *et al*. (1953). Baker *et al*. (1950, 1951) reported dwarfism in Shorthorns and Aberdeen-Angus herds respectively. In both breeds dwarfism was believed to be conditioned by a single autosomal recessive gene. Johnson *et al*. (1950)

reported 'snorter' dwarfism in Hereford cattle. Chambers et al. (1954) observed that either the genes causing dwarfism in 'compress' and conventional Herefords and Angus may be allelic or that the 'compress' also carried a similar dwarf recessive gene of noncompress type at a high frequency. Stonaker (1954) described 'compress' dwarfism, which he considered to be due to an incompletely dominant gene. This was different from the conventional dwarfism in Herefords caused by an autosomal recessive gene. The 'compress' gene in the homozygous dominant condition produced extreme type of dwarfism. Koger et al.(1955) studied different forms of dwarfism prevalent in Florida beef cattle herds. 'Snorter' dwarfism was believed to be the same in both Hereford and Angus breeds. The mode of inheritance in 'Long-headed' Angus, 'Ant-eater' Angus, 'stumpy' Shorthorns, 'midget' Brahmans, 'Guinea' crossbreds and native cattle was also described.

Gregory (1955) conducted mating tests among dwarfs of the different breeds and observed that more than one pair of genes was involved in producing different morphological forms of dwarfism. Gregory (1956) and Gregory and Carroll (1956) observed that all phenotypes tested were part of the same genetic complex in Herefords and Angus breeds and that modifying genes were involved in differentiating specific dwarf phenotypes. They suggested that this type of

dwarfism could also occur in Shorthorns and Holsteins.

Burris and Priode (1956) showed that recessive genes causing dwarfism in Angus and Hereford cattle were either the same gene or alleles with similar effects. They also noted that crossbred dwarfs had greater vigor than purebred dwarfs. Dollahon et al. (1957) made experimental crosses between Herefords, Angus, Shorthorns, Natives and Brahmans. Results of each cross were listed. Midget Brahmans mated inter se produced midget progeny only.

Julian et al.(1959) classified all the dwarfs into 4 categories, namely, brachycephalic (short headed), dolicho-cephalic, 'intermediate' and compressed varieties. The brachy-cephalic dwarfs were the same as 'snorter' dwarfs. The doli-chocephalic (long headed) were slightly larger than 'snorter' dwarfs. They were characterized by long heads and commonly occurred in Aberdeen Angus herds. The intermediate dwarfs were not clearly defined with respect to their morphology. They usually possessed the features of both of the former types. The compressed variety was characterized by definite subnormal size ranging from 65-90% of the normal. Dwarfism was recognized as a genetic complex (Julian et al. 1959; Gregory et al. 1964; Gregory et al. 1966). The latter showed that achondroplastic mutants, both dominant and recessive were interrelated and were components of the same genetic complex. Their results indicated that the major dwarf condi-

tioning gene is recessive and not dominant as described earlier by Crew (1923, 1924).

Gregory (1954) reported a high incidence of 'wry' calves in California herds. He classified them into two varieties depending upon their apperance. The first type was somewhat uniform in appearance and occurred in Angus and Hereford breeds. The calves were characterized by crooked forelegs. The second type occurred in calves with Hereford background. They did not possess dwarf features, but had crooked spines, forelegs and enlarged knee joints. The second type was supposed to be non-genetic in origin and presumably caused by faulty nutrition of the dam during gestation.

Andrews and Fransen (1958) tried endocrine therapy on dwarf beef cattle. The administration of thyroactive iodinated protein, testosterone propionate, and diethylstilbesterol singly or in combination brought slight improvement in the rate of gain and a doubtful increase in the height at withers.

Gregory _et al_. (1963) reported a reduction in the weight and dimensions of Herefords of the California Experimental Station herd from 1920 to 1940. They attributed this reduction to the effects of intensive selection. All the evidence accumulated on dwarfism by different investigators has pointed out a hereditary involvement.

The reason for a higher incidence of dwarfs from 1950

to 1960 has been attributed to the favouring of heterozygous animals as future parents although Baker *et al.* (1951) and McCann (1956) did not consider this as a valid reason. It remained a controversial hypothesis for a few years but Bovard and Hazel (1963), Gregory *et al.* (1964) and Marlowe (1964) clearly showed that selection preference for heterozygotes was a major cause of the higher incidence of dwarfs during the period already referred to.

B. Tests for Dwarfism

The different tests evolved to identify dwarfism may be arbitrarily classified for convenience.

1. Tests based on pedigree analysis. Dinkel and Gregory (1965) observed that pedigree discrimination has greatly reduced dwarfism in recent years. Lush and Hazel (1952) showed that both parents were responsible for the dwarf offspring. No amount of inbreeding would increase the incidence of dwarfism if the parents were free of the dwarf-gene. They also cautioned that it would be wrong to attribute guilt of dwarfism to a particular breed, family, or line simply because one or two animals produced dwarfs.

2. Progeny test. Pahnish *et al.* (1955b) considered the possibility of using dwarf animals in breeding experiments. They noted that dwarf bulls had difficulty in performing natural service and suggested the use of a special breeding pit. The dwarf heifers were slow to conceive and those that did conceive had dystocia. Lush and Hazel (1952, 1955) pointed

out the practical difficulty in testing cows since a cow can have only a few calves whereas a bull can sire many calves. They proposed a method for testing a prospective sire and suggested that known carrier cows can be best used to prove a young bull. If a bull were mated to more than 10 carrier cows and produced no dwarf offspring, one can be fairly certain that the bull is free of dwarf gene. Emmerson and Hazel (1956) suggested that the best method to test heifers is to mate them to dwarf bulls. According to them, if a dam fails to produce dwarf calves in 4 consecutive gestations, one may classify the cows as free of the dwarf gene.

Gregory _et al_. (1957) considered the validity of using carrier cows as testers for the major dwarf conditioning gene. They observed that the comprest dwarfs, dwarfs of brachycephalic, dolichocephalic, intermediate and other varieties belong to the same genetic complex. Progeny testing a bull only for brachycephalic dwarfism may prove futile since there were other varieties of dwarfism to contend with. Their results indicated that specific modifying genes or a combination of modifiers were the cause of different types of dwarfism. Gregory _et al_. (1964) attributed the lack of effectiveness of various tests to the fact that achondroplasia was a complex resulting from interaction of alleles at several loci instead of being conditioned by a recessive

allele at a single locus. They doubted their earlier hypothesis (Gregory _et al_. 1953) that hereditary dwarfism is conditioned by a single autosomal recessive gene with complete penetrance. Julian _et al_. (1959) described morphological characters of different types of dwarfs and recommended the use of a progeny test for solving the dwarf problem effectively. Lasley (1963) referred to a situation wherein a bull sired 600 normal calves but when the progeny were mated _inter se_ many dwarf calves were produced. He emphasized a need for a well planned progeny test. He cited another instance wherein a young bull was proven to be dwarf gene free, yet his sire was a carrier. Bovard and Priode (1965) practised pedigree selection, limited progeny testing and lateral lumbar radiography for selecting against dwarfism in Aberdeen Angus cattle and recommended the use of these methods especially for selecting replacement calves and young males. Since the laws of inheritance are based on probability, no single fool-proof test could be evolved. Berge (1953) derived formulae to evaluate homozygosity under different mating systems practised in livestock breeding. Kidwell (1951) presented a method of estimating the minimum number of progeny required to test a male for heterozygosity for a single autosomal recessive gene.

3. Tests based on phenotypic expression. Attempts were made to identify the different dwarf genotypes at a young age instead of waiting for the results of pedigree analysis

or progeny test. The aforesaid genetic tests are not only time-consuming but also involve a certain probability of error.

(a) Biochemical tests. Lorincz (1961) believed the snorter dwarf condition to be similar to Hurler's syndrome in humans. He noted a greater amount of urinary mucopoly-saccharides in dwarfs than in normal animals. McIlwain and Eveleth (1962) observed higher excretion of chondroitin sulphate B in dwarfs than in normal animals. The carbozol to napthoresorcinol ratio was 1.38 for 'snorter' dwarfs whereas the similar C/N ratio was 9.96 for normal animals. They indicated the feasibility of using this ratio for identifying dwarfs. Mayes et al. (1964) however did not find any abnormal excretion of urinary mucopolysaccharides in dwarfs. Dorfman (1966) recorded this disagreement between different workers in his article on Hurler's syndrome.

(b) Haematological tests. Cornelius et al. (1956) studied different blood constituents of short headed Angus and Hereford dwarfs of both sexes. Serum electrolytes and protein levels were run to aid interpretation of osteological lesions. They estimated cholesterol and protein bound iodine values to assay thyroid function. They found that serum proteins, calcium, magnesium and phosphorus of short headed dwarfs were within normal range. They reasoned that since serum cholesterol and protein bound iodine levels were within

normal range, the dwarfs cannot be primarily thyroid cretins. They further observed that all the haematological values were apparently normal except the differential count. Dinkel (1955) made circulating eosinophil counts after injecting ACTH as a test for adrenal cortical insufficiency. Dwarfs showed lower initial eosinophil levels than normals but wide variations encountered in the initial eosinophil levels prevented the classification of dwarfs and normals. It was postulated that varying eosinophil levels may vary with rations, worm infestation, and gestation and may thus confuse the test. Deyoe et al. (1957) determined the blood glucose and plasma free glutamic acid, glycine and histidine values after insulin induced stress in dwarf and normal cattle. They observed no significant differences in these responses. However, they observed an increase in total leucocyte count in heterozygotes and dwarfs but not in animals from dwarf free herds. Fransen and Andrews (1958a) observed lower levels of plasma cholesterol in dwarf cattle than in normal cattle. The cholesterol levels of dwarf bulls were higher than those of dwarf heifers.

Dollahon et al. (1959a) studied different blood constituents of Hereford heifers of 4 age groups which included both known dwarf carrier animals and a group assumed to consist of non-carrier animals. Their results indicated that mean corpuscular volume was significantly higher in

the dwarf carrier group. The RNA content of plasma protein was higher in the dwarf carrier group but the DNA content of the plasma protein was higher in the non-carrier group. However, they did not observe significant differences between carrier and non-carrier groups for haemoglobin, mean corpuscular haemoglobin, haematocrit, erythrocyte number, leucocyte number, blood cell fragility, blood serum albumin, blood globulin fractions, blood copper or blood zinc levels. In spite of the fact that several factors were significantly different, they doubted the feasibility of using them for detecting carriers, because of the overlapping between the groups considered. Lucas and Turman (1959) measured several haematological values with a view to comparing them between different dwarf genotypes. Their study included erythrocyte fragility, haemoglobin, haematocrit, erythrocyte number, specific gravity of plasma, electrophoretic analysis of plasma proteins, and leucocyte differential counts. They found no significant differences between genotypes in values for haemoglobin, haematocrit, erythrocyte number, specific gravity of plasma, plasma protein fractions, or leucocyte differential counts. They noted, however, an increase in the mean values for haemoglobin, haematocrit, and erythrocyte number with increasing age up to 3 months from birth and a decrease in the plasma specific gravity during the same period. Hafez _et al_. (1959) found no significant

difference between dwarf and control animals for the characteristics of rectal temperature, pulse rate, blood specific gravity, prothrombin time, sedimentation rate, red cell count, electrophoretic pattern of serum proteins, serum creatine and creatinine and Ca/P ratio in hair samples. The dwarfs had lower respiration rate, lower haemoglobin, haematocrit and leucocyte count than normals.

Stufflebeam _et al_. (1965) compared the rate of alkali denaturation of haemoglobin of the 3 genotypes for snorter dwarfism in Herefords. They determined the haemoglobin denaturation values at different temperatures and different periods of exposure to 1/12N sodium hydroxide. Although the degree of denaturation differed among the genotypes at certain temperatures, there was considerable overlap of values between the individuals which prevented its use as a diagnostic aid.

(c) _Physiological tests_. Carroll _et al_. (1951) observed smaller pituitaries in dwarfs than in normal animals. Their investigations indicated thyrotropic deficiency in dwarfs. However, they found that growth hormone and gonadotropic hormone were present in dwarf pituitaries. Marlowe and Chambers (1954) conducted bioassay studies to determine the potency of thyrotropic, adrenocorticotropic, and growth hormones obtained from the pituitaries of normal and dwarf calves of Angus and Hereford breeds. They observed

normal potency for thyrotropic and adrenocorticotropic hormones in dwarfs but the growth hormone of dwarfs showed greater potency than normals. Fransen and Andrews (1954) noted hydrocephalus with distended lateral ventricles as a common feature in most dwarfs. Bioassay studies indicated normal thyrotropic and pituitary activity in dwarfs. Electrocardiographic studies indicated normal cardiac activity in dwarfs. Hormonal therapy stimulated daily rate of gains to some extent in dwarfs but the bone growth was unaffected. Foley et al. (1956) developed the insulin tolerance test, with a view to separating carriers from homozygous normal animals. All 3 genotypes of Aberdeen Angus and Herefords were included in the test. They determined blood sugar levels at regular intervals after intravenous injection of insulin and estimated the leucocytes to test the adrenal hormonal activity. The dwarfs showed greater decline in the blood sugar levels than the normal animals. The dwarfs responded little whereas the normals responded rapidly showing high leucocyte count and the carriers were intermediate in their response. They suspected abnormal pituitaries or adrenal response to stress in dwarfs. In view of the disagreement between the findings of Carroll et al. (1951) and Marlowe and Chambers (1954), Crenshaw et al. (1957) conducted experiments on dwarf and normal animals by administering I^{131}. They inferred that in dwarfs

the rate of release of thyroid hormone was within the normal range and concluded that the dwarf condition was not due to dysfunction of the pituitary-thyroid axis of the endocrine system. Deyoe et al. (1959) studied physiological responses to insulin induced stress in Herefords of the 3 genotypes. They observed a greater increase in neutrophils of dwarfs and carriers than in normals, following insulin induced stress. The overlapping values did not permit definite classification of several animals into normals, carriers and dwarfs. Plasma-free glutamic acid, glycine and histidine were determined before and 5 hours after insulin injections. The magnitude of the drop in the values of the 3 amino acids was similar in dwarfs and normal animals.

Taylor and Turman (1959) studied the changes in blood glucose levels of dwarfs and non-dwarfs of Angus and Hereford breeds after administering epinephrine, insulin and glucose. The experiments were conducted on fasted and non-fasted animals. They recorded smaller increases of blood glucose in non-fasted and fasted yearling dwarfs than in non-dwarfs after epinephrine injections.

Foley et al. (1960) measured blood sugar values by using Nelson-Somogyi and Folin-Wu methods. The values for normal, carrier and dwarf animals were compared and a significant difference between the two methods of estimation was found. The sensitivity of insulin was determined by measuring

the fall in blood glucose level after insulin injection. The dwarfs registered a greater fall in blood glucose level than the carrier and normal animals. They suggested the 'snorter' dwarf gene in some way affected carbohydrate metabolism and suspected impaired function of the hypothalamus but concluded that blood sugar determinations were not of much use in distinguishing between the normals and carriers because of the overlap between the 2 groups. Curl *et al*. (1961) subjected the animals of the 3 genotypes to hormone induced stress by injecting insulin, adrenalin and ACTH and blood samples were collected from each animal immediately before and after the injection of each hormone. Blood sugar levels were determined. The genotypes differed significantly in eosinophil numbers, before and after the injection of ACTH. Significant differences were observed in neutrophil counts, blood glucose levels, and total number of leucocytes before and after the injection of insulin. The authors concluded that although genotypic differences existed for several characteristics, none of them either singly or in combination was found to be of sufficient magnitude to use in identifying the individuals of different genotypes.

(d) Cerebrospinal fluid pressure studies. Fransen and Andrews (1958b) observed high cerebrospinal fluid pressure at atlanto-occipital or lumbo-sacral joints of some dwarfs.

But, because of variable results, this was not enough to detect the animals carrying the dwarf gene. Dollahon _et al_. (1959b) described a method for measuring the cerebrospinal fluid pressure at the atlanto-axial and at lumbar regions. The cerebrospinal fluid pressures at the atlanto-axial joint in dwarf-carrier cattle were lower than those in non-carrier cattle. However, an area of overlap for these values between the 2 types did exist which prevented its use as a discriminating factor.

(e) _Anatomical studies._ Different organs were studied to elucidate the phenotypic expressions of different dwarf conditions in dairy cattle caused by a dominant mutation. There was general reduction in the skeletal development in homozygotes, exhibited by severe achondroplasia. The mutants were more susceptible to teat injuries since the udders were closer to the ground. They observed no overlap between the normals and mutants with respect to height at withers. Arthaud _et al_. (1957) made body measurements on 95 pedigree clean and 29 carriers of the 'snorter' dwarf gene. Both sexes were included in the study. The characteristics studied were cannon bone length, cannon width, length of first five lumbar vertebrae, cannon length/width, cannon length/circumference and cephalic index. All differences were highly significant except for the cephalic index and lumbar vertebrae length. When the discriminate functions

were applied the overlap between pedigree clean and carrier calves was 37% for males and 38% for females. Bovard and Hazel (1963) studied the growth patterns of 'snorter' dwarf and normal Hereford calves. The dwarf gene primarily affected the skeletal development and affected the development of muscles and internal organs less. They noted that the dwarf gene exerted a restraining influence on skeletal growth, especially on length.

The anatomical studies were conducted on head, lumbar vertebrae, limbs, endocrines and heart.

Head form. The head form has been studied both in living and dead animals. Gregory et al. (1951) reported mid-forehead bulging in brachycephalic Hereford dwarfs. Gregory and Brown (1952) developed an instrument known as a profilometer to record with precision the median head profile or contour of an experimental animal. Klussendorf (1953) recommended the profilometer for field use on young bulls and advocated culling border line cases to improve the efficiency of the test. Gregory et al. (1952, 1953) elaborately described the use of the profilometer in identifying heterozygote bulls. They observed that dwarf carrier bulls were introduced unconsciously and consistently into herds. Stonaker (1954) rejected the hypothesis that the profilometer predicts the genotypes of sires and their unselected yearling sons.

Stratton et al. (1956) studied the head-profile measure-

ment of Hereford bulls at different ages. They found that those bulls which were classified as dwarf-gene free at 6 months of age remained in that same classification at 12 months of age. A high percentage of those individuals which were classified in the overlapping zone and carrier genotype at 6 months of age moved in a favourable direction when reclassified at 12 months of age. Schoonover and Stratton (1958) studied the relationship between head form and economic traits in horned Hereford heifers. According to them, bone measurements as a measure of growth were found to be more stable than weight upto 30 months of age. The mid-forehead prominence characteristic of the dwarf gene was also observed.

Dinkel and Gregory (1965) evaluated the use of the profilometer as an aid in detecting heterozygous Hereford 'snorter' dwarf bulls, and they observed that on the basis of median head profile readings, the profilometer did have a predictive value for distinguishing between heterozygous and homozygous normal animals. However, they felt that this accuracy was too low to be useful in identifying genotypes for this condition.

Skulls have been studied by various research workers. Buchanan et al. (1956) observed two types of 'pinching' in the occipital condyles of dwarf beef cattle which was not observed in normal animals. Julian et al. (1956, 1957) examined the skulls of short-headed Hereford dwarfs and normal animals and detected premature closure of spheno-

occipital synchondrosis in 'snorter' dwarfs. The closure of spheno-occipital synchondrosis occurred in the first week of post-natal life whereas the closure occurred between 24-36 months of age in phenotypically normal animals. The fusion occurred between 8-17 months in some apparently normal animals. They explained that the persistent bulge on the foreheads of dwarfs was due to tendency toward internal hydrocephalus. Tyler et al. (1959) observed 2 projections into the cranial cavity of dwarfs. The projections were of variable length and seemed to indicate arrested development and differential growth rates due to achondroplasia.

Vertebrae. Buchanan et al. (1956) made a detailed study of the osteological lesions of lumbar vertebrae of dwarfs. In general the vertebrae of dwarfs were shortened. Bovard et al. (1956) studied the effect of the 'snorter' dwarf gene on lumbar vertebrae and metacarpal bones of Hereford calves. On the basis of sex and radiographic findings the calves were classified as normal, slightly irregular and definitely abnormal and on the basis of pedigree or progeny test results were classified as known hetrozygotes, possible heterozygotes, and presumed homozygous normals.

Hazel et al. (1956) examined calves of different genotypes for snorter dwarfism radiographically and found severe longitudinal compression of thoraco-lumbar spinal region

in dwarfs. The abnormality was less marked in carriers. The longitudinal compression was accompanied by irregular protrusion below the body of the vertebrae. The normal homozygous animals were sometimes classified as abnormal because of minor variations in vertebral development. In general the vertebral classification did not exactly fit the genotypic classification. Emmerson and Hazel (1956) conducted radiographic studies on thoraco-lumbar vertebrae of 1-10 day old calves and noted the longitudinal compression in dwarfs and dwarf gene carriers but not in dwarf gene-free calves. They considered the radiographic checking method to be more useful than the profilometer method with respect to early detection of carriers. Most calves were radiographed at 1 week of age or younger. The radiographs were taken with animals on lateral recumbent position. High et al. (1959) conducted an extensive survey to identify animals heterozygous for the 'snorter' dwarf gene. Radiographs were made from 1500 calves of Angus and Hereford breeds. The X-ray method of diagnosing different genotypes seemed to be more accurate in Herefords than in Angus. The X-ray method was highly accurate in identifying 'snorter' dwarfs. However, there was considerable overlap between presumed 'clean' and 'carrier' animals with respect to vertebral morphology. The X-ray method was not advocated as a test for identifying the different genotypes because of this overlap. Rankin et al. (1959) tested the accuracy of the X-ray method of identifying

the 3 genotypes, namely, the normal, carrier and dwarf. Both Hereford and Angus calves were included in the study. According to them, the X-ray method identified 78% of dwarf carriers, 74% of dwarf free animals, and 96% of dwarf calves. Vertebral abnormalities were more frequent in males than in females. They concluded that the X-ray method was not highly accurate in identifying carriers and non-carriers of the dwarf gene, but it was highly accurate in identifying dwarf calves.

Limbs. Baker et al. (1950) noted that the achondroplastic condition was more marked in forelegs than in hind legs of 'stumpy' Shorthorns. Tyler et al. (1956, 1957) examined the skeletal material of short-headed dwarfs and phenotypically normal Herefords. Long bones of dwarfs of all age groups were disproportionate with respect to both length/width relationship and diaphyseal length/epiphyseal length. The dwarfing process was supposed to be initiated in prenatal life. Tyler et al. (1961) derived three indices from the measurements made on the metacarpal bones. The measurements of total length (TL), diaphyseal length (DL), and diaphyseal diameter (DD) were used for deriving the 3 indices, namely, TL/DD, TL/DL and DL/DD. They pointed out that these indices were more useful than the absolute measurements. These indices possessed an additional advantage of not being influenced by age, breed or sex and hence, no correction factors were needed. Only 3% of the dwarf values overlapped

with those of controls.

Endocrine glands. Craft and Orr (1924) observed under-developed pituitary, thyroid and parathyroids in a Hereford dwarf calf and assumed this abnormality to be due to endocrine deficiency. Hafez and Rupnow (1960) observed that dwarfs had shorter thoracic and abdominal cavities, humeri, ulnae and radii than the controls. Thyroid glands showed a wide range of weight differences and thereby no differences between the genotypes were observed. In general dwarfs had more blood, heavier feet, less abdominal fat, and smaller loin eye area at 12th rib than the normals.

Heart shape. Eveleth et al. (1956) reported a hypertrophied heart condition in a dwarf calf at post-mortem. Dinkel et al. (1960) conducted radiographic studies in an attempt to separate heterozygous and homozygous normal calves. They correctly diagnosed 71% of the normal homozygotes, 67% of the carriers and 100% of the dwarf calves. Although heart shape was ovoid in dwarfs, extreme variation in the shape limited its use in separating heterozygous and homozygous normal calves. Similarly electrocardiographic studies were not useful in identification of carriers and normals. Hafez et al. (1959) also observed spheroid hearts in most of the dwarf calves.

Cytology. Chromosomal studies were formerly conducted on squash preparations or on paraffin sections. These techniques were too laborious and prone to producing misleading

artifacts. Moreover, they have limited application in studies pertaining to higher mammals. Important technological contributions during the last 2 decades have given an impetus to the systematic study of cytogenetics. The tissue culture medium 199 was introduced by Morgan et al. (1950). Several tissue culture media were tried and few modifications were made to the original medium 199. The proprietary preparations are marketed by different firms.* Hsu (1952) showed that treatment with hypotonic solution facilitates spreading of chromosomes. Ford and Hamerton (1956) introduced the use of colchicine in mammalian chromosomal studies. It was not only useful in arresting mitosis, but it also helped in diverging sister chromatids. Nowell (1960) showed the mitogenic effect of phytohaemagglutinin (PHA) on human leucocytes which till then was known to separate the erythrocytes from blood samples. These 3 basic contributions made chromosomal study less cumbersome and more informative. Thus when routine laboratory techniques were available it became apparent that certain syndromes may be caused by chromosomal abnormalities. These abnormalities may be structural or numerical.

The scope of cytogenetics in medicine was extensively reviewed by Hischhorn and Cooper (1961). The chromosomal abnormalities associated with different syndromes were listed

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*For example, Connaught Medical Research Laboratories, Toronto, Canada.

by Meyer-Robsich et al. (1967). Makino et al. (1962) felt similar investigations should be undertaken to diagnose some hereditary diseases (whose aetiology was not clearly known) in domestic animals. Knudsen (1961) reported clumping of chromosomes in seminiferous epithelial cells of a few bulls exhibiting testicular hypoplasia. Gustavsson and Rockborn (1964) observed 59 chromosomes in 3 cases of lymphatic leukaemia of cattle. Gustavsson (1966) observed 58 and 59 chromosomes in apprently normal bulls, besides the usual 60 chromosomes. He attributed this to intra-specific chromosomal polymorphism due to centric fusion or translocation of Robertsonian type. Herschler et al. (1966) presented a useful technique for diagnosing bovine free-martinism, based on chromosomal studies.

Leuchtenberger and Shrader (1955) observed exceptionally low DNA content in the cells of a dwarf bull. The low DNA content was slightly more than half of somatic tissue DNA content of a normal bull. The DNA content of spermatozoa found in the epididymis and seminal vesicles of a dwarf bull also showed similar low values in comparison to the spermatozoa of normal bulls. However, the DNA ratio between somatic cells and spermatogonia cells of the dwarf (2:1) was similar to that in normal bulls. Leuchtenberger et al. (1956) further investigated DNA values and chromosomes in dwarfs and normal bulls. They found that the DNA content of the spermatogenic tissue of dwarfs varied considerably more

than that of normal bulls. There was a variable tendency towards reduction in the amount of spermatogenic tissue of dwarfs. Cytological examination of dwarf testes revealed no deviation from normal with respect to number and behaviour of the chromosomes. Jubb and McEntee (1955) noted evidence of hyperactivity of acidophils (pituitary) indicated by hypergranulation. These hypergranulation cells were seen in cows with cystic ovaries, in cows treated with parentral administration of estrogens and in Hereford dwarfs. However, no abnormalities were detected in β and δ cells of dwarfs. Yosida and Lamontain (1964) studied the chromosomes of normal and dwarf Hereford cattle. Using skin tissue culture technique, they found 29 pairs of telocentric autosomes and one pair of sex chromosomes. The X chromosome was sub-metacentric and the Y chromosome was metacentric. They found no difference between the karyotypes of dwarf and normal animals.

Histology. All the histological observations made on dwarfism have been mainly concerned with endocrine glands or gonads. Systematic studies on skin structures were not conducted although Crenshaw *et al.* (1957) and Bone (1963) assumed that dwarfs possess thick skin. Hafez *et al.* (1959) observed that skins of typical dwarfs were thinner than those of normal animals.

Johnson *et al.* (1950) made histological examination of dwarf pituitary, thyroid and adrenal glands. The thyroid

glands presented a normal appearance with colloid in the acini. The seminiferous tubules showed very little spermatogenesis and few spermatozoa were located in the sections of ampulla of the ductus deferens. Lindley (1951) studied the histological preparations made from pituitaries, thyroids, adrenals, ovaries and testes of Hereford dwarfs. He observed large cystic spaces with reddish hyalin material in pituitary glands. The acini of the thyroid were dilated but the adrenals and ovaries were apparently normal. He observed a few sperms in the sections of one testicle whereas the other one showed hypoplastic symptoms of germinal epithelial cells and interstitial cells. Gregory et al. (1951) noted no abnormalities in the sections of pituitary, thyroid and adrenal glands of dwarfs. The male genital tract exhibited little spermatogenic activity. However, some spermatozoa were observed in the sections of ampulla and ductus deferens. Tyler et al. (1956) conducted microscopic examination on the matacarpal bones of normal and dwarf animals and observed hypoplastic achondroplasia symptoms.

EXPERIMENTAL

Bone (1963), while briefly reviewing dwarfism in cattle, advocated the need for further chromosomal studies along similar lines to those followed in human chromosomal studies. The present investigation was undertaken to determine whether there are any noticeable karyotypic or histological abnormalities among normal, heterozygous and dwarf Herefords. The leucocyte culturing technique was used for the chromosomal study.

A. CYTOLOGICAL STUDIES

Materials and methods

Experimental animals. The experimental animals consisted of 10 horned Hereford cows and 2 heifers. The 3 genotypes, namely, the normal, the carrier and the dwarfs were represented by 4 animals from each genotype, making up a total of 12 animals. The presumably normal animals were pedigree clean Hereford cows. Furthermore, these cows dropped 3 or more normal calves each when mated to different phenotypically normal sires. The carrier or heterozygous cows were phenotypically normal and were dams of dwarf calves. The dwarfs considered in this study belong to the commonly described 'snorter' class.

Materials and culturing technique. All the glassware, syringes, hypodermic needles and corks used in the experiment were sterilized by autoclaving for 30-40 min. The

sterilizing and drying were done about 5 hours prior to the actual use of instruments. Culture medium CMRL-H-597 (Connaught Medical Research Laboratories, Toronto, Canada) buffered with 3% sodium bicarbonate solution was used. Ten ml. of culture medium were dispensed into the required number of culture bottles. Ordinary 100 ml (3 ozs) prescription bottles were used as culture bottles. The bottles were closed with tight fitting silicone rubber corks (Bell Co., Vineland, N. J., U. S. A.) before and after the addition of necessary ingredients. Heparin (0.5 ml) was placed in each centrifuge tube (12 ml Pyrex centrifuge tubes) an hour before collecting the blood sample. Usually 2 animals were bled each afternoon, as it was found unwieldy to handle too many samples under the restrictions imposed by experimental requirements. Duplicate samples of 10 ml of blood were collected from the jugular vein of each animal, directly into a centrifuge tube containing 0.5 ml heparin. The tubes were corked, gently agitated to facilitate mixing, and kept at room temperature for 3-4 hours. Usually the volume of plasma obtained after this period was insufficient to draw and inoculate into the culture medium. Stepwise centrifugation was carried out. The plasma so collected at intervals was aspirated with a sterile Pasteur pipette and inoculated into culture medium.

The samples were spun at 600 r.p.m. for 10 min and the supernatent leucocyte rich plasma was collected and added

to the culture medium. The procedure was repeated after centrifuging at 800 r.p.m. for 10 min. and 1000 r.p.m. for 10 min. The opening and closing of culture bottles was done over the flame to avoid air-borne contamination. Reconstituted phytohaemagglutinin*(0.2 ml) was added to the contents of each culture bottle to initiate mitosis. The culture bottles were tightly corked and transferred to the incubator, preset at 37°C. After 72 hours of incubation 0.2 ml of colcemid (Ciba, Summit, N.J., U.S.A.) was added to each culture bottle to arrest mitosis and incubation was continued for a further 3 hours. Following this incubation period the contents of each culture tube were decanted into a centrifuge tube and spun at 700 r.p.m. for 5 min. The supernatent fluid was discarded and 4 ml of hypotonic solution (0.075 M KCl) previously incubated at 37°C were added to each tube and incubated at 37°C for a further 5 min. The supernatent hypotonic solution was poured off, following 5 min spinning at 700 r.p.m. The freshly prepared fixative, consisting of 1 part of glacial acetic acid and 3 parts of absolute methanol, was added to the tubes to prevent further swelling and bursting of cells under the influence of hypotonic treatment. The centrifuge tubes containing cell suspension and fixative were transferred to a cold room set at 5°C and left there

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*Burroughs Wellcome, La Salle, P.Q. Canada.

overnight. They were then centrifuged next morning at 700 r.p.m. for 5 min. after which the supernatent fixative was discarded and about 5 ml of freshly prepared fixative was added and the tubes centrifuged. The cells were centrifuged twice with 2 changes of fixative as outlined. Finally about 0.5 ml of fixative was added to each tube to make a dense suspension of cells. This suspension was used for making smears on slides previously wetted with cold tap water. The spread of the smear was adjusted by altering the dropping height from the pipette to the surface of the slide. The slides were rapidly dried over a Bunsen flame, labelled and kept ready for staining. Aceto-orcein* was used for staining smears. No. 1 coverglasses of 22 x 40 mm were used to cover the stained smears.

Each animal's identity was carefully maintained throughout by assigning a coded number to the centrifuge tube while collecting blood. The stained preparations were scanned under low power. Well spread non-overlapping fields were photographed under oil immersion objective. High contrast copy, 35 mm film was used for photomicrography. Out of the photomicrographs made for all experimental animals, one animal from each genotype was karyotyped as a representative of that genotype. Four blood samples were collected from phenotypically normal Hereford bull

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*One gramme of orcein (Esbe Laboratories, Toronto) dissolved in 45 ml of acetic acid and 55 ml of distilled water and filtered.

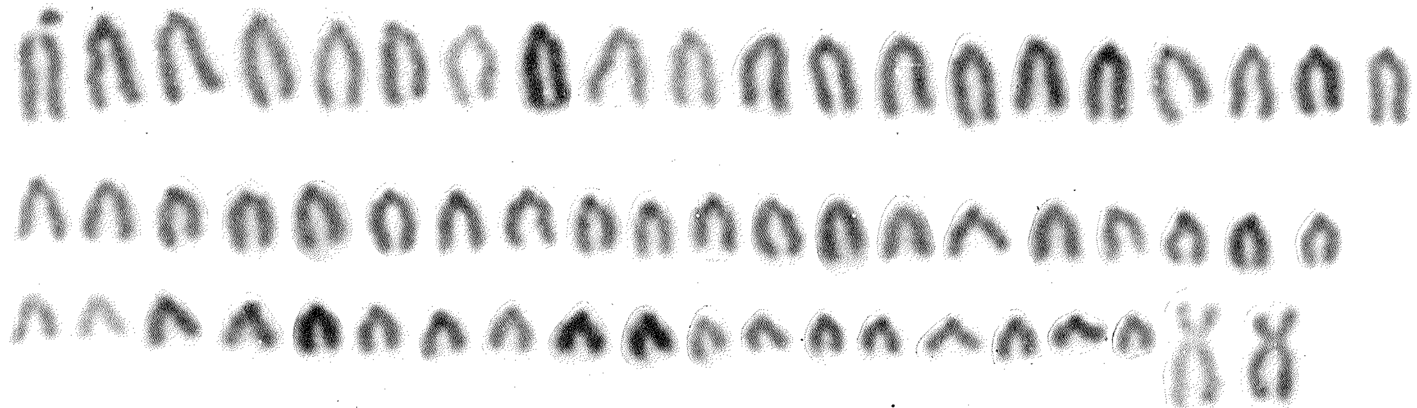
calves of unknown pedigree and those samples were processed along similar lines already described for females.

In addition to CMRL-H-597, two other culture media, CMRL-HB-597 (Connaught's) and TC Medium 199 (Difco) were tried on 6 samples.

RESULTS

The diploid number of chromosomes for each genotype was found to be 60 (2n = 60) consisting of 29 pairs of autosomes and one pair of sex chromosomes in both sexes (Figure 1). The XX and XY types of sex determination were noted in Hereford cattle. These results are in general agreement with earlier observations made by other workers (Sasaki and Makino, 1962; Crossley and Clarke, 1962; Nichols et al. 1962). The X chromosome of both sexes in the present study was submetacentric and about the size of the largest chromosome. In the male, th Y chromosome was submetacentric and was about 1/3 the size of the corresponding X chromosome.

All the autosomes appeared to be acrocentric. Crossley and Clarke (1962) pointed out that the autosomes do not show wide enough morphological variation to arrange them strictly into homologous pairs. However, they can be arranged in descending order based on size starting with the largest (Figures I & II). No differences were found in the karyotypes of the 3 different genotypes considered.



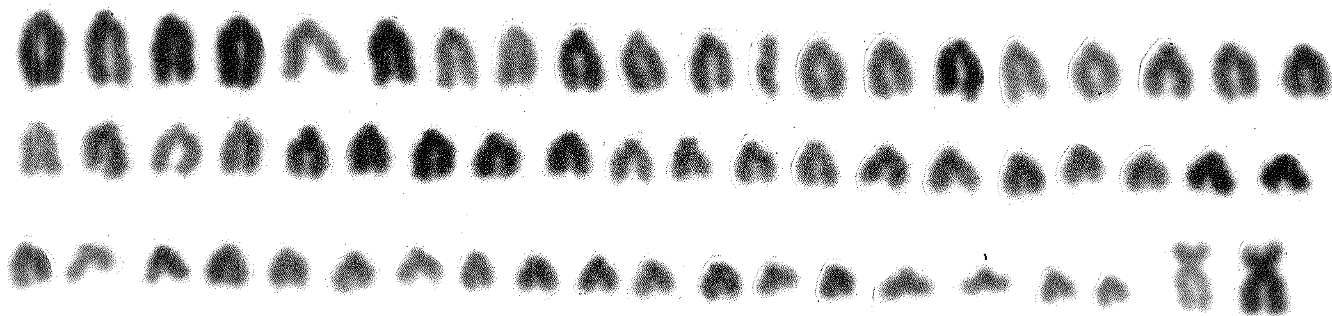
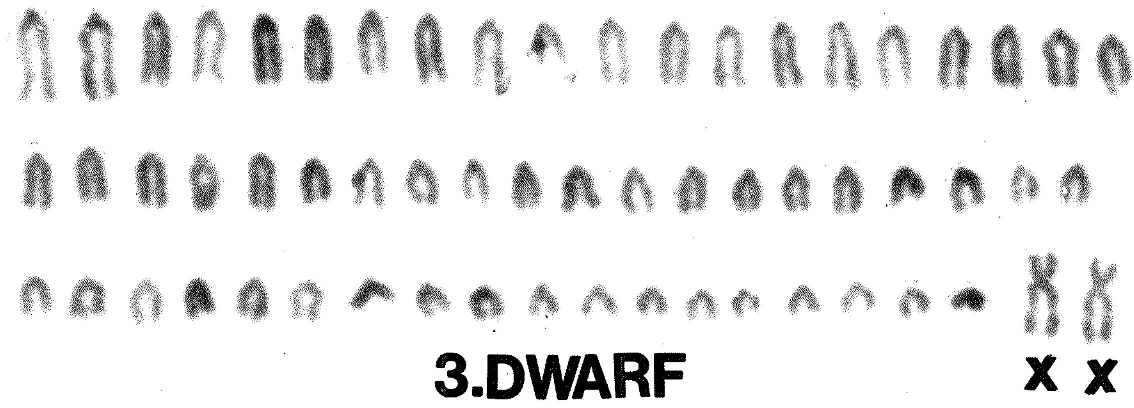


Fig. I. Karyotype of the metaphase chromosomes in Hereford cattle (from a normal and a carrier animal) X 2600 (approx)



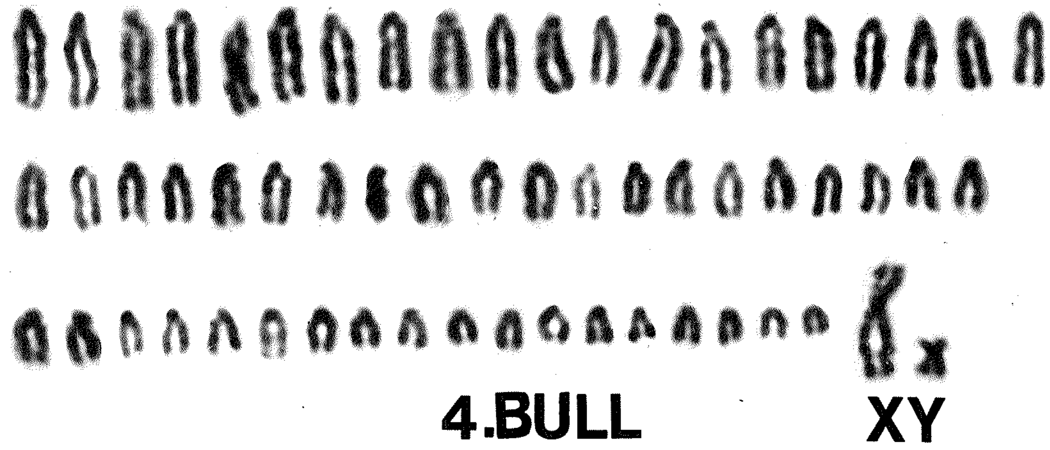


Fig. II. Karyotype of the metaphase chromosomes in Hereford cattle(from a dwarf and a bull calf) X 2600 (approx)

DISCUSSION

Methodology. Moore and Hay (1963) pointed out the undesirability of using anticoagulants such as oxalates, as they might be toxic to leucocytes. Heparin was successfully used by several workers (Crossley and Clarke, 1962; Nichols *et al*. 1962). Crossley and Clarke (1962) found that the amount of heparin used on human blood samples was not sufficient for cattle. However, too much heparin interferes with the growth of the culture. Walford (1960) observed that excess heparin might clump the leucocytes or the preservative (phenol in this preparation) in the commercial preparation may inhibit their growth. In the preliminary work quantities of more than 0.5 ml per 10 ml of blood were found to be unsuitable.

One of the formidable problems encountered in bovine chromosomal work is the slow sedimentation rate of red blood cells. Biggers and McFeely (1963) noted the poor tendency of rouleaux formation in cattle blood. Several workers have observed that phytohaemagglutinin while useful in agglutinating human erythrocytes is of doubtful value in cattle (Biggers and McFeely, 1963; Nichols *et al*. 1962; Ulbrich *et al*. 1963). Undoubtedly it is useful for stimulating mitogenic activity of leucocytes.

In the present experiment chilling and warming of blood samples was avoided as these were presumed to be detrimental to the proliferative ability of cells.

Ford and Hamerton (1956) reported a shortening effect of

colchicine on chromosomes. In the present investigation 0.2 ml of colcemid was added to each culture and incubated for 3 hours. Kleinfield and Sisken (1966) experimentally showed colcemid to be less toxic and more effective than colchicine. Hence, this particular preparation was preferred. Hypotonic treatment with 0.075M KCl (Hungerford, 1965) was found to be satisfactory.

Besides, CMRL-HB-597 (Connaught's) is buffered and reinforced with two other antibiotics (Neomycin and Polymyxin B) in addition to streptomycin. Because air borne contamination is possible during collection of blood samples in open barn yards or pens it is advisable to use this medium. However, it should be remembered that leucocyte growth will be better in the absence of too many potent antibiotics which presumably interfere with protein biosynthesis. TC Medium 199 (Difco) was also used in combination with Bactophytohaemagglutinin (Difco) proving satisfactory when used on a few samples. Ulbrich _et al_. (1963) and Herschler _et al_. (1966) used TC Medium 199 satisfactorily.

The emphasis should be more on combating contamination at every stage rather than on the choice of a particular medium. Even the best culture medium can give mediocre results if it is partially or fully contaminated.

Chromosomes. Hereditary dwarfism is known to be an autosomal recessive trait (Johnson _et al_. 1950; Lush and Hazel,

1952; Gregory et al. 1951 & 1953; Pahnish et al. 1955b) although in latter years it was recognized as being a genetic complex (Gregory et al. 1964, 1966) involving several modifying genes. Dwarfism is also known to be non-sex linked (Baker et al. 1951; Lush and Hazel, 1952). Thus the 'dwarf' genes are expected to be located on one of the 58 autosomes.

The present results are in agreement with the findings of Leuchtenberger et al. (1956) and Yosida and Lomantain (1964). The present investigation indirectly supports the hypothesis that dwarf mutation is genic and not chromosomal. A mutation may easily be eliminated, unless it is favoured by either natural selection or artificial selection, or recur frequently.

Chiarelli et al. (1960) described the Y chromosome to be acrocentric in *Bos taurus*. Crossley and Clarke (1962) repeated the latter work and disagreed with the description of the Y chromosome. According to Crossley and Clarke (1962) the Y chromosome in *Bos taurus* was submetacentric. In the present study the Y chromosome of the Hereford male was submetacentric and it was about 1/3 the size of corresponding X chromosome (Figure II). Recently Keiffer and Cartwright (1968) showed that the Y chromosome of *Bos indicus* is acrocentric and that of *Bos taurus* is submetacentric. All the autosomes and X chromosomes of both species were identical. The Y chromosome of *Bos indicus* resembled one of the smaller (acrocentric) autosomes. Although the Y chromosome looked very much like one of the autosomes,

according to Keiffer and Cartwright (1968) it can be identified by auto-radiographic studies. This observation admirably settles the earlier dispute between Chiarelli *et al*. (1960) and Crossley and Clarke (1962). Apparently Chiarelli *et al*. (1960) sampled *Bos indicus* animals but assumed them to be *Bos taurus*. In fact if this correction is made there is no disagreement between the two groups of workers.

B. HISTOLOGICAL STUDIES

Skin affords a protective covering and also plays a dynamic role in aiding the animal to adjust to environmental changes. The environment could be internal or external. The present study was undertaken to observe whether any differences exist among normal, heterozygous and dwarf animals with respect to different skin characteristics, namely, skin thickness, number of sweat glands, volume of sweat glands and their depth from the skin surface.

Materials and methods. The animals sampled for histological study were the same as those used for chromosomal study, except that the data of two young dwarf heifers were not considered for 3 characteristics (sweat gland volume, sweat gland density and the depth at which these glands are situated from skin surface). All the animals were housed in the same barn, kept on the same feed and sampled within a 10-day period. Three animals, that is, one from each genotype were sampled on the same day. All animals were Herefords.

Skin biopsy and histological techniques. Skin thickness measurement was made on the left lateral neck region immediately behind the tip of the ear. An area of about 6 cm^2 was clipped and skin fold measurements were made on 6 locations selected at random within the clipped area. Tuberculin callipers (Hauptner, Solingen, West Germany) were used for all measurements, since they have a built in spring device which regulates the pressure

of the bite. Skin thickness was accurately measured upto 0.1 mm.

Skin biopsies were made on the skin over the 12th rib which approximately corresponds with the M_2 region (midside) described by Dowling (1955a). The trephine used for skin biopsy was similar to that used by Carter and Dowling (1954). Circular discs of skin were cut after inducing local anaesthesia with xylocaine hydrochloride 2% (Astra, Cooksville, Ontario). The pieces were cut with the aid of the trephine. The internal diameter of the cutting edge was exactly 1 cm. The known area of skin enables one to apply a correction for shrinkage due to histological processing. Five biopsied samples were cut from the midside region of each animal. Two pieces were collected in 5% formol-saline and the remaining 3 pieces were collected in Bouin's fixative. The procedure of processing the formol-saline fixed samples were, with slight modifications, similar to that described by Nay and Hayman (1956). The mounting medium used was neutral Canada balsam and not 'Siro'. The sections were cut at 1/3 to 2/3 mm thick. Since the sections were rather thick the coverslip had to be propped up by small glass pieces at 4 corners. The length and width of 10 undamaged sweat glands were measured for each animal, using the eye piece micrometer. The depth at which these glands were located from the skin surface was also measured. In this experiment the scale was 1 eye piece micrometer division equalled 10 microns. The approximations suggested by Nay and Hayman (1956) were followed in esti-

mating the volume of sweat glands.

The skin samples fixed in Bouin's fixative were used for microtome sections. The pieces of skin were treated with a saturated solution of lithium carbonate made in 70% ethanol, for one or two days. The pieces were dehydrated in ascending grades of ethanol, cleared in cedarwood oil, embedded in paraffin and sectioned. Vacuum embedding technique was used to shorten the paraffin infiltration time. The paraffin sections were stained as per routine histopathological techniques. Delafield's haematoxylin and eosin, Weigert's haematoxylin and Van Gieson's stain were used for staining purposes. The latter combination gives a better contrast. Duplicate samples were used for vertical sections. Out of these 2 horizontal sections one was selected for estimating the hair follicle density of each animal. The horizontal sections were cut at 12μ, and the vertical sections were cut at 7μ and were used for studying skin structures in general.

The area of the mounted section was obtained from the outline of its shadow projected on a graph paper. Photo enlarger (Pixur, Japan) was used for getting the shadow of the sections on graph paper. Since it was known that each hair follicle is accompanied by a sweat gland (Carter and Dowling, 1954), the count of one will automatically provide the count of the other. It was found convenient to count hair follicles. Ten random fields were counted from each section to obtain an estimate of the number of hair follicles/cm^2 for each animal. All the counts

were made under 40X objective, 8X eye piece. The following correction factor was used to estimate the number of hair follicles.

$$\text{No. of hair follicles/cm}^2 = \frac{100na}{Ay}$$

where,

n = is the average number of hair follicles counted from 10 random fields(in a horizontal section)

a = area of the mounted section

A = area of the biopsied piece of skin

y = area covered by microscope objective

The histological data were analysed by the method outlined by Fryer (1966) for nested classifications.

RESULTS

Skin thickness. The mean skin fold thickness of normal, heterozygous and dwarf animals are presented in table 1A. Normal cows had the thickest skin and the dwarfs had the thinnest skin. The heterozygous or carrier animals showed intermediate values. While there was considerable variation among the animals of the same genotype (table 2), the differences among genotypes were significant ($P \leq 0.05$).

Number of sweat glands. The estimated density of sweat glands/cm^2 of skin was least in normal cows (table 1B). The young dwarfs however showed very high values. This was expected since the density is inversely proportional to body size. The analysis of variance showed no significant ($P \leq 0.05$) difference between the genotypes (table 3).

Sweat gland volume. The mean values of the sweat gland volume with standard errors (table 1C) indicate that dwarf and carrier cattle had smaller sweat glands, when means alone were considered. The mean values of normal, dwarf and carrier cattle were 12811.9 ± 873.76, 10167.8 ± 907.08 and 9793.2 ± 533.72 cubic eye piece units respectively. However, statistical analysis of the values revealed no significant differences ($P \leq 0.05$) for this characteristic (table 4).

Depth of sweat gland from skin surface. A comparison of the depth of sweat glands below the skin surface in dwarf, carrier and normal cattle (table 1D) showed that there was no marked difference in the values of dwarfs (114.3 ± 5.44 e.p.u.) and heterozygote cows (107.1 ± 3.53 e.p.u.). The sweat glands of the normal animals seemed

to be located further away from skin surface (130 ± 3.47 e.p.u.). Analysis of variance showed no difference between genotypes for this characteristic (table 5).

TABLE 1

MEANS AND STANDARD ERRORS OF FOUR SKIN CHARACTERISTICS

	Table 1A		Table 1B		Table 1C		Table 1D	
	Skin fold thickness (mm)		No. of sweat glands/cm^2 of skin		Volume of sweat glands in cubic eye piece units (1eye piece unit equals 10μ)		Depth at which the sweat glands are located from the skin surface in eye piece units;(1 eye piece unit equals 10μ)	
	Mean ±	S.E.	Mean ±	S.E.	Mean ±	S.E.	Mean ±	S.E.
Normals	10.4	0.29	711	19	12811.9	873.26	130.2	3.47
Carriers	8.5	0.14	782	34	9793.2	533.72	107.1	3.53
Dwarfs	7.8	0.15	914	261	10167.8	907.08	114.3	5.44

TABLE 2

ANALYSIS OF VARIANCE FOR SKIN FOLD THICKNESS (IN MILLIMETERS)

Source of Variation	d.f.	M.S.	F
Total	71		
Between genotypes	2	41.77	5.75*
Between animals Within genotypes	9	7.26	74.80*
Within animals Within genotypes	60	0.097	

*Significant at $P \leq 0.05$

TABLE 3

ANALYSIS OF VARIANCE FOR SWEAT GLAND DENSITY (NUMBER OF SWEAT GLANDS/CM^2)

Source of Variation	d.f.	M.S.	F
Total	9		
Between genotypes	2	27421.1	1.24
Between animals Within genotypes	7	22051.6	

TABLE 4

ANALYSIS OF VARIANCE FOR THE SWEAT GLAND VOLUME (CUBIC EYE PIECE UNITS; 1 EYE PIECE UNIT=10μ)

Source of Variation	d.f.	M.S.	F
Total	99		
Between genotypes	2	101426382.8	1.164
Between animals Within genotypes	7	87109767.7	5.851*
Within animals Within genotypes	90	14887221.9	

*Significant at $P \leq 0.05$

TABLE 5

ANALYSIS OF VARIANCE FOR THE SWEAT GLAND DEPTH FROM SKIN SURFACE (EYE PIECE UNITS; SCALE, 1 EYE PIECE UNIT EQUALS 10μ)

Source of Variation	d.f.	M.S.	F
Total	99		
Between genotypes	2	5466.1	1.66
Between animals Within genotypes	7	3279.7	11.09**
Within animals Within genotypes	90	295.7	

**Significant at $P \leq 0.01$

DISCUSSION

Skin thickness and structure. Yamane and Ono (1936) intensively studied the skin characteristics of different breeds of cattle and discussed their relative suitability for tropical adaption. Nay and Hayman (1956) found that sweat glands were superficially located from the surface in more heat tolerant zebu breeds. Dowling (1955b) ranked Devon, Hereford, Aberdeen Angus and Shorthorn breeds of cattle in descending order of skin thickness. Among the European breeds, Jerseys had the thinnest skin. He also observed that well-fed animals have thicker skin than under-fed animals. However, he did not consider age to be an important factor influencing skin thickness measurement. But Tulloh (1961) noted an increase in skin thickness with age upto 26 months. He did not, however, find any correlation between live weight and skin thickness. Goldsberry and Calhoun (1959) noted that the skin of Herefords was thicker than Aberdeen Angus. They also noted that among Angus, males had thicker skin than females. Benjamin and Pillai (1963) observed that Hariana bullocks (a zebu breed) possessed thicker skin than cows.

Of all the parameters studied in the present investigation skin thickness was the only characteristic that differed significantly among the various genotypes. This finding is in agreement with the observation made by Hafez *et al*. (1959) who also observed thinner skin in dwarfs than controls. If this

were to be true in a larger population, it can be used as a convenient test. The advantages are that the information is available when the animals are still alive and the test would not involve great expense or sophisticated laboratory techniques. One has to eliminate or minimize the influence of different factors like sex, age, season and breed on skin characteristics before making valid comparisons. As Dahlberg (1949) pointed out, it would be necessary to have a clear concept of normal values, before proceeding to compare with abnormal values. The skin thickness data of young dwarf heifers were also included for comparison, since the t-test showed no significant differences between adult dwarfs and young dwarf heifers.

Density of sweat glands. Although sweat glands in cattle were observed by Gurlt (1835), the functional significance was not fully comprehended till Ferguson and Dowling (1955) experimentally showed them to be functional. The dwarfs showed high values for the characteristic, number of sweat glands/cm^2 (914 ± 261) compared to heterozygous (782 ± 34) and normal (711 ± 19) animals. Carter and Dowling (1954) showed that younger animals have a greater number of hair follicles/cm^2 of skin, and that hair follicle density decreases from birth to maturity. This decrease was attributed to stretching of skin over the body surface as the animal grows. After attaining maturity the hair follicle density remained fairly constant. Nair and Benjamin (1965) observed greater density in cows than in bulls. They also

observed greater density in Kumoani hill bulls (a zebu breed of small size). They attributed this difference to the smaller body size of females and hill bulls.

In the present experiment the dwarf animals used were comparatively smaller in body size than the normal and carrier cows. The higher average sweat gland density data of young dwarf heifers were not included in the comparison with other cows as they were found to be different (t-test) from dwarf cows.

The average density obtained for normal Hereford cows in this study is in general agreement with those values obtained for European breeds like Shorthorn and Red Poll (Carter and Dowling, 1954; Dowling 1955a). The difference between means of the genotypes as regards the sweat gland density, though large were not statistically significant.

Sweat gland volume. The analysis of variance did not reveal any significant difference between genotypes for this characteristic (table 4). The mean volume with standard errors are shown in table 1C.

Depth at which the sweat glands are located. Nay and Hayman (1956) showed that sweat glands were more superficially located in zebu cattle than in the European breeds of cattle. The depth of sweat glands in different genotypes for 'snorter' dwarfism of Herefords considered in the present study, indicated that these glands were located more superficially in dwarfs

(114.3±5.44 e.p.u.) and in carriers (107.1±3.53 e.p.u.) than normal (130.2±3.47 e.p.u.) cows. The importance of this finding cannot be evaluated since the number of animals used was small. The data of young dwarf heifers were not comparable with the rest of the experimental cows as there was difference between young dwarf heifers and dwarf cows for this characteristic.

Standard errors for all characteristics except for skin thickness were higher in dwarfs than normal and carrier cows, indicating a wide variation for each parameter. Leuchtenberger *et al*. (1956) noticed a wide range of variation in the DNA content of dwarf cells. Hafez and Rupnow (1960) noted a similar variation in the thyroid gland size of dwarfs.

The sweat glands of all Herefords studied appeared to be folded and twisted. Nay (1959) observed similar coiling sweat glands in *Bos taurus*.

It is interesting to note that the skin characteristics studied justify the claim that Herefords are well suited for the cold Steppe regions of U.S.S.R. (Ritchie, 1960).

Myoepithelial structure of sweat glands was similar to that observed in zebu cattle and Indian water buffaloes (Benjamin, 1960).

SUMMARY AND CONCLUSIONS

1. No karyotypic abnormalities were detected among the three genotypes considered in this experiment.

2. Karyotyping was not useful for distinguishing the dwarfs, carriers and normal animals.

3. Four characteristics, namely, the skin thickness, the number of sweat glands per square centimeter of skin, the volume of sweat glands and the depth at which the sweat glands were located, were investigated in the 3 genotypes of Hereford cattle.

4. Of the 4 characteristics studied only skin thickness differed significantly among the 3 genotypes. The dwarfs had the thinnest skin and the normal animals had the thickest skin. The heterozygote showed intermediate values. The other 3 characteristics did not reveal any significant differences among the 3 genotypes. The difference between the 3 genotypes for skin thickness was significant ($P \leq 0.05$). Measurements of skin thickness may be a worthwhile procedure for identifying genotypes. However, it is suggested that additional studies should be conducted to provide further evidence.

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