THE HEREDITARY DEFECTS
CONGENITAL DROPSY OF CATTLE AND
ATRESIA ANI OF SWINE

by

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A THESIS SUBMITTED IN PARTIAL FULFILMENT
OF THE REQUIREMENTS FOR THE DEGREE
OF
MASTER OF SCIENCE IN AGRICULTURE
in the Department
of
Animal Husbandry

We accept this thesis as conforming to the standard
required from candidates for the degree of
MASTER OF SCIENCE IN AGRICULTURE

The UNIVERSITY OF BRITISH COLUMBIA

October, 1951
ABSTRACT

Two hereditary defects, Congenital Dropsy of cattle and Atresia ani of swine, are investigated. The introduction makes reference to the evolutionary significance of lethal and sub-lethal characters and compares it to the importance of such factors to the practical breeding of livestock. Some reviews published on the hereditary defects of farm animals are listed.

Part one is concerned with the congenital dropsy defect which was observed and studied in the Ubyssey herd of registered Ayrshire dairy cattle owned and bred by the University of British Columbia.

The history of the herd and the breeding practices employed since the herd's foundation are outlined.

A single-factor recessive genetic hypothesis to account for the occurrence of the ten defective calves is formulated and tested. Genetic analyses of the pedigrees of 153 of the 501 calves born in the herd up to September 30, 1961 indicated the average theoretical probability of the defect occurring to be 0.0853. A test for the "goodness of fit" was applied and showed that the sample studied fits the hypothesis.

The etiology and pathogenesis of the defect are investigated under a working immunogenetic hypothesis based on the two assumptions: 1) that the defect, congenital dropsy, is the counterpart of the hereditary disease of new-born infants, Erythroblastosis fetalis; 2) that the Rhesus isoimmunization theory which serves to explain the familial incidence of the disease in humans may be adapted to the genetics of cattle populations. The immunogenetic studies undertaken to test this hypothesis are described and an explanation of the results, aberrant to the hypothesis, is offered.
Part two deals with a more complex hereditary defect, Atresia ani of swine. The literature is reviewed. The histories and pedigrees of three abnormal litters born in a local herd of registered Yorkshire swine are presented. Two explanations of the possible mode of inheritance of the defect are put forward and tested on the sample available for study.

The recommendations made to the breeder which would enable him to rid his particular herd of breeding stock of the defect are quoted.

The conclusion is a brief discussion of the problems confronting the breeder of registered livestock in whose herds or flocks a hereditary defect occurs.

The appendices include explanations of the methods used to calculate the coefficients of inbreeding and of probability. The chi-square test for "goodness of fit" is outlined. The procedures for the serological reactions employed in the immunogenetic study of cattle are also presented.
ACKNOWLEDGEMENT

For suggestions and guidance in undertaking the genetic studies included in this thesis, the writer is greatly indebted to Dr. J. C. Berry, Professor in The Department of Animal Husbandry, The University of British Columbia.

Special thanks are due Dr. S. N. Wood, Professor in The Department of Animal Husbandry, The University of British Columbia, for his invaluable assistance in the immunogenetic phase of the work.
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INTRODUCTION

Variations in the form and function of organisms enable populations to meet the force of selection, whether it be natural selection or the selection practiced by breeders. If populations lack sufficient variation to meet changes in the environment they become extinct. No environment is static.

The governor of the evolutionary process, selection, continually makes use of variation in evolving the forms best adapted to the environment. If an individual form exceeds the limits of variation allowed by the environment in which it must live, it is unable to reproduce and the germ plasm which it carried is eliminated. This is selection.

Nature's passive selection has, for many species, been superseded by man's active selection. Though the living entity is not immutable but rather prone to vary, man's selection is based on the static concept of the gene. For the practical breeder, the static concept suffices. To the biologist the dynamic concept is necessary to account for the variations in heredity.

In the practice of selection breeders assume that the offspring of the parents selected will receive a sample half of the parent's heredity. If the progeny vary from the expected beyond the limits allowed by the breeders' standards it is usually right to assume that one or both parents carried undesirable germ plasm. If several offspring deviate greatly in form there is extremely little chance the genetic control was altered by a mutation.

Though natural selection is passive, the forms it has allowed to evolve are well adapted to existing environments. Deviations from the normal
are for the most part less adapted to survival and are therefore deleterious.

The abnormalities are referred to as hereditary defects and said to be under the control of lethal or sub-lethal genes. Natural selection has largely eliminated the single-factor dominant lethals and sub-lethals. There are still large numbers of single-factor recessive lethals in each population. The genes controlling the latter are capable of phenotypic expression only when they occur in the homozygous state. Sewall Wright (1931) has shown that a large population mating at random can support a large number of recessive genes. But with the domestication of species, the creation of the races known as breeds, and the division of the population into isolates, true random mating is non-operative. The number of recessive genes that may be carried in the population is much reduced. Inbreeding which of necessity must occur in isolates increases the incidence of the homozygous recessive genotype and the lethal or sub-lethal phenotype. As the practice of breeding within closed populations continues, more and more homozygous recessives will be segregated.

Individuals of reliable pedigree, high individual merit and good breeding worth may be developed by the application of inbreeding in a line breeding program, but the breeder must be willing to risk producing a greater number of homozygous recessives. Not all, but most homozygous recessives are deleterious.

The ease with which a population can be rid of the genes causing a deleterious phenotype varies with not only the basic mode of inheritance but also with the fecundity and prolificacy of the animals. The selection pressure that may be applied is governed by the reproductive rate as well as the economic value of each individual.

The hereditary defects of livestock were reviewed by Hadley and Warwick (1927). Later reviews include those of The Imperial Bureau of Anim-
al Genetics (1931), Hutt (1934), and Batén (1937). Lerner (1944), and Rice and Andrews (1951) have compiled lists of all reported lethals and sub-lethals of livestock and man. The mode of inheritance of most defects is known. Of the thirty-four characters for which the hereditary control has been determined, only two are of the dominant type. The more recent list includes only nine characters of unknown heredity.

Hereditary defects in animals are either anatomic or physiological alterations manifested as changes from the expected either in appearance or in function of body parts involved. Aberrant embryological development may cause similar defects, but they may not be hereditary. Breeding tests must be conducted, or family histories must be secured in order to determine whether or not a given defect is heritable.
I Edematous Calves in the Ubyssey Ayrshire Herd

A The Ubyssey Ayrshire Herd

The University of British Columbia Ayrshire herd of dairy cattle has as its foundation, twenty-four head imported from Scotland in 1929. The present herd of seventy-five head is linebred to three foundation imported cows. These cows were:

- Rainton Rosalind V -130259-
- Ardgowan Gladness II -130282-
- Lochinch Lassie-130269-

All animals in the herd trace from two to six times to one or more of these cows. Three recent herd sires are sons of these cows. These sires are:

- Ubyssey Rosalind's Admiral -228521-
- Ubyssey Governor's Spitfire -246791-
- Ubyssey White Cockade -289047-

Admiral is a son of Rosalind and a grandson of Gladness, While Spitfire is a son of Gladness and a grandson of Rosalind. White Cockade is a son of Lassie and is by Spitfire.

The present herd sire is Ubyssey Admiral's Commodore -307558-, a son of Admiral out of a daughter of Spitfire. The junior herd sire, Ubyssey Cockade's Senator -339850- is a double grandson of Lassie, by White Cockade and out of a daughter of Admiral. Commodore has sired eighty calves. Eight of his calves have been abnormal at birth. All eight, four males and four females, suffered from accumulations of body fluids in the subcutaneous fascia manifested as acute edema. Only two calves born in the herd previously had shown somewhat similar abnormalities. They were sired by Admiral.

The high incidence of abnormal calves among those sired by Commodore led to the undertaking of a genetic analysis of the problem. It
seemed more than coincidental that Commodore, the half-sib of two abnormal calves, had sired eight similarly abnormal calves.

B Congenital Dropsy in Dairy Cattle

The anomaly observed in the Ubyssey calves is comparable to conditions reported to have been observed in certain Scandinavian dairy cattle herds. In his check-list of the lethal genes of cattle, Lerner (1944) has included 'Congenital Dropsy'. The condition in Swedish Fresian cattle was studied by Larsson (1935) and has been reported by Johansson (1939) to the International Congress of Genetics. Korkman (1940) has described similar cases which occurred among the Ayrshire cattle of Finland. The first cases he observed occurred in the progeny of the bull Dunlop Talisman, imported from Scotland.

Johansson reported that the congenital dropsy shows wide variation in manifestation, some fetuses being greatly enlarged through accumulations of fluid in the subcutaneous fascia and in the body cavities, while others are only slightly abnormal. He states that gestation periods in cases of fetal dropsy average 226 days, with a range from less than 150 days to 277 days. Early dropsical degeneration is followed by early abortion, whereas the gestation period may be normal if the degeneration starts late in intra-uterine life. Johansson reports further that pronounced dystocia is the rule if pregnancy exceeds 200 days. The dystocia results from the enlargement of the fetus to more than twice normal weight. The slightly defective animals are born alive and may live for several days or weeks. The extremely defective are stillborn or die shortly after birth.

From a study of Johansson's descriptions and from observations of the cases in the Ubyssey herd, it would seem reasonable to assume that the disease is the same as that occurring in Scandinavian herds. The nomenclature adopted by Lerner, congenital dropsy, therefore seems applicable to the cases observed at the University of British Columbia.
Though Lerner (1944), and Rice and Andrews (1951) have listed congenital dropsy with other defects controlled in heredity by single, recessive factors, there are not, according to Johansson, sufficient cases on record to establish the mode of inheritance. Johansson notes that the cases he observed occurred only among the progeny of some consanguineous matings and suggests the control is by a single recessive gene. Korkman's analysis of the occurrence of the abnormality indicated that a single recessive gene controls the inheritance. No investigation into the etiology or pathogenesis of the disease has been reported.

C Erythroblastosis fetalis in Humans

The disease erythroblastosis fetalis in humans has a marked familial incidence, but the mode of inheritance governing its expression is not known. The study of the disease is further complicated by the manifold clinical manifestations commonly observed. In most instances the disease takes the form of an edematous condition in the new-born infants. According to Weiner (1946) there are two clinical syndromes: the first, congenital hemolytic disease with anemia and/or hydrops; the second, icterus gravis (acute yellow atrophy of the liver). Davidson (1945) states that all clinical entities of the disease are related genetically and etiologically, but none of the syndromes are pathognomonic (peculiar to the disease). He has outlined a principle for the pathogenesis of erythroblastosis fetalis which is based on the isoimmunological reactions of the Rhesus (Rh) group of antigens which occur in approximately 85 per cent of Americans. The Rh-positive husband of a Rh-negative woman transmits, as a mendelian dominant, the Rh factor(s) to the fetus. The Rh-antigenic substances pass from the Rh-positive fetus through the placenta and incite the production of Rh antibodies in the mother. The latter pass from the mother through the placenta to the fetus where they act as hemolytic agents and initiate a sequence of interlocking pathological changes. Similar explanations have been offered
by Weiner (1946), Levine, et al (1939, 1941), Potter and Wilson (1945), and Irwin (1947).

The Rh isoimmunization theory of erythroblastosis fetalis can explain the familial incidence of the disease. While the above authors accept that the human placenta is permeable to agglutinins and antibodies, at least in the later stages of pregnancy, Ranstrom (1947) regards a placental lesion as an indispensable prerequisite to an isoimmunization. He advances the theory that the primary element in the disease is an endocrine dysfunction in the mother which induces a placental change. Any resultant placental lesion paves the way for an Rh isoimmunization if the appropriate serological conditions are present. Potter and Wilson (1945) suggest that an abnormality of the placenta permits fetal erythrocytes to escape to the maternal circulation. Ranstrom's theory affords explanation of the low incidence of the disease. Erythroblastosis fetalis has an incidence of one in 300 to 400 births while one in every eleven pregnant women is Rh-negative and is carrying a Rh-positive fetus (Potter and Wilson, 1945). Irwin (1947) states that approximately one in 50 of the Rh-negative women involved in the critical mating becomes sensitized.

There is nothing to explain why antibodies to the Rh group of antigens should primarily be involved. On the other hand, Levine (1947, 1948) proposes that there may also be an interaction between the mother and the fetus of the A and B antigens. Crew, et al (1947) state there have been obstetrical disasters in cases in which the father was P-positive and the mother P-negative.

The Rh-isoimmunization theory raises a number of questions for which there is no satisfactory answer. Ranstrom has questioned the absence of Rh isoimmunization in many cases of the disease; and the presence of Rh isoimmunization without manifestation of the disease. Levine, et al.
(1941) state there are cases of hemolytic disease in infants when the mother is Rh-positive.

Davidson (1945) has listed the conditions which influence the severity of erythroblastosis fetalis.

i) The age of the fetus when the Rh antibodies become active in its blood stream.

ii) The length of time during which the fetus is exposed to this action.

iii) The strength of the Rh antibodies of which the titer of anti-Rh agglutinins in the maternal blood may not be measured (there being no relation between the titer of these antibodies in the mother and the severity of the disease in the infant).

iv) Permeability of the placenta, there being quantitative differences in different women or the same woman at different times.

He concludes that hemolytic anemia due to the action of Rh antibodies is not only the initial cause of erythroblastosis fetalis, but also it explains the genesis of the complex manifestations of the disease.

D Immunogenetic Study of Cellular Antigens in Cattle

The term immunogenetics was proposed by the Wisconsin immunologists to designate studies in which the techniques of genetics and immunology were employed jointly (Irwin, 1947). The term indicates the study of genetic characters as yet only detectable by immunological reactions.

1 The Antigen Theory: Agglutination and Hemolytic Tests

Basic to the study of immunology is the antigen theory. This theory presumes that the production of antibodies is incited when foreign antigens are introduced into an organism. Findings in the field of immunohemistry have proved that antigens recognizable by immunological techniques are definite chemical compounds. Difference between antigens involve their chemical structures and not simply their physical states. Landsteiner (1931) discovered that substances of simpler form than proteins could be antigenic if attached to proteins. These substances have been
named haptens. The application of serological reagents led to the discovery that the proteins in animals and plants are different and specific for each species.

The antibody is specific for the antigen which incited its production. The presence of these antibodies in the blood serum may be demonstrated. If the red blood cells of a cow possessing the three antigens, A, B, and C are inoculated into a second cow whose red blood cells contain none of these antigens, the production of an isoimmune serum containing three highly specific antibodies to antigens A, B, and C would be expected. The antibodies are as qualitatively distinct as the antigens they define. When this isoimmune serum is tested, while fresh, with the washed corpuscles of individuals having antigens A, B, and C, or any combination of the three, the corpuscles are agglutinated into clumps visible to the eye.

If the fresh, immune serum is heated for one hour at 56° centigrade, or aged for several days, it loses its ability to agglutinate the cells having the antigens. This is due to the loss of a third component called complement, a heat-labile, non-specific substance in all normal sera. The addition of fresh, normal beef serum, containing complement, will restore the ability of the isoimmune serum to agglutinate the cells. A more sensitive test is possible if fresh normal rabbit serum is used as the source of complement. When immune serum is mixed with cells carrying any of the respective antigens in the presence of complement serum, the cells are hemolyzed. Hemolysis of red blood cells is easily observed in vitro because of the free hemoglobin pigment liberated from the cells. Complement combines with antibodies only when the latter are absorbed on the cells by virtue of their union with their specific antigens. Thus the three essential components of a positive hemolytic test are: antigen, its specific antibody, and complement.
Over forty antigens are demonstrable in the cells of cattle. Most of these can be detected singly, so the number of different combinations possible in the bovine species is at least $2^{40}$. If each antigen is a chemical entity, biochemical individuality is possible in the cattle species.

Although the antigens may be detected independently, many are linked in inheritance. For example, of the antigens called B, G, and K, B and G may occur singly or together, whereas K is not found except in combination with B and G. There are genetically two kinds of animals possessing B and G together, namely those in whose offspring B and G segregate as if the parent were heterozygous, and those whose offspring carry either both or neither antigen. There must therefore be linkage of the causative genes of these three substances or they are controlled by genes in an allelic series. Analyses by the Wisconsin workers (Ferguson, 1941; Ferguson, et al., 1942; Stormont and Cumley, 1943) indicate that the causative genes are located on ten of the thirty pairs of cattle chromosomes. There appears to be at least two sets of multiple alleles each including several factors. This reduces the number of loci involved and it is still possible that most of the antigens, each determined by a dominant gene, are independently inherited. The breed differences in the frequencies of the genes responsible for the control of the cellular antigens offer the most detailed information yet available for the genetics of cattle populations. Owens, et al. (1944) found the breeds Holstein and Guernsey differ only in the frequencies of the thirty antigens studied.

E Hypotheses to Account for the Occurrence of Congenital Dropsy

1 A Genetic Hypothesis

Accurate pedigrees of all members of the population under study enable a reliable analysis of a genetic problem. The pedigrees of registered
livestock are highly reliable and are complete in most instances for several generations. Complete records of ancestry are available for each individual in the Ubyssey Ayrshire herd. These records enabled genetic analysis of the edematous-calf problem. A working hypothesis was formulated based on the assumption that the heredity of the defect is controlled by single-factor recessive genes.

The pedigree of each animal in the herd was examined and analysed. Probability coefficients were calculated to establish the likelihood of certain events occurring if the hypothesis was correct. Comparison of the theoretically expected with the observed results by application of a test for "goodness of fit" offered a fairly reliable test of the working genetic hypothesis.

2 An Immunogenetic Hypothesis

A second hypothesis to explain the etiology and pathogenesis of the disease was tested. This hypothesis was based on the similarity which exists in the manifestations of congenital dropsy in cattle and those of erythroblastosis fetalis in humans. If the Rh isoimmunization theory, accepted in eugenics as an explanation of the familial incidence of erythroblastosis fetalis, has a counterpart in cattle genetics, a hypothesis based on the knowledge of bovine cellular antigens would serve to explain the occurrence of congenital dropsy in cattle.

Concurrently with the pedigree analysis undertaken to test the genetic hypothesis, immunogenetic studies using the techniques of Ferguson (1941) were made to test the isoimmunization hypothesis.

Breeding tests which might conclusively establish the mode of inheritance of the disease have not been deemed possible nor practical. Statistically significant results which would establish the mendelian seg-
regation could be obtained only following large numbers of test matings. Breeding tests with cattle are of necessity long-term experiments. To obtain sufficient data would not only be costly but would reduce the average breeding merit of the herd. The deleterious gene would be disseminated throughout the herd in the heterozygous condition. Subsequent matings of the heterozygotes could cause increased incidence of the defect. Several generations of breeding would be necessary to reduce the frequency of the gene to its present value. The low viability of defective calves would increase the difficulty of not only the actual conduct of breeding experiments but also the interpretation of the results.

II Formulation of a Genetic Hypothesis

As related previously, the edematous calves born in the Ubyssey herd were close, collateral relatives. This familial incidence together with the reports of Larsson (1935), Johansson (1939), and Korkman (1940), led to the assumption that the condition was hereditary.

Pathological examinations at post mortem gave no conclusive evidence that the disease might be caused by a specific infection. Also there had been no alteration of feeding methods. Feeding practices during the pregnancies, which resulted in the production of edematous calves, were substantially the same as followed for a period of several years. It seems unlikely therefore that a nutritional deficiency was the predisposing factor.

A Pedigrees and Calving Records

The pedigrees of edematous calves born in the Ubyssey herd are presented in figures 1 to 9, inclusive. The calves have been assigned Roman numerals, being enumerated in the order in which they were born. Calves I and II were by the sire Admiral, and Calves III to X, inclusive, were by his son Commodore. Calf X was a full sib to Calf VI. From extended pedigrees of each of these calves, arrow type diagrams to show lines of descent from
Figure 1. Arrow diagram drawn from pedigree of edematous calf I.

Admiral ——— Galahad ——— Gaiety

I

Jessie

Chaplain

Figure 2. Arrow diagram drawn from pedigree of edematous calf II.

Admiral ——— Galahad ——— Gaiety

II

Lily ——— Daphne

Rosalind V

Chaplain
Figure 3. Arrow diagram drawn from pedigree of edematous calf III.

Commodore — Admiral — Galahad — Gaiety

Natalie — Spitfire — Gladness

Governor — Rosalind — Chaplain

Jezebel — Florence — Brownie

Mainspring

Joanne — Amy

Man of War

Figure 4. Arrow diagram drawn from pedigree of edematous calf IV.

Commodore — Admiral — Galahad — Gaiety

Natalie — Spitfire — Gladness

Governor — Rosalind — Chaplain

Jezebel — Florence — Brownie

Amy

Man of War

Gardenia

Bunice — Mainspring
Figure 5. Arrow diagram drawn from pedigree of edematous calf V.

Admiral — Galahad ———— Gaiety
Commodore

Natalie — Spitfire ———— Governor ———— Mainspring ———— Chaplain

V

White Cockade

Quaker

Jezebel ———— Morven

Kathy ———— Geraldine ———— Elvy ———— Carrie

Figure 6. Arrow diagram drawn from pedigree of edematous calf VI.

Admiral — Galahad ———— Gaiety
Commodore

Natalie — Spitfire ———— Governor ———— Mainspring ———— Chaplain

&

X

White Cockade

Regina ———— Jezebel

Mabel ———— Florence

Brownie

Man of War
Figure 7. Arrow diagram drawn from pedigree of edematous calf VII.

Admiral — Galahad — Gaiety
Commodore
Gladness — Rosalind
Natalie — Spitfire — Governor — Mainspring — Chaplain

Florence — Brownie
Primrose — Jezebel — Morven
Kathy — Geraldine — Elvy — Carrie

Figure 8. Arrow diagram drawn from pedigree of edematous calf VIII.

Admiral
Commodore — Rosalind
Natalie — Spitfire — Governor

Galahad — Gladness — Mainspring
Jezebel — Morven
Ramona — Gladgovan
White Cockade
Precious — Lenora — Juanita
Gem

Man of War
Figure 9. Arrow diagram drawn from pedigree of edematous calf IX.

Admiral

Commodore

Natalie — Jezebel

IX

Spitfire

Rennie

White Cockade — Governor — Morven

Lucretia ———— Heather
<table>
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<tr>
<th>Calf</th>
<th>Sex</th>
<th>Date</th>
<th>Sire</th>
<th>Dam</th>
<th>I.C.</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>M</td>
<td>21/9/41</td>
<td>Admiral</td>
<td>Jessie</td>
<td>12.5</td>
<td>Born dead, enlarged with fluid</td>
</tr>
<tr>
<td>II</td>
<td>M</td>
<td>27/10/43</td>
<td>Admiral</td>
<td>Lily</td>
<td>20.3</td>
<td>Born dead, enlarged with fluid</td>
</tr>
<tr>
<td>III</td>
<td>F</td>
<td>16/6/49</td>
<td>Commodore</td>
<td>Lucy</td>
<td>19.5</td>
<td>Head and legs edematous</td>
</tr>
<tr>
<td>IV</td>
<td>M</td>
<td>3/7/49</td>
<td>Commodore</td>
<td>Ophelia</td>
<td>18.2</td>
<td>Edematous</td>
</tr>
<tr>
<td>V</td>
<td>F</td>
<td>22/11/49</td>
<td>Commodore</td>
<td>Quaker</td>
<td>7.1</td>
<td>Edematous</td>
</tr>
<tr>
<td>VI</td>
<td>F</td>
<td>8/1/50</td>
<td>Commodore</td>
<td>Regina</td>
<td>9.8</td>
<td>Edematous, died at birth of suffocation</td>
</tr>
<tr>
<td>VII</td>
<td>F</td>
<td>7/4/50</td>
<td>Commodore</td>
<td>Primrose</td>
<td>16.4</td>
<td>Stillborn Edematous</td>
</tr>
<tr>
<td>VIII</td>
<td>M</td>
<td>4/9/50</td>
<td>Commodore</td>
<td>Ramona</td>
<td>17.3</td>
<td>Edematous, living, active</td>
</tr>
<tr>
<td>IX</td>
<td>F</td>
<td>31/12/50</td>
<td>Commodore</td>
<td>Rennie</td>
<td>7.8</td>
<td>Aborted at six months. Serous edema, hydrodrops</td>
</tr>
<tr>
<td>X</td>
<td>M</td>
<td>15/1/51</td>
<td>Commodore</td>
<td>Regina</td>
<td>9.8</td>
<td>Clear edematous fluid in subcutaneous fascia -- gaseous edema.</td>
</tr>
</tbody>
</table>

I.C. -- Inbreeding Coefficient (per cent)

Note: Galahad, not Morven is believed to be the sire for Jessie. Both sires served the cow during the same oestrous.

Table I: Data and Records Concerning the Edematous Calfs Born in the Ubyssy Herd.
common ancestors were drawn. These diagrams are presented in figures 10 to 18, inclusive. The coefficients of inbreeding were calculated using the arrow diagrams; and they together with other data and records concerning each edematous calf are presented in Table I.

The calving records, dating back to the founding of the herd were examined and revealed the following information. There have been 539 calves born to 146 dams. There were eight sets of twins. The names of 12 sires and the number of calves sired by each are presented in Table II.

<table>
<thead>
<tr>
<th>SIRE</th>
<th>NUMBER OF CALVES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Auchenbrain Royal Chaplain -130252-</td>
<td>64</td>
</tr>
<tr>
<td>Grandview Man O' War -159659-</td>
<td>15</td>
</tr>
<tr>
<td>Grandview Mainspring -165506-</td>
<td>39</td>
</tr>
<tr>
<td>Ubysssey Royal Gaiety -166532-</td>
<td>18</td>
</tr>
<tr>
<td>Fintry Morven -178672-</td>
<td>37</td>
</tr>
<tr>
<td>Ubysssey Gaiety Sir Galahad -202516-</td>
<td>33</td>
</tr>
<tr>
<td>Ubysssey Rosalind's Governor -202521-</td>
<td>14</td>
</tr>
<tr>
<td>Ubysssey Morven Gladgowan -218123-</td>
<td>7</td>
</tr>
<tr>
<td>Ubysssey Rosalind's Admiral -226521-</td>
<td>93</td>
</tr>
<tr>
<td>Ubysssey Governor's Spitfire -246791-</td>
<td>38</td>
</tr>
<tr>
<td>Ubysssey White Cockade -269047-</td>
<td>66</td>
</tr>
<tr>
<td>Ubysssey Admiral's Commodore -307558-</td>
<td>79</td>
</tr>
</tbody>
</table>

Table II: Sires Used in the Ubysssey Herd and the Number of Calves Sired by Each.

**B Formulation of the Hypothesis**

1 Basic Assumptions

For purposes of the analysis, it was assumed that the pheno-
typic expression, observed as edema in new-born calves, was possible only in genotypes carrying both recessive genes. That is, there are three genotypes possible in the population: the homozygous dominant (AA), the heterozygous (Aa), and the homozygous recessive (aa). Because only two phenotypes have been observed, namely Normal and edematous, we must assume complete dominance. The genotypes AA and Aa having the same phenotypic expression, Normal; and the genotype aa the phenotypic expression, edematous.

From an examination of the arrow diagrams prepared from the pedigrees of the edematous calves, and considering also the breeding practice and resultant lines of descent most common in the herd, certain ancestors of these calves come under suspicion of being heterozygous for the character.

In a population of Normal animals there are only three types of matings possible, viz.

i) homozygous dominant X homozygous dominant

ii) homozygous dominant X heterozygous

iii) heterozygous X heterozygous

The frequency of each type of mating in a randomly mating population will be a function of gene frequency. The mendelian segregation from matings of these two genotypes will be as follows:

i) Parents AA X AA
   Gametes all A
   F₁ all AA

ii) Parents AA X Aa
    Gametes all A 1/2 A, 1/2 a
    F₁ 1/2 AA, 1/2 Aa

iii) Parents Aa X Aa
    Gametes 1/2 A, 1/2 a 1/2 A, 1/2 a
    F₁ 1/4 AA, 1/4 Aa, 1/4 aa
Only under experimental conditions would matings involving a homozygous recessive individual be likely to occur.

2 Heterozygotes in Pedigrees

It is therefore possible to obtain a homozygous recessive individual only when two heterozygotes are mated. In mating two known heterozygotes, only one of every four offspring is expected to be homozygous recessive. The probability of obtaining a homozygous recessive offspring from the mating of two animals of unknown genotype chosen at random from a population is extremely low. To increase the probability of such an event occurring to levels suitable for practical breeding experiments it is necessary to mate animals of known pedigree and genotype.

Since all matings in the Ubyssery herd were of Normal phenotypes, those matings which produced edematous calves were matings of two heterozygous animals. The sires and the dams of any edematous calves were heterozygotes. The names of these eleven known heterozygotes are included in Table I. Further, by examination of the pedigrees of these animals, certain other ancestors of the edematous calves can be assumed, within fair limits of probability, to have been heterozygotes also.

The line of descent from Chaplain, to Gaiety, to Galahad, to Admiral is common to all the edematous calves. The line extends from Admiral to Commodore in all but two of the pedigrees (I and II). In all but two of the pedigrees (VIII and IX), one or more of these sires is a common ancestor. It is therefore probable that the five sires in this line of descent were heterozygous. Because there are at least two lines of descent from at least one of these sires in all pedigrees except VIII and IX, the single recessive hypothesis is acceptable. To account for edematous calves of pedigree VIII or IX it is necessary to incriminate as being heterozygous, animals other than those on lines of descent from any of these five sires. Though Admiral
is heterozygous, and having assumed his sire to have also been heterozygous, the genotype of his dam, Rosalind, may be assumed to have been heterozygous at a probability of one-half. Assuming Rosalind to have been heterozygous, it is possible to account for the occurrence of edematous calves of pedigree VIII or IX. Rosalind is a common ancestor in all but pedigree I.

Calculation of the probability of the recessive genes being passed down both lines of descent in each pedigree from one or more of the heterozygous (assumed) ancestors serves to test the hypothesis and allows a test for goodness of fit.

The hypothesis formulated from examination of the pedigrees of the edematous calves may be outlined as follows:

i) assumed the character to be under control of a single genic pair.

ii) assumed complete dominance.

iii) assumed a line of descent from Chaplain, to Gaiety, to Admiral, to the edematous calf; or from Admiral to Commodore to the edematous calf. All these sires assumed to have been heterozygous.

iv) assumed Rosalind to have been heterozygous.

C Testing the Hypothesis

The pedigrees of all animals born in the herd were checked to determine which calves had Chaplain, Gaiety, Galahad or Admiral as a common ancestor. There are 153 pedigrees in which one of these sires appears as a common ancestor. Chaplain is a common ancestor in 123 pedigrees; Gaiety in 13 pedigrees; Galahad in 26 pedigrees; and Admiral appears in 27 pedigrees. There are 37 pedigrees in which two or more of these sires are common ancestors. Arrow diagrams were constructed from each pedigree and the probability of each offspring being homozygous recessive was calculated.
1 Probabilities of Homozygous Recessives

The average probabilities of obtaining edematous calves together with the assumptions under which they were calculated are presented in outline form.

i) Assuming Chaplain was Aa, ignoring that the calves have other common ancestors:
   - ignoring that Gaiety, Galahad, and Admiral were Aa;
     average probability -- 0.0132.
   - assuming that Gaiety, Galahad, and Admiral were Aa;
     average probability -- 0.0526.
     (based on 123 pedigrees)

ii) Assuming that Gaiety was Aa, ignoring that the calves have other common ancestors:
   - ignoring the Admiral, Galahad, and Commodore were Aa;
     average probability -- 0.0189.
   - assuming that Galahad, Admiral, and Commodore were Aa;
     average probability -- 0.0697.
     (based on 13 pedigrees)

iii) Assuming Galahad is Aa, ignoring that the calves have other common ancestors:
   - ignoring that Admiral and Commodore were Aa;
     average probability -- 0.0396.
   - assuming that Admiral and Commodore were Aa;
     average probability -- 0.0865.
     (based on 26 pedigrees)

iv) Assuming Admiral is Aa, ignoring that the calves have other common ancestors:
   - ignoring that Commodore is Aa;
     average probability -- 0.0549.
   - assuming that Commodore is Aa;
     average probability -- 0.0914.
     (based on 27 pedigrees)
v) Assuming all the sires, Chaplain, Gaiety, Galahad, Admiral, and Commodore were Aa. (This follows naturally from the basic assumption that a line of descent from Chaplain was common to all calves by Commodore.)

average probability — 0.0772.
(based on 146 pedigrees)

vi) Assuming all the sires, Chaplain, Gaiety, Galahad, Admiral, and Commodore were Aa; assuming also that Rosalind was Aa;

average probability — 0.0853.
(based on 91 pedigrees)

2 Goodness of Fit

The chi-squared \( (\chi^2) \) test of "goodness of fit" was applied to the data obtained from the pedigree analysis. From the formula:

\[
\chi^2 = \sum \left[ \frac{(f_o - f)^2}{f} \right]
\]

in which \( f_o \) is the observed frequency and \( f \) is the theoretical frequency, the value chi-squared may be obtained. By reference to a table of chi-squared (Snedecor p190, 1949) the goodness of fit may be evaluated. In the tables of chi-squared, the values indicate the probability of obtaining a fit, due to chance alone, as poor as or worse than the one obtained by calculation. If this probability is small, the likelihood that the disparities between the observed and calculated data are due to chance is also small. If such is the case, the hypothesis under test is probably applicable with fair accuracy to the solution of the problem under investigation.

The average probability of obtaining an edematous calf in any one of the 146 pedigrees analyzed is 0.0853. On the basis of the hypothesis, it would be expected that of the 153 calves born, 13 calves would be edematous. The actual frequency of the edematous calves in the pedigrees under analysis is ten in 153, or expressed as a probability, 0.0653. From these two frequencies, the value of chi-squared can be calculated. Entering the tables of
chi-squared under one degree of freedom (one less than the number of classes) a value for P may be obtained by interpolation. When chi-squared is 0.00469, P is 0.9325 under one degree of freedom. In other words, in approximately 93.25 per cent of similar cases, as great or greater deviations from the theoretical probability would be found, and the present population fits the hypothesis.

\textbf{D Discussion of Results}

Though the observations and data drawn from this fairly large sample support the working hypothesis as it was originally formulated, the hypothesis is not confirmed. More evidence to support the hypothesis could be obtained by test matings and back crosses if it were possible to raise the homozygous recessive to reproductive maturity. The maintenance of complete calving records and accurate pedigrees, as required with registered cattle, will however enable further analysis to test the hypothesis.

The types of matings possible in a population of Normal individuals have already been outlined. There are three similar types of matings possible if the population contains edematous individuals. If the mating of two homozygous recessives was possible, the hypothesis would receive an absolute test, since only edematous offspring could be produced. Unless all the offspring from two homozygotes are themselves homozygotes, the character is not under control of a single genic pair.

There are at present only six animals of proven genotype in the Ubyssey herd. These are the sire, Commodore, and the five dams of edematous calves remaining in the herd. The cows to which Commodore can be mated are either homozygous dominant or heterozygous. The descendants of Rosalind and especially the descendants of Admiral are more likely to be heterozygous than homozygous dominant. Mating Commodore to collateral relatives increases the likelihood of obtaining edematous calves. The probability of the homozygous recessive occurring in each case would be at least or greater than 0.25. If a female edematous calf by Commodore is born alive and is fertile
at maturity, she could be mated to her sire, in which case the probability of obtaining an edematous calf would be 0.50. Such a mating would be of genetic interest only. Such a female calf would not ordinarily be raised. She could however be used to test cross other bulls of unknown genotype. Such test crosses would not likely be warranted. Because of the long gestation period and the small number of offspring it would take several years to prove the genotype of one bull. The number of sires which could be test crossed to one female is very limited.

If a male edematous calf was fertile at maturity and was successfully mated to the cows known to be heterozygous, the probability of obtaining an edematous calf in each case would be 0.50.

One other method of testing the hypothesis, the least likely to become available, is to mate two edematous individuals. The likelihood of obtaining two calves of opposite sex whose periods of sexual maturity and fertility would coincide is very small.

It has been assumed that the condition observed in the newborn calves and named congenital dropsy is due to the presence of a single pair of recessive alleles. Also it has been assumed that there is complete dominance, that one recessive allele is overshadowed completely by the phenotypic expression of the dominant allele.

The condition of the fluid balance of the immature bovine is then assumed to be under control of a single genetic pair. If one or both dominant alleles are present, the individual is a normal phenotype. If both recessive alleles are present, the individual is edematous. The fluid balance of an organism must, by the number of factors exerting influence on the balance, be under a complex control; but, because of the delicate nature of this balance, it may be upset by a single, simple change in the physiological processes which control the accumulation and distribution of body fluids. If but one step in one of these processes is dependent on the
presence and phenotypic expression of at least one dominant gene of an allelic pair, the absence of both dominant genes will prevent the initiation of the series of events necessary to produce a system of control for the fluid balance of the organism.

Though pedigree analysis has shown the heredity of congenital dropsy to be very probably under the control of a single recessive gene, the wide variations in manifestation may seem to contradict this assumption and to indicate rather, a complex mode of inheritance. Since in each individual the genes exert their separate influences at equivalent stages of development, a single genetic pair would be expected to produce the same syndrome in all individuals possessing it. However, the intrauterine environments may be of sufficient variation to cause the various degrees of severity in the defect observed at birth.

If a linkage group, or a number of genes, were responsible in part or in total for the controls of the fluid balance, it is possible a weakness in this group is that which would be inherited and the weakness could be affecting different genes within the group in each individual. Again assuming that each step of the physiological processes is under control of a separate gene, that step in the process which would be deleted would depend on which gene was affected by the hereditary weakness.

III Formulation and Testing of an Immunogenetic Hypothesis

A review of the literature on the congenital diseases of infants makes evident the similarity between the manifestations of erythroblastosis fetalis in humans and those of congenital dropsy in cattle. The Rhesus-isoimmunization theory, accepted by many as an explanation of the disease erythroblastosis fetalis, has already been outlined. The immunogenetic studies of the cellular antigens of cattle have been reviewed. Considering the Rhesus-isoimmunization theory and the information available on the cellular antigens of cattle, there is a possibility that the
explanation of the human disorder could serve to explain the disorder in cattle. One, or a group of cattle antigens may function, as the Rh antigens are thought to function in humans, to produce an edema of the new-born. An analysis to determine the antigen complement of individuals in the families producing defective calves would show whether or not an isoimmunization theory could explain the incidence of the edematous calves.

A working hypothesis based on the following assumptions was formulated.

1) Assumed that the erythroblastosis fetalis is the counterpart of congenital dropsy in cattle.

2) Assumed that the Rhesus-isoimmunization theory explains the incidence of erythroblastosis fetalis.

3) Assumed that a similar isoimmunization theory applies to the inheritance and interaction of the cellular antigens of cattle.

A. Formulation of the Hypothesis

The hypothesis, as it applies to the cases in the Ubyssey herd, may be stated as follows. The sire, Commodore, possesses certain cellular antigen or group of antigens that is not possessed by the dams of the edematous calves. If the fetus by Commodore is positive for antigens for which the dam is negative, and the antigens traverse the placenta to enter the blood stream of the dam, the production of antibodies specific to each antigen is incited. These antibodies, carried in the serum of the dam, may traverse the placenta to enter the serum of the fetus. The antibodies of the dam cause a hemolysis of the fetal erythrocytes or erythroblasts and initiate the chain of developments leading to the manifestation of congenital dropsy.

Commodore will pass on to his offspring a sample half of the inheritance governing the phenotypic expression of the antigens for which he
is positive. It is not necessary that all the dams of edematous calves be negative for the same antigens. If the blood of Commodore is not compatible to the blood of each of the dams of edematous calves the theory accepted in human genetics may be applicable to cattle genetics.

B Testing the Hypothesis

The hypothesis was tested by conducting blood hemolysis tests using the washed cells and the sera of the seven cattle available and four normal rabbits. The bloods of Commodore, Lucy, Ophelia, Primrose, Quaker, Regina, and an edematous calf out of Primrose were tested. Fresh, normal rabbit serum was used as the source of complement in all hemolytic tests. Detailed procedures for conducting hemolytic tests are presented in the appendices.

Commodore-immune sera were produced by repeatedly injecting intravenously into normal rabbits suspensions of washed cells from Commodore. Suspensions of the washed blood cells of each cow were also prepared. Hemolytic tests were conducted using the immune rabbit sera separately and suspensions of the washed cells of each cow. Similar tests were conducted using the so-called isoimmune serum of each cow and suspensions of the washed cells of Commodore. These latter tests served to test the belief that the fetus can inherit certain antigens from its sire that will incite the production of antibodies in the dam.

All isoimmune and immune sera were used in three dilutions and adequate controls were prepared in all tests. The tests were conducted in triplicate and repeated twice.

None of the so-called isoimmune sera produced a hemolysis of Commodore cells. The Commodore-immune rabbit sera each produced hemolysis in all cases when tested with suspensions of the washed cells of the cows.
These results show that Commodore's cells possessed antigens that incited the production of antibodies in the rabbit sera. These antibodies were specific for at least one antigen in each of the cows' cells, showing that Commodore has at least one cellular antigen in common with each cow.

Tests using the serum (antibodies) of each of the seven animals and the cells (antigens) of the other six animals showed that the serum of Lucy contained antibodies which would lyse the cells of Ophelia, Quaker, and Regina. None of the other sera contained antibodies to the antigens of the cells of the other animals.

C Discussion of Results

While the tests employed were not sufficiently extensive to confirm that the hypothesis is incorrect, it would seem probable that the iso-immune theory is not applicable to the incidence of congenital dropsy.

It would be expected that the production of antibodies incited in the dam by the paternal antigens of the fetus would have resulted in the accumulation of sufficient amounts of the antibodies to cause a hemolysis of the cells of Commodore. Especially in the case of Primrose would this be expected since her serum was used in tests shortly after the birth of her edematous calf when the antibody titre would likely be at a maximum. None of the cow sera produced hemolysis of Commodore cells, therefore none of the sera was carrying antibodies for the antigens of Commodore.

The serum of Primrose did not cause a hemolysis of the cells of her calf, showing that she had not produced any antibodies specific to the antigens the fetus had inherited from Commodore. It is possible, but highly improbable, that Primrose would carry the same antigens on her cells that the fetus inherited from Commodore. If the hypothesis was correct, the
serum of Primrose should have caused lysis of both the cells of Commodore and the cells of her calf.

The results of tests using the Commodore-immune rabbit sera seem consistently in aberrance to the hypothesis. However, considering the large number of cattle antigens and recognizing that all the animals are of similar inheritance, it is unlikely that any would have an antigen complement entirely different from that of Commodore. The tests using cells and sera of each animal showed that Lucy carries antibodies for the antigens of three other cows. Lucy must have been sensitized by antigens common to each of these cows, but not found in the other animals. Since her serum would not lyse the cells of Commodore, she could not have been sensitized by the fetus sired by Commodore.

A thorough test of the hypothesis would necessitate a complete antigenic analysis of many individual animals. Such an analysis would require the production of isoimmune as well as immune sera. The Wisconsin workers have cited cases of anaphylactic shock during the production of iso-immune sera. The risk involved is too great when working with valuable breeding stock. Any solution would be very involved because of the large number of cellular antigens demonstrable in cattle.
II. **Atresia ani** in a Yorkshire Swine Herd

A second hereditary defect has been observed in a registered swine herd. While pedigrees of all the animals are available, the complex mode of inheritance of the defect does not make genetic analysis possible without extensive breeding tests. Because of the high prolificacy and lesser value of each individual, breeding tests would be much more easily conducted with swine than with the other classes of livestock. However, no such breeding tests have been undertaken.

An examination of the pedigrees made it possible to make certain recommendations to the breeder, recommendations that would enable him to rid his swine herd of the defect.

A Review of Literature

The defect observed in these registered Yorkshire swine is similar to the condition described by Berge (1941) as occurring in the same breed in Norway. Berge also cites the writings of Kinzelbach (1931) and Cartens, et al., (1937) which describe the condition occurring in Yorkshire swine in Germany. The abnormality is named **Atresia ani** and is included in Lerner's (1944) list of lethal and sub-lethal characters observed in swine. Rice and Andrews (1951) also list the condition as a lethal, but do not state the mode of inheritance of the defect.

Berge states that the malformation is found to be different in sows and boars. Typical in boars is the closed anus. The pigs are usually short-lived because of the obstruction of the rectum. In the sows, he continues, the anus is absent but usually an opening is found in the ventral wall of the rectum with a communication to the vagina, resulting in a type of cloaca. Some of these sows live to maturity and are fertile.
B History of the Occurrence of Atresia ani in the Local Herd

The conditions observed in the local swine herd seem very similar to those described by Berge. There were some cases of Atresia ani previous to 1951, but they were not recorded in detail nor studied by the breeder. Recently there have been born three litters of which some piglets were abnormal. In one litter of eleven males and five females all were abnormal. The dam of the litter was abnormal but had been bred to a normal boar that had sired several normal litters. She had previously dropped some abnormal pigs.

A second litter of abnormal pigs (eight males and six females) was sired by an imported boar and was out of a sow that had previously given birth to two normal litters. However, the dam of this sow had shown abnormal formation of anus and vulva.

The third litter of abnormal pigs was also by the imported boar. Of the eighteen pigs, ten were males and eight were females, four of the females being stillborn. The sow had previously given birth to five litters of normal pigs. The imported boar had not been mated to either of these sows previously.

All three of the dams of abnormal pigs are by the same boar. They are also out of half sisters. Two of the grandams were themselves out of half sisters. There is no inbreeding in any of the pedigrees, at least as far back as five generations.

C Pedigree Analyses

In his work at the Aas Breeding Station in Norway, Berge test mated boars known carriers of a factor causing atresia ani. The two boars were both apparently normal, but transmitted atresia ani in different manners. One had 21.2 per cent atresia ani in litters containing abnormal pigs, while the other had 14.3 per cent. The percentages effected in both sexes
were equal.

Two full sisters, daughters of boar A, with cloaca reached maturity and were bred to boar A, boar B, and an unrelated boar, C. The results are tabulated in Table III.

<table>
<thead>
<tr>
<th>SIRE</th>
<th>SOW I</th>
<th>SOW II</th>
<th>Per cent</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal</td>
<td>Abnormal</td>
<td>Normal</td>
</tr>
<tr>
<td>A</td>
<td>6</td>
<td>1</td>
<td>15</td>
</tr>
<tr>
<td>B</td>
<td>2</td>
<td>3</td>
<td>9</td>
</tr>
<tr>
<td>G</td>
<td>4</td>
<td>0</td>
<td>12</td>
</tr>
</tbody>
</table>

Table III. Incidence of Atresia ani in Two Inbred Litters and One Outcrossed Litter (Berge, 1941.)

The number of offspring was not large enough to justify calculation of the segregation ratio, though it was clear that a monogenic mode of inheritance was not in control.

Berge has suggested a two-factor hypothesis, but he states it is impossible to say if both, one or neither of the factors is chiefly dominant. In conclusion he states that when the mode of inheritance is so irregular, the purchasing of breeding stock would be impossible if the buyer required the animals to be free of genes causing the deleterious character.

The pedigrees of swine with Atresia ani in the local herd are presented in Figures 10, 11 and 12.

D Genetic Hypotheses
1 Simple Mendelian Recessive

Since in the cases cited all pigs in each litter were abnormal, it is very unlikely that this defect is under control of a simple mendelian recessive factor. So many sub-lethal and lethal characters studied in farm animals are of this type, this may seem the most likely hypothesis to test. The pedigrees examined under the hypothesis show that two animals, C and Q
Figure 10.

Pedigree I

X (zox 29e)
first mating to A

8 males
6 females
2 fem. s-b
Atresia ani

A (arr 264c)
two normal litters previously

B (qual 15a)

G (qual 19b)
Atresia ani

F (HRP)
Figure 11.
Pedigree II

X (zox 29e)
first mating
to M

11 males
5 females
Atresia ani

M (arr 68b)
five normal
litters
previously

B (qual 15a)

F (HRP)

N

O (Jub.)

P
Figure 12.

Pedigree III

Y (hnf 107c)

had sired no
abnormal pigs
previously

10 males
4 females
4 fem. s-b

Atresia ani

B (qual 15a)

Q (arr 9b)

Atresia ani,
abnormal offspring

F (HRP)

R

O (Jub.)

T
in addition to the pigs in the three litters, are homozygous recessive. The animals, X,Y,A,M,B,F,G, and R must therefore also be heterozygous. In pedigrees I and II, since both parents are heterozygous, it would be expected that only one offspring in four would be homozygous recessive. If dominance is assumed, the phenotypes should appear in the ratio, three normal to one abnormal. In pedigree III, since one parent is homozygous recessive and one is heterozygous, it would be expected that equal numbers of normal and abnormal offspring would be obtained. Since no normal offspring were born in all forty-nine births it is reasonable to assume that a more complex mode of inheritance is in control.

2 Two-factor Dominant

If the condition Atresia ani is under the control of a two-factor dominant character, as Berge states is likely, it is possible to account for the results in the above pedigrees. Phenotypic expression is possible when the genotype is AABB, AAbb, AaBB, or AaBb. These are abnormal phenotypes. Phenotypic expression is not possible when the genotype is aabb, aaBb, Aabb, AAbb, or aaBB. These are normal animals.

The types of matings possible under the two factor hypothesis may be outlined as follows.

i) Of normal animals

There are ten possible matings of normal phenotypes. In only four of these can any abnormal animals be obtained. In two cases the probability is 0.50; in one case the probability is 0.25; and in one case all offspring will be abnormal.

ii) Of abnormal animals

There are six possible matings of abnormal animals. In four cases all offspring will be abnormal. In two cases the probability of abnormal offspring is 0.75. It is not likely that this type of mating would be made except for experimental investigations.
iii) Of normal to abnormal animals

There are twenty possible matings of this type. In seven cases all offspring will be abnormal. In eight cases the probability is 0.50. In two cases the probability is 0.75 and in another two cases the probability is 0.375. In the final cases the probability of obtaining abnormal offspring is 0.25.

In pedigree I there is a normal to normal mating. Neither A nor X is aabb genotypically. If animal A or animal X is Aabb or aaBb then the other is neither AAbb nor aaBB. If either animal is Aabb and either is aaBb then the probability of obtaining abnormal offspring is 0.25. If either A or X is AAbb or aaBB and the other is Homozygous dominant on the other locus, the the probability of obtaining abnormal pigs is 1.00. The weighted average probability of obtaining an abnormal offspring is 0.233. In the same pedigree, the mating of B to C is an abnormal to normal mating. No matter what the genotype of these animals there is always a chance of abnormal offspring being born. Since A was normal, seven of the twenty possible matings may be omitted. In the remaining types, the probabilities of obtaining abnormal animals range from 0.75 to 0.25. The weighted average probability is 0.50.

In pedigree III the mating of Y to Q is also an abnormal to normal mating, but unlike the mating of B to C, there have been no normal offspring produced. Therefore the mating may have been any of the twenty types in this group. The weighted average probability of obtaining abnormal offspring in this type of mating is 0.75.

B Recommendations to the Breeder

The following recommendations, made after an examination of the pedigrees and the literature on the defect, were offered to the breeder to enable him to take the steps necessary to rid his herd of the defect.
i) Stop service of boar X immediately. Butcher him now or de­lay final judgement until the sows already bred to him have farrowed.

ii) Butcher all sows which have born defective offspring and the progeny of these sows.

iii) Refrain as much as possible from using females closely re­lated to the above sows. For example, descendants or any close relations of the boar B.

iv) Any boars in service now or to be in service in the future should themselves be free of the defect and be chosen from families known to be free of the defect.

It is highly probable that the boar X is a carrier of the factors causing Atresia ani since the sows A and M have previously had no abnormal pigs by other boars. If the boar Y is used on normal sows from families free of the defect, the probability of obtaining abnormal offspring is rather low. However, none of his offspring should be considered as breeding stock. In the selection program there should be considerable discrimination against descendants and relatives of the boars B and F because they are closely related to the dams of abnormal litters.

These are severe recommendations, but rather drastic steps must be taken to control this type of defect in breeding stock. It will be necessary to maintain close observation and culling practice must be very severe for several generations before the herd is rid of this defect.
CONCLUSION

Linebreeding, which is often referred to as applied inbreeding, enables the breeder to maintain a high degree of relationship to ancestors highly meritorious for the hereditary traits on which he bases his selection practices. His selections are made easier and more accurate if he is able to evaluate his stock on the merit of the ancestory as well as on the merit of each individual in the herd.

Continued linebreeding will increase the inbreeding in his animals to significant levels. By making more gene loci that were formerly heterozygous homozygous, he increases the prepotency of each individual. In payment for the increase in prepotency he must risk the greater likelihood of homozygous recessives occurring. Not all, but most homozygous recessives are less desirable than either the heterozygote or homozygous dominant.

The breeder who is following a line breeding program should expect, sooner or later, to be confronted with a hereditary defect. Defects such as the absence of one or all dew claws in cattle are of no practical significance since the performance of the animal is not effected. But the breeder should be gravely concerned with severe defects classed as lethals or sub-lethals which interfere with reproductive efficiency and individual performance.

Considerable income may accrue to the progressive breeder from the sale of breeding stock. If his breeding stock, because of its homozygosity, is prepotent, it will be much in demand. If the breeding to produce prepotency also allows the expression of genes (formerly carried in the heterozygous state) which control deleterious characters, the sale value and demand for his stock immediately suffers.

To rid his herd of the defect he may have to sacrifice animals proven to be of high breeding worth for the characteristics of
economic importance. The development of such individuals would likely require several generations of careful selection and breeding.

If the facts concerning the occurrence of the defect are not publicized, and the breeder is judicious in his choice of animals fit for sale as breeding stock, the reputation of his herd may be maintained while the defect is eradicated. If he continues to sell animals which could possibly be carriers of the defect, sooner or later, the genes will segregate as homozygous recessives and the defect will be uncovered. Meanwhile the deleterious genes may have been spread to several herds. The incidence of even a lethal character may be so low that it is of no practical concern to any breeder. The maximum probability of the occurrence of the defect calculated from the genetic theory of the mode of inheritance is seldom reached in small families. The establishment of the true mode of inheritance and the segregations that may be expected should be available to the breeder to enable him to deal with the problem in a method least deleterious to the breed and at the same time economically possible for him to follow. Defects under control of single genic pairs may be eliminated more easily than those having more complex genetic controls.

With the less prolific and less fecund classes of livestock the elimination of even a single-factor recessive is difficult. A large fraction of the animals in a linbred herd come under suspicion when a defect occurs. Breeding tests to prove the genotypes of the suspected heterozygotes are not practical. The breeder can only apply a somewhat passive selection pressure against the individuals under suspicion since the economic characteristics must receive selection priority if his enterprise is to remain profitable. The expense of replacing the suspected with other animals of equal worth can be prohibitive in the less prolific classes of livestock. The salvage value of animals culled from the herds or flocks of these animals is too low to defray the expense of replacement.
With swine, however, it is possible to take rather drastic steps to rid a herd of a hereditary defect. The salvage value of mature stock is in line with the purchase price of immature replacements of equal worth. The rapid maturity, high prolificacy and greater fecundity enable the breeder to quickly replace his herd. In order that he may replace his herd with animals free of the same or another defect, he should have available the mode of inheritance and the segregation ratios pertaining to the hereditary defects of swine. Breeding tests and backcrosses are possible with swine and should be carried on by qualified geneticists who would thus increase the knowledge of the heredity of swine as well as aiding the practical breeder in his work.
REFERENCES CITED


Ferguson, Stormont and Irwin, (1942), *Journal of Immunology*, vol 44, pp 147-164.

Hadley, F. B. and B. L. Warwick, (1927), *Journal the American Veterinary Medical Association*, vol 70, pp 492-504.


Kinzelbach, (1931), *Vererb*, vol 60, p 84.

Korkman, (1944), *Biological Abstracts*, vol 22, abstract 10625.


Levine, P., (1947), cited in _Advances in Genetics_ vol 1, pl133.

Levine, P., (1948), cited in _Advances in Genetics_ vol 1, p 133.


Levine, Katzin, and Burnham, (1941), _Journal the American Medical Association_, vol 116, p 825.


Ranstrom, S., (1947), _Journal the American Medical Association_, vol 134, num 13, p 1136.


Wright, Sewall, (1931), _Genetics_, vol 16, pp 97-159.
APPENDICES

A Calculation of the Coefficient of Inbreeding

The coefficient of inbreeding is a measure of the amount of decrease in heterozygosis to be expected from a certain amount of consanguinous mating. It indicates the amount of heterozygosity of the whole group of genes in an individual animal. The coefficient is exactly one-half of the relationship between the two parents of an individual unless the parents themselves are inbred.

The formula is as follows:

\[ F_x = \frac{1}{2} \cdot S \left[ \left(\frac{1}{2}\right)^n (1 + F_a) \right] \]

where \( F_x \) is the inbreeding coefficient of animal \( x \); \( n \) is the number of generations in a line by which sire and dam are related; \( F_a \) is the inbreeding coefficient of the common ancestor \( A \) out of whom that line of descent divides; and \( S \) is the summation of separately evaluated results. The factor 1 plus \( F_a \) corrects for the greater likeness which can be expected between the gametes of an inbred individual.

B Calculation of Probability Coefficients

In the calculation of the probability of obtaining a homozygous recessive from the mating of two unknown genotypes it is assumed that the likelihood of the genotype of the parent being heterozygous is \( \frac{1}{2} \) raised to a power equal to the number of generations the parent is removed from the ancestor common to both parents that is assumed or known to have been heterozygous. If both parents are known heterozygotes, the probability of the offspring being homozygous is \( \frac{1}{2} \cdot \frac{1}{2} = 0.25 \); of being homozygous recessive is 0.25. If a common grandparent is known to be heterozygous, the probability of the genotype of the descendants one generation removed being homozygous recessive is one-half raised to the fourth power, or one-sixteenth.
C Test for Goodness of Fit

Biometricians measure the goodness of fit by adding together the proportional deviations of each class, obtaining a constant known as chi-squared, and this determining the probability that a deviation as great or greater will occur by chance. Chi-square is obtained by squaring the deviation of each class from the theoretical expectation of that class, dividing this by the theoretical expectation for that class, and adding together the results from all classes. From prepared tables it is possible to obtain for a given value of chi-square and a given number of classes the value F, which measures the probability that a deviation as great or greater will occur by chance, in other words, the percentage of cases in which such a deviation may be expected by chance.

D Procedures for Serological Studies

Preparation of Immune Serum

Source of Antigen

Collection of Blood Sample

Materials: cotton-batten, alcohol, hypodermic needle fitted to rubber tube, 200 ml sterile flask with stopper, 75 ml isotonic citrate solution (2% sodium citrate -- 0.5% sodium chloride), 50 ml tube fitted with stopper and containing glass beads.

Method: the blood is drawn from the jugular vein using the hypodermic needle and rubber tube. Approximately 75 ml of blood is collected in the 200 ml flask containing the 75 ml of isotonic citrate solution. The flask is stoppered and refrigerated. Approximately 25 ml of blood is drawn into the 50 ml flask. The flask is quickly stoppered and shaken for five minutes to defibrinate the blood sample. Defibrinated blood is used in the preparation of cell suspensions. It must be heated to 56° C for one half hour to destroy the complement.
Preparation of Erythrocyte Suspension

Materials: One litre Gelatine Locke's Solution, 50 ml physiological saline solution (0.85%), sterile 15 ml pipette, 4 → 20 ml sterile graduated centrifuge tubes, sterile graduate, 50 ml sterile flask with stopper, 60 ml citrated blood sample.

Method: pipette 5 ml of citrated blood sample into each of the four centrifuge tubes. Centrifuge to ten minutes. Decant or withdraw with a pipette the citrate-serum mixture. Add 5 ml of gelatine Locke's solution to each tube, shake thoroughly, recentrifuge for ten minutes. Repeat washing four times. Transfer all cells to one tube, pack cells by centrifuging, record volume of packed cells. Transfer cells to sterile 50 ml flask using equal volume of sterile physiological saline solution. Place 3.5 ml of the 50% suspension in each of nine vaccine bottles fitted with rubber stoppers.

Inoculation of Rabbits

Inoculations

Materials: three mature normal healthy rabbits, razor, cotton-batten, alcohol, sterile 5 ml hypodermic syringe, erythrocyte suspension, 3 sterile hypodermic needles (No. 22).

Methods: the hair over the marginal vein is dry shaved. The area is sterilized with alcohol. The syringe is filled aseptically and one ml is injected into the vein. Each rabbit is injected intravenously three times weekly for three weeks with one(1) ml of the 50% suspension.

Drawing the Immunized Blood

The rabbit is held in a bleeding stock on its back with the head caught in a well-rounded notch and the four feet held by thongs tightened to draw the limbs to full extension. Blood is drawn by cardiac puncture technique. The blood is expelled into sterile tubes with cotton stoppers and allowed to clot. The immune serum is withdrawn using a sterile pipette.
Complement serum may be obtained using the same technique and normal healthy rabbits. The blood should be refrigerated as soon as clotted and the serum must be used shortly after the blood is drawn.

Test Suspensions of Washed Cells

Blood samples are drawn into defibrinating flasks as described above, 25 ml of blood is a large enough sample to supply cells for the 2.5% suspensions used in the tests. At the same time, 40 ml of blood is drawn into sterile tubes fitted with cotton stoppers in which it is allowed to clot before being refrigerated.

Note: To insure strict aseptic conditions each tube is fitted with a hypodermic needle attached by a plastic tubing. The assembled equipment is autoclaved at 15 pounds for thirty minutes and is not disassembled until the serum is to be withdrawn by a sterile pipette.

The preparation of the 2.5% suspensions of washed cells is parallel to that of the 50% suspensions.

Hemolytic Tests

Immune Sera Dilutions

Materials: undiluted rabbit sera in 25ml flasks, 9 -- 20 ml test tubes, physiological saline, 3--- 10 ml pipettes.

Method: place five ml of serum A in tube I, and in tube II. Add 10 ml of saline solution to tube II. Remove 5 ml of solution from tube II and place it in tube III. Add 10 ml of saline solution to tube III.

Test Tubes

Each tube should contain:

- 1 drop of 2.5% suspension of washed cells
- 2 drops of undiluted or diluted serum from one Immunized rabbit
- 1 drop of complement serum

Control tubes and replicate tests should be prepared under the same conditions.