

6, 10, 14 and 18 weeks of age in 1958 and on 16 inbred Suffolk lambs at 4, 6, 8, 10, 14 and 18 weeks of age in 1959. In a second experiment, 16 line cross Suffolk lambs were serially sacrificed in groups of four each at 2, 4, 6 and 8 weeks of age. The rumen, reticulum, omasum, and abomasum were removed immediately after slaughter and washed. Volume and wet and oven-dry weight determinations were made after exterior adipose tissue had been manually removed.

Multiple linear regression analyses by years revealed that among the Suffolk lambs urea and uric acid concentrations were significantly affected by age ($P < .01$) and that urea, amino acid nitrogen and creatinine were significantly affected by weight ($P < .01, .05, .10$, respectively). Among the crossbred lambs only uric acid was significantly affected by age ($P < .05$). Although breed, sex, and birth type were not shown to significantly influence the concentrations of the blood constituents, it is believed that differences due to these factors do exist but that they were small.

Stepwise discriminant functions were computed at each time period to find as early in lamb age as possible that combination of weight and/or blood constituents which most effectively distinguished between the more desirable lambs which were saved and the inferior lambs which were culled. Among the crossbred lambs the most effective discriminators were weight and uric acid at 10 weeks and those variables plus creatinine at 14 weeks of age. The most effective discriminators among the Suffolk lambs were: weight, creatinine, and amino acid at 10 weeks, urea, creatinine, and amino acid at 14 weeks, and weight, urea, and

amino acid at 18 weeks of age. In both years the coefficients of determination, R^2 , reached a minimum value at six weeks of age then climbed to a maximum at 14 weeks. The problem of optimising the choice of time period at which to make selections based on the discriminant function was noted, but was not answered in this study.

Simple product-moment correlations showed that wet and oven-dry weights of the rumen were highly correlated ($r = .99$) and that both were highly related to volume ($r = .97$). On a dry weight basis the rumen comprised 35.9 per cent of the total weight of the four stomach compartments at two weeks of age, and increased linearly to 63.9 per cent at eight weeks. At the same time the abomasum decreased from 47.8 per cent to 19.5 per cent of the total dry weight. Rumen dry weight was significantly correlated with uric acid ($r = .52$) and creatinine ($r = .66$) concentrations, but the relationship between rumen dry weight and urea nitrogen concentration was borderline ($r = .42$, $P < .10$). The amino acid nitrogen concentration was independent of rumen size ($r = .02$). The physiological significance of these relationships was discussed.

NONPROTEIN NITROGENOUS CONSTITUENTS OF THE BLOOD
IN RELATION TO RUMEN DEVELOPMENT AND GROWTH
IN SUCKLING LAMBS

by

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NON-PROTEIN NITROGENOUS CONSTITUENTS OF THE BLOOD IN RELATION TO
RUMEN DEVELOPMENT AND GROWTH IN SUCKLING LAMBS

I. INTRODUCTION

Statement of the Problem

It has long been recognized that individual animals of a given breed and species differ in their ability and efficiency to gain weight or to produce milk or wool. In nearly every instance studied, it has been demonstrated that the animals with the propensity for a rapid rate of growth or production required less feed per unit of production and were therefore economically more efficient than their slower performing relatives. It is also well established that to some degree these differences in rate and efficiency of gain are inherited. In practically all cases it can be demonstrated that this is a quantitative type of inheritance where many pairs of genes are presumed to influence the attributes which contribute to such differences. If we accept the theory that genes exert their effects biochemically by controlling the production of specific proteins, it is logical to conclude that the inherited portion of the differences in rate and efficiency of production must be due to genic control of the various metabolic processes.

The blood in particular, and the circulation system in general, is probably more intimately associated with all aspects of metabolism in multicellular animals than any other single physiological system. Of the many physiological processes which must be involved in the production of differential growth rates, it seems logical to suppose that at least four are both of primary importance and are capable of being

measured: 1) absorption of nutrient materials from the digestive tract; 2) withdrawal of the absorbed nutrients from the blood by the tissue cells; 3) oxidation and metabolism within the cell; and 4) excretion of waste products of tissue metabolism into the blood. This is to say that an animal superior for rate and economy of gain will also be superior in one or more of these four attributes.

If the preceding hypothesis and assumptions are true, differences in growth rate and efficiency should be reflected by corresponding differences in the various blood constituents. Growth in the young animal is characterized largely by deposition of proteins and the purine and pyrimidine containing nucleo-proteins. Consequently the blood constituents investigated here have been restricted to those nitrogenous ones involved in the metabolism of proteins and nucleo-proteins, specifically, urea nitrogen, amino acid nitrogen, uric acid, and creatinine. These variables have previously been studied in young growing beef cattle (500 to 800 pounds body weight) on feed efficiency-rate of gain tests (41, 48, 70). It is the intent here to parallel this line of research with suckling lambs.

The general objective of this research project has been to describe the blood picture of these non-protein nitrogenous constituents in suckling ruminants (birth to weaning age) in terms of growth manifested both by overall weight and by physiological changes in the digestive system. At birth the digestive system of the ruminant may be considered equivalent to that of the single stomach mammals. At this age the abomasum is proportionately large and performs nearly all

stomach functions, while the rumen is small and practically inoperative. The rumen grows much more quickly than the abomasum however, and rapidly assumes an ever increasing share of the digestive functions. This change in type of digestive system no doubt is associated with metabolic changes, and conceivably could result in changes in quantity or quality of the nutrients absorbed which in turn affect the growth rate of the animal. For this reason, studies of ruminal development have been included to determine whether relationships exist between rumen development, nitrogenous blood constituents, and growth rate in young lambs.

Finally, the hypothesis of rapid growth as an indication of efficient metabolism plus the study of non-protein nitrogenous constituents as related to growth offer the opportunity to provide a more definitive basis for selection of the more desirable animals in a breeding program. As currently practiced, selection of the more desirable individuals from a flock of farm animals is largely a subjective process. If objective measurements are recorded, they consist primarily of body weight or weight of the product produced (milk, wool, etc.), with very few breeders recording any other types of measurements. The assigning of "scores" to various traits is a fairly common but still subjective procedure. Research workers frequently utilize a selection index based on such scores plus a few measurements. As a general statement, however, it is safe to say that more efficient and objective methods of

selection, particularly of the meat producing animals, are needed and desired. This research has been extended to explore the possibility of improving the current practice of selection.

Specific Objectives

The specific objectives of this study were: 1) to determine whether the non-protein nitrogenous (NPN) constituents of the blood of lambs are significantly affected by genetic and environmental factors such as age, weight, sex, breeding, and birth type; 2) to determine whether the concentrations of the blood constituents are related to growth and if so, to ascertain how these relationships may be incorporated into a more objective selection procedure; and 3) to determine whether a relationship exists between the blood constituents and the growth and development of the rumen.

The current knowledge impinging on this problem has been indicated in the following section, the review of literature. A historical perspective narrows to specific status of measurement techniques and interpretation of the values observed.

II. REVIEW OF LITERATURE

General Considerations

Blood is the most important and most vital of the body fluids. It comes in contact, directly or indirectly, with all tissues of the body. The functions of blood include: 1) conveyance of absorbed nutritive materials from the digestive tract to the body tissues, 2) removal of the waste products of cellular metabolism, 3) transport of oxygen from the lungs to the tissues and the return of carbon dioxide from the tissues to the lungs, 4) maintenance of water content of tissues, 5) regulation of body temperature, 6) regulation of metabolic functions by transporting the hormones of the endocrine glands, and 7) protection of the body through production of various antitoxins, lysins, and other antibodies (7, p. 1-8). Thus it can be readily seen that the blood is intimately involved in or associated with all metabolic functions, and has therefore been a favorite subject of study by physiologists, pathologists, nutritionists, pharmacologists, and more recently by geneticists and others.

Although the bulk of the blood studies have been concerned with the human or with experimental animals such as the dog and rat, there appears to be some information available on nearly all known species of animals. Most of the early workers attempted to establish the composition of the blood in the healthy individual and to compare it with the composition found under various pathological conditions. Of the

work prior to 1900, Folin (22, p. 460-478) states that the analytical methods for most nitrogenous constituents were necessarily so crude that the results were little more than garnishments to good, logical reasoning ability. Urea was the exception in that "reasonably plausible figures" for its content in different kinds of blood were published as early as 1850 to 1860 (22, p. 460-478). Kjeldahl published his method for the determination of nitrogen in 1883, but it was not used extensively nor successfully for the study of the non-protein nitrogen of the blood until 1902 when Strauss attempted to classify the various forms of nephritis on the basis of non-protein nitrogen found in the blood serum of a large number of patients. Volhard in 1914 repeated the work of Strauss with greatly increased detail and accuracy. During the period 1912 to 1922, Folin and his students at the Harvard Medical School (22, 23, 24, 25) and Van Slyke and co-workers at The Rockefeller Institute for Medical Research (65, 66) brought out a series of papers describing the preparation of protein-free filtrates of blood and the quantitative determination of total non-protein nitrogen, urea nitrogen, ammonia, amino acid nitrogen, uric acid, creatine, and creatinine content of blood (32, p. 543-567).

With the advent of these reliable quantitative methods of chemical analysis, the number of metabolic studies involving determination of the composition of the blood under normal, diseased, and experimental conditions rapidly expanded. Such studies helped establish "normal" or average values, and were useful in clinical and diagnostic work. However the genetic aspects of metabolism were scarcely considered at that

time, and most studies were not designed to distinguish differences between various classes of normally healthy individuals. Exceptions to this can be found in the case of sex and age characteristics in that some workers reported separate values for males and females, while other investigators distinguished between juvenile and adult values. Dukes (4, p. 48-49) summarized the results of several workers and prepared a table showing the normal range of some of the blood constituents in mature domestic animals. Table 1 lists some of the values reported by Dukes (17, p. 49) for domestic animals and for man as shown by Best and Taylor (7, p. 8).

Table 1. Usual normal range of some blood constituents of mature humans and mature domestic animals. (Selected from Table 6 of Dukes (17, p. 49) and Table 2 of Best and Taylor (7, p. 8).

Species	Sugar	Total NPN	Urea Nitrogen	Uric Acid	Preformed Creatinine	Amino Acid Nitrogen
(milligrams per 100 milliliters whole blood)						
Humans		28 - 39	9 - 15	2.0 - 5.0*	1 - 1.5*	6 - 8
Cow	40 - 70	20 - 40	6 - 27	.05- 2.0	1 - 2	4 - 8
Sheep	30 - 50	20 - 38	8 - 20	.05- 2.0	1 - 2	5 - 8
Goat	45 - 60	30 - 44	13 - 28	.3 - 1.0	1 - 2	---
Pig	45 - 75	20 - 45	8 - 24	.05- 2.0	1 - 2	8
Horse	55 - 95	20 - 40	10 - 20	.9 - 1.0	1 - 2	5 - 7
Dog	60 - 80	17 - 38	10 - 20	.00- 0.5	1 - 2	7 - 8
Hen						
lay	130 - 290	20 - 35	.4 - 1.0	1.0 - 7.0	1	4 - 9
non-lay	130 - 260	23 - 36	.4 - 1.0	2.0	1	5 - 10

*plasma instead of whole blood.

The discovery of blood types and the fact that they are genetically controlled resulted in multitudinous studies that have described the inheritance and distribution of the different blood types in nearly all human population groups and in some of the domestic and laboratory animals. However, the inheritance of metabolic patterns as reflected in quantitative analysis of blood constituents has been largely neglected. Exceptions to the above are noted at the Oregon Agricultural Experiment Station where Tether (64), MacDonald (41, p. 1-136), Williams (70, p. 1-83) and Price (48, p. 1-89) have found significant breed and sex differences in certain blood factors in rabbits and beef cattle. The present study is a continuation of this line of research.

Nitrogenous Constituents of Blood

Amino Acid Nitrogen. Amino acids are the chief products of protein digestion found in the portal blood, and from this fact it is generally agreed that, with certain exceptions, ingested protein must be broken down through the processes of digestion into its component amino acids in order to be absorbed from the small intestine (4, p. 649-658). The disappearance of the amino acids from the blood is rapid, but is never complete, even in starvation. Therefore there must be two sources of blood amino acids: exogenous or that obtained from digested food protein and endogenous or that derived from hydrolyzed tissue proteins. It is also known that the concentration of amino acids is greater in

tissues than in blood. Van Slyke and Meyer(65, p. 399-410) as quoted by Bard, (4, p. 649-658) injected amino acid nitrogen into dogs and showed that muscle, kidney, intestines, pancreas, and spleen behaved in the same manner in that there was a rapid rise in amino acid concentration followed by a gradual return to normal levels. In blood and liver the initial rapid rise in concentration was followed by rapid decline to the normal level. Van Slyke and Meyer also injected amino acids into hepatectomized dogs and observed that the amino acids accumulated in the blood while the urea concentration decreased. This was evidence that the liver is the chief site of metabolic changes in the amino acids. Longenecker and Hause (39, p. 46-59) investigated the relationship between plasma amino acid levels in dogs and the composition of the ingested protein. They concluded that the plasma amino acid levels are dependent on the rate of absorption from the intestine into the blood and on the rate of removal from the blood by the body tissues. Furthermore, if it is postulated that individual essential amino acids are removed from the blood by the body tissues at rates proportional to the requirements, then it is evident that changes in the plasma concentration after a meal are dependent on the amino acid composition of the ingested protein.

Charkey and co-workers (12) found age and species differences in the amino acid content of chick blood. Two week old chicks deprived of food for twenty-four hours showed increased blood levels of all amino acids tested. These effects varied inversely with age of the chicks

however, and at six weeks of age only lysine was consistently increased in the blood as a result of fasting. The authors believed this indicated that "a greater degree of maturity was required for the development of metabolic capabilities toward lysine, similar to that developed earlier in life toward other amino acids." A similar experiment applied to adult humans (13, p. 469-480) caused increased blood levels of leucine and valine, but reduced the concentrations of lysine and other amino acids studied. From the results of these two studies, and from examination of the literature, the authors concluded that there was a positive association between the amino acids "exhibiting increased blood levels due to fasting and those metabolically unavailable by precursor amination". They suggested that these relationships may occur in a number of different species. If this is so, genetic implications can be inferred. On the other hand, Salander and Fisher (55) found no significant difference in the plasma amino acid nitrogen between five week old growing cockerels and laying hens.

Sutton and Clark (63, p. 53-65) analyzed the morning urine of male and female Chinese and Caucasians, and found that the Chinese individuals had significantly higher values for alanine, β -amino isobutyric acid, histidine, leucine, lysine, tyrosine, and uric acid. Males had higher values for creatine, while females were higher in specific gravity, β -amino isobutyric acid, glycine, and histidine. There was a statistically significant interaction between race and sex for specific

gravity. The authors believed that these results were evidence that chemical as well as morphological differences between races are genetically controlled. Reed (51) found differences in the excretion level of taurine, lysine, and methionine sulfoxide between inbred strains of rats. Pratt (47) reported differences in the free amino acid content of the blood of seven species of insects.

Using a paper chromatographic technique, Hrubant (35, p. 591-608) studied blood levels of amino acids in three inbred strains of mice. He found significant differences between genotypes in the levels of glutathione, glycine, valine-norvaline, α -alanine, aspartic acid, lysine, and isoleucine-leucine. He also found within strain differences between sexes for arginine, lysine, valine-norvaline, and glutamic acid. However when animals of all strains were pooled by sex, the significant sex differences disappeared. The three strains differed significantly in their relative proportions of glutathione, homocysteine, cysteine, taurine, glycine, lysine, arginine, α -alanine, β -alanine, valine-norvaline, and isoleucine-leucine. Although many significant differences among strains were found, a fairly standardized metabolic pattern could be derived for each strain.

Tether, et al (64) found sex, age, and breed differences in blood amino acid nitrogen content among Polish, New Zealand White, and Flemish Giant rabbits. Adult males varied more than adult females, and the blood amino acid nitrogen levels were similar for males of all three breeds. Polish females had higher levels than New Zealand females, but

differences were not statistically significant between Polish and Flemish Giant or between Flemish Giant and New Zealand females. In young, growing rabbits of both sexes, the Polish breed had higher blood amino acid nitrogen values than the New Zealand Whites. Young rabbits of both of these two breeds and of both sexes showed higher levels than their adult counterparts, but within breeds there were no differences between young males and young females. However in adult Polish rabbits, the females had greater amounts of blood amino acid nitrogen than the males. There was a greater change in blood amino acid nitrogen as males reach maturity than there was for females.

MacDonald (41, p. 1-136) and MacDonald, et al (42, p. 1-34) reported wide variations in the blood constituents of fed beef cattle examined at 500 and 800 pounds of body weight. In all cases, individuals were found that ran counter to the average trend. Herefords had a lower concentration of blood amino acid nitrogen than Angus at both weights, and males were lower than females at both weights. As rate and efficiency of gain increased the concentration of blood amino acid nitrogen decreased in a linear manner. MacDonald and White (43) did not find a statistically significant difference in the concentration of blood amino acid nitrogen between steers and heifers on pasture, although steers averaged higher than heifers (9.4 vs 9.1 mg o/o). A similar relationship held for the non-protein nitrogen content of the blood.

Williams (70, p. 1-83) found sex differences in beef calves on feed at 500, 600, 700, and 800 pounds body weight, with the heifers having higher average concentrations of amino acid nitrogen than the bulls. Breed and line differences were not statistically significant at these weights. Feed intake was found to significantly affect blood amino acid nitrogen, but rate of gain and age did not. Blood amino acid levels were more stable at 800 pounds than at 500 pounds body weight.

Price, et al (49) correlated rate and efficiency of gain with blood and urine constituents from thirty-nine beef calves on feed test from 500 to 800 pounds body weight. They reported that the blood amino acid nitrogen at 800 pounds was inversely related ($r = -.53$) to rate of gain per day, but was positively correlated at both 500 and 800 pounds ($r = .47$ and $r = .53$, respectively) with feed required per pound of gain. Since faster gaining calves generally require less feed per pound of gain, these authors believed that the low levels of amino acid nitrogen in the blood of these faster gaining calves had a definite physiological significance. One explanation was the possibility that faster gaining calves are "superior in their ability to utilize the amino acids for protein syntheses", and thus withdraw larger quantities from the circulating blood.

Urea Nitrogen. Urea nitrogen is the most abundant non-protein nitrogenous constituent of the blood, and comprises some forty to fifty per cent of the blood's total non-protein nitrogen. In mammals, and

especially herbivorous ones, urea is the chief end product of protein metabolism. A large percentage of the nitrogen released by the catabolism of amino acids is excreted as urea in the urine, and in man on a normal mixed diet, from eighty to ninety per cent of the urinary nitrogen is in the form of urea nitrogen. Dukes (17, p. 49) shows ranges of 20 to 38 mg of total non-protein nitrogen and 8 to 20 mg of urea nitrogen per 100 milliliters of whole blood of sheep. Although minute amounts of urea have been shown to be produced in the brain (62); mammary gland, and a few other tissues, the only important site of urea production is in the liver. According to the ornithine cycle which was described by Krebs and Henseleit in 1932, ornithine is converted to citrulline by the addition of one molecule each of ammonia and carbon-dioxide. Citrulline in turn is changed to arginine by the addition of a second molecule of ammonia, and arginine is then hydrolyzed directly (without preliminary deamination) by the action of arginase into urea and ornithine to complete the cycle (7, p. 622-642).

Until quite recently then, urea was regarded as mainly, if not solely, a waste product of protein metabolism which was only excreted. The one important exception occurred in ruminants where urea and certain other non-protein nitrogenous substances could be utilized in the diet as a substitute for part of the protein nitrogen. Annison and Lewis (2, p. 92-120) prepared the following chart showing the various pathways of nitrogen metabolism in ruminants (Figure 1).

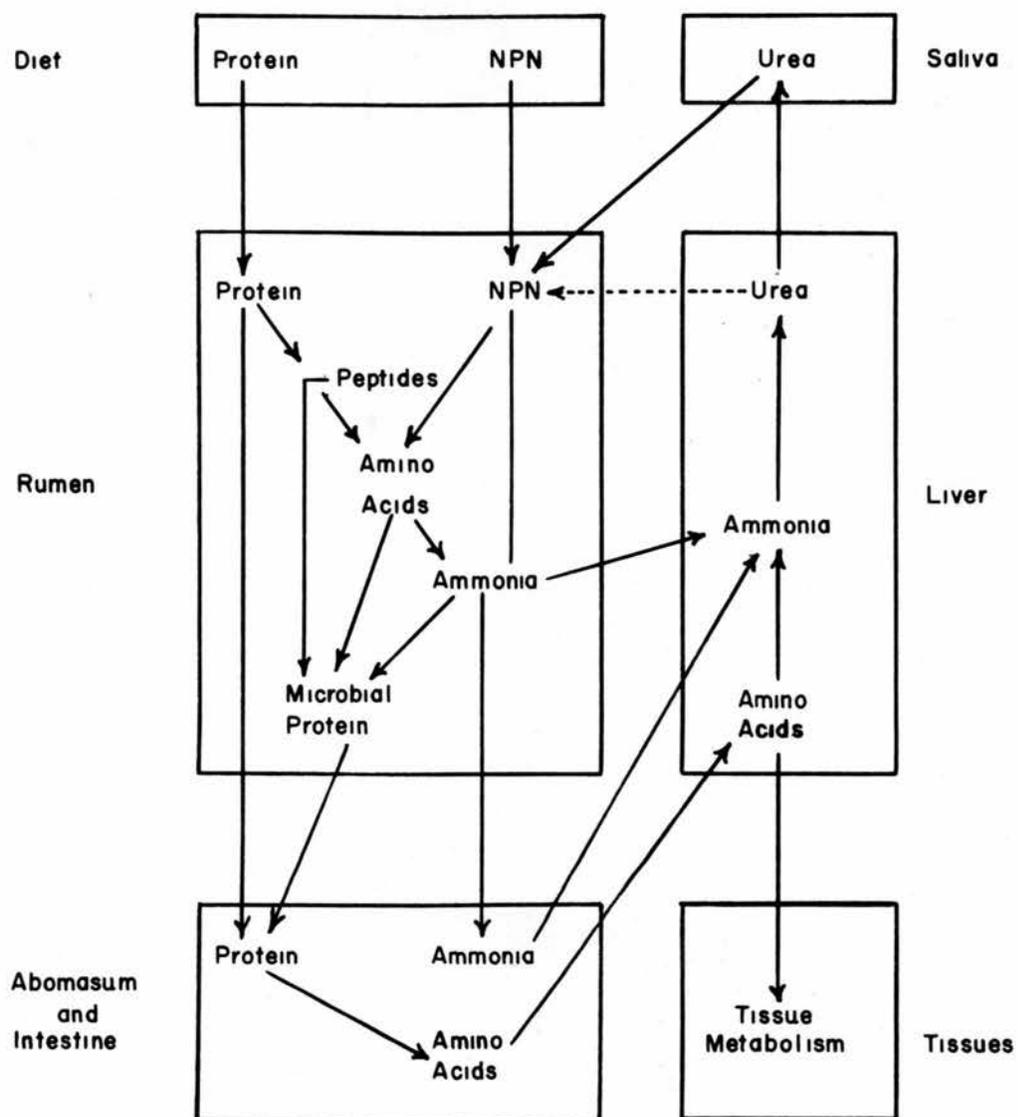


Figure 1. Pathways of nitrogen metabolism in ruminants. Adapted from Figure 4 of Annison and Lewis (1, p. 94).

To this can be added one additional pathway (dotted line) which has been derived from a report by Houpt (34). Working with sheep, Houpt showed that under the conditions of a low protein, carbohydrate supplemented ration, blood urea will diffuse into the rumen. Once in the rumen, this transferred urea serves the same function as dietary urea, and acts as a source of nitrogen for microbial synthesis of protein. The ruminant would thus have a distinct advantage over the monogastric mammal under unfavorable nutritional conditions in that the blood urea, which is normally a waste product of protein metabolism, could be utilized as a source of protein. Houpt has termed this phenomenon a "protein regeneration cycle".

Greatorex (28) studied the blood urea of calves and adult cattle. In calves he found the blood urea content was higher the first three months of life, then lower from four to six months of age, and then gradually increased to adult level. In adult cattle, Ayrshires, Friesians, and Shorthorns had approximately the same level of blood urea nitrogen, while Guernseys were somewhat lower. Higher blood urea values were found when cows were not pregnant, but there was no correlation between blood urea nitrogen and lactation. Highest values were found in August and lowest values in January, February, and March.

MacDonald, et al (42, p. 1-34) found that the blood urea nitrogen level in calves on feed was lower in males than in females, and that among the animals studied the Herefords averaged slightly lower than the Angus. They also reported that the blood urea nitrogen averages

were lower at 800 pounds body weight than they were at 500 pounds, and that the results were less variable at the heavier weights. Price, et al (50, p. 1-23) also reported sex differences in blood urea nitrogen in cattle. Comparisons were made between 500 and 800 pounds body weight. In all cases the males had lower values, but only at 500 pounds were the differences statistically significant ($P < .01$). On an over all basis, a significant difference was not found between the Angus and Herefords, but among the Herefords, the males of the Lionheart line had higher levels of urea than the David line males at 500 pounds; while at 800 pounds the Lionheart males were higher than the Prince males ($P < .01$). The Prince and David lines did not differ significantly from each other at either weight. Male calves were consistently superior to female calves in rate and efficiency of gain, but only in the David line was the difference sufficiently great to be significant. As in many other studies, rate and efficiency of gain were significantly correlated ($r = -.87$). Blood urea nitrogen was correlated at the 5 o/o level with rate of gain ($r = -.31$) and at the 1 o/o level with feed per unit of gain ($r = .38$). Therefore lower levels of blood urea nitrogen were associated with faster growth rate and greater efficiency per pound of feed consumed.

Uric Acid. Uric acid in the blood of mammals is the product of purine metabolism. Consequently it is also intimately associated with the metabolism of nucleoproteins. As with most of the other non-protein nitrogenous constituents of the blood, uric acid is derived from

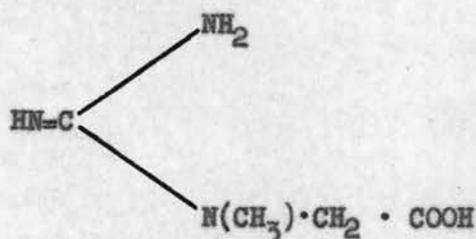
endogenous (metabolism of the nucleic acids of body tissues) and exogenous (absorbed products of nucleic acid digestion) sources. In man, the chimpanzee, and the Dalmatian dog, uric acid is largely excreted in the urine as urates of sodium, potassium, and ammonium, and in the free state. In most other mammals it is first oxidized to allantoin before passing into the urine. This process occurs in the liver where it is catalyzed by the enzyme uricase. Those mammals which do not convert uric acid to allantoin lack the enzyme uricase (17, p. 565-571). In the Dalmatian dog at least, and probably in the other species as well, this is a genetically controlled trait.

In humans, Morgan, et al (46, p. 671-685) found age and sex differences in the uric acid content of whole blood. Men had higher mean levels than women in all age groups, although the "differences were not always significant". Blood uric acid concentrations were relatively constant in the 18 to 40 year age group, but from 50 to 75 years there were increases in concentration. After 75 years of age the uric acid content of the blood declined. Bell, et al (5, p. 355-364) reported that uric acid levels in the blood plasma of chickens (Brown Leghorns) were related to sex and to the physiological and nutritional states of the birds.

In beef cattle, Williams (70, p. 1-83) found sex and line differences in the uric acid content of whole blood of calves between 500 and 800 pounds weight. He also reported that feed intake significantly affected the uric acid content of the blood. MacDonald, et al

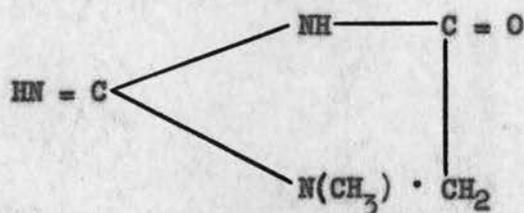
(42, p. 1-34) also showed sex and line differences in uric acid content of calves blood. With the increase in body weight from 500 to 800 pounds: Angus females showed an increase in blood uric acid, Hereford females showed no change, while males of both breeds showed a decrease. Price, et al (50, p. 1-23), working with similar cattle did not find statistically significant differences in blood uric acid content between sexes and lines. Their one exception to this was in the case of the Angus calves at 500 pounds weight where the males had significantly less ($P < .05$) blood uric acid than the females (1.38 to 1.91 milligrams uric acid per 100 milliliters of whole blood for males and females, respectively). However they did find that uric acid content of the blood was negatively correlated with rate of gain ($r = -.30$ and $r = -.35$ at 500 and 800 pounds, respectively) and positively correlated with feed consumed per unit of gain ($r = .37$ and $r = .52$ at 500 and 800 pounds).

Preformed Creatinine. Creatine, $C_4H_9N_3O_2$ (methyl glycoamine or methyl guanidine acetic-acid), has a structural formula (7, p. 634) which may be represented as



It is not a waste product of protein metabolism as was once believed, but rather serves an important function in the transfer of energy required for muscle contraction. This function can be simply summarized as follows: creatine (C) plus adenosinetriphosphate (ATP) in the presence of actomyosin forms adenosinediphosphate (ADP) plus creatine phosphate (CP) plus energy for muscle contraction $[C + ATP \rightleftharpoons ADP + CP + \text{energy}]$ (7, p. 716-725). The capacity of muscle tissue for contraction is related to its creatine phosphate content, while the force of the contractions is proportional to the extent of the creatine phosphate breakdown.

On the other hand, creatinine, $C_4H_7N_3O$ (methyl glycoamidine) which is the anhydride of creatine is considered to be largely a waste product. Its structure (7, p. 634) may be represented by the formula:



In normal humans, the amount of creatinine excreted via the urine in a 24 hour period is remarkably constant (1.5 to 2.0 grams for men and 0.8 to 1.5 grams for women), and appears to be essentially independent of diet, urine volume, and perhaps muscular activity. This last point is open to question, however. Some investigators believe that exercise has

no effect on the level of urinary creatinine, while others think that although the daily elimination is constant, the urinary creatinine is increased during periods of muscular activity--the increase being derived from creatine released during such periods (11, p. 148-151).

Because it is derived from creatine, the amount of creatinine excreted in urine is considered to be an indicator of the level at which the body maintains its creatine phosphate. This is usually expressed as a creatinine coefficient which is the ratio of the number of milligrams of creatinine (plus creatine) nitrogen excreted in a 24 hour period to the number of kilograms of body weight. Thus the creatinine coefficient is assumed to be an index of the amount of active muscle tissue in the body (11, p. 180-182).

Many workers have attempted to make use of these relationships between creatine, creatinine, and muscle tissue for varied purposes. Ashworth and Brody (3, p. 1-18) found that the creatinine coefficient was the same in dairy cattle as it is in humans (9.5), and that in cattle it was constant for the age period studied (7 to 40 months). Fluctuations in the coefficient were explained by deviations in the relative degree of fatness; fatter animals having lower coefficients. They also found that the ratio of basal metabolism to creatinine excretion declined with increasing live weight in about the same manner as did the ratio of basal metabolism to live weight. Morgan, et al

(46, p. 671-685) reported that blood creatinine levels in 553 human males and females over fifty years of age were not affected by age or sex. However, the mean values of 1.53 ± 0.11 and 1.50 ± 0.08 mg per cent for aged men and women respectively were twenty to thirty per cent higher than those reported for young adults (1.27 ± 0.10 and 1.18 ± 0.07 mg per cent for young men and women, respectively). By analyzing first morning urine samples of Chinese and Caucasian men and women, Suttan and Clark (63, p. 53-65) found that men had greater amounts of urinary creatinine than women, but that there were no differences between races.

Correlations between various carcass measurements with live probe, urinary creatinine coefficient, and blood creatinine levels have shown that all three variables can be utilized to predict leanness in swine (52). The live probe measurement was the most useful predictor, however, while blood creatinine level was the least useful (53).

Blincoe (8) reported no significant differences in blood creatinine levels between healthy Nevada lambs and those showing symptoms of white muscle disease. However diseased lambs did show elevated non-protein nitrogen levels as well as changes in other constituents of the whole blood or serum. On the other hand, Ellis and Oldfield (18) reported that plasma creatinine levels were reduced in cows but increased in ewes and lambs that were fed a diet of cull beans and alfalfa hay calculated to induce white muscle disease. Kandutsch and

Russell (36) compared the muscle, serum, and urinary content of creatine and creatinine in normal mice with that of mice with hereditary muscle dystrophy. Dystrophic muscle tissue contained 20 per cent less creatine than normal tissue, but when corrected for water and lipid content the difference was only 14.7 per cent less than normal. Serum creatine levels were not elevated in dystrophic mice, as contrasted to the results found in humans, and there was some evidence that dystrophic mice could excrete exogenous creatine (intraperitoneal injection) more rapidly than normal mice. Interpretation of such increased creatinuria in dystrophic mice was hampered however by several complicating factors.

In beef cattle, MacDonald, et al, (42, p. 1-34) found that blood creatinine levels in growing calves at 500 and 800 pounds weight were significantly affected by breed and breed by sex interaction but not by sex. Hereford calves were generally found to have higher creatinine levels at 800 than at 500 pounds body weight, while Angus calves showed the reverse picture.

Rumen Development

The importance of cattle, sheep, and other ruminants to the world's economy as a source of food, clothing, and work has ensured the continuous investigation of their nutritional requirements, which, as is now clearly recognized, are profoundly influenced by the presence of the rumen (1, p. 705-714). Most investigations of rumen biology however,

have been concerned with the adult rumen. Only recently has a certain amount of effort been directed towards the study of rumen development in young animals (67). With dairy calves it is readily apparent that it would be economically advantageous if their feed could consist mainly of roughages, but early development of rumen function may also be an advantage with beef calves and lambs. Not only would the strain on the lactating dam be relieved, but in addition there may be some connection between poor development of rumen function and unthriftiness in suckling and weanling animals. Lambs previously fed milk only have been shown to have a lower percentage digestibility of dry matter (56.6 o/o) than lambs grown on limited milk plus alfalfa hay ad. lib. (64.7 o/o). However, the differences between the two groups disappeared after twelve days of feeding alfalfa hay. This suggests that development of rumen function in the lamb may not be as slow a process as has been generally believed (67). Using grazing lambs slaughtered at weekly intervals from birth to 112 days of age, Wardrop and Coombe (68) reported that the abomasum was the largest of the four stomach compartments at birth, but that it had been surpassed in size by the rumen at twenty days of age. By fifty-six days the weights of the four stomach compartments had achieved their approximate adult proportions.

Studying rumen development in seventy-five feed lot lambs fed a constant diet, Sinclair, et al (59) found a significant correlation ($r = .608$) between total rumen weight and average daily gain, and between relative length of rumen papillae and average daily gain

($r = .583$). The partial correlation of feed lot gain with papillary length, independent of rumen weight was also significant ($r_{gp \cdot r} = .423$), but papillary width was not associated with gain ($r = .10$). However the cause and effect relationships responsible for these correlations were not entirely clear. In a more detailed study, Sinclair and Kunkel (58) were able to verify the working hypothesis that ruminal development is in part related to rate of gain. They concluded that the significant correlations between rate of gain and rumen characteristics was "strong supportative evidence that growth and development of the animal was related to and may be dependent upon rumen development." Length and density of rumen papillae appeared to be a definitive criterion for evaluating mucosal development, and no doubt served as an evaluation of surface area available for absorption or other rumen functions. An unexpected and unexplained finding was a significant relationship between mucosal color and gain over the last twenty-eight days of the feeding trial.

In a series of studies with cannulated dairy calves, Flatt, Warner, and co-workers (20, 21, 30, 69) introduced a number of natural and purified diets which included such items as plastic or cellulose sponges and solutions of fatty acids into the rumen. These workers concluded that the development of rumen papillae was stimulated by the end products of rumen fermentation rather than by the coarse nature of the diet. Brownlee (10), while agreeing that development of rumen papillae is not predetermined but varies with diet, concluded that the factors

governing the degree of development are likely to be the energy value of the food or the rapidity with which it is broken down into absorbable fractions. He also found that the rumen as a whole can increase in weight up to six times its estimated weight at birth without a corresponding growth of papillae. Therefore he concluded that "rumen muscle development is not pari passu with papillae development".

III. MATERIALS AND METHODS

Flock Management

In order to provide data suitable for the objectives of this research project and typically representative of breeding practices, the procedures described below for mating and lambing were followed in the years 1958 and 1959 in the experimental sheep-breeding flock maintained at Corvallis, Oregon. The experimental lines were produced in accordance with the provisions of the Oregon Agricultural Experiment Station project 157 entitled "Improvement of Sheep Through Application of Breeding Methods". The breeds available at Corvallis were representative of those of major importance in both commercial and research activities of the Pacific Northwest. Thus the conclusions to be drawn can be considered to have general applications within this climatic region. The specific breeds and lines have been shown in Table 2 where breed and line crosses are given in paradigm form.

Management of the breeding flock was conducted in accordance with the recommended commercial practices for the "hill pasture" region of western Oregon. Ewes running on the dry pastures of late summer and early autumn were flushed with one pound of grain per head per day for approximately one month prior to and during the six weeks breeding season which began on September 20th of each year. The ewes were brought in from pasture twice daily (6:00 A.M. and 6:00 P.M.). Vasectomized teaser rams were used to detect those ewes in estrus. Each ewe so found was individually placed with the assigned ram and

observed until mating occurred. At the completion of the breeding season the grain supplement was discontinued.

Beginning approximately one month prior to the lambing period, the ewes were again given a supplemental feeding of one-half pound of grain per head per day. Upon the arrival of the lambs in early February the ewes were placed on a full feeding of chopped hay or hay and grass silage supplemented by one pound of grain per head per day. Ewes with lambs were kept on this ration until the new growth on the pastures was adequate -- usually between the middle and latter part of April.

The ewes were allowed to lamb in a small pasture near the barn by day, or in the barnyard at night. Ewes with their new-born lambs were picked up every six hours or oftener and placed in individual pens where the lambs were weighed and identified. As the lambs progressed, the ewes and lambs were first turned into small mixing pens of four to six ewes each, then into progressively larger mixing pens, and finally into the "wet band" of ewes and lambs.

Due to the advanced age of the Columbia ewes lambing in 1958, (10 to 12 years) their lambs were given a supplemental creep feeding of up to one-half pound of mixed grain per head per day throughout the suckling period. Lambs were not fed in other years when the ewes had a more normal distribution of ages. In all years however, the lambs did have access to the feed troughs of their dams, and as the lambs increased in age and size the amounts of hay and grain that they consumed from these troughs also increased. The numbers of lambs on which detailed

measurements were taken for this project are shown in Table 2 where they are classified according to breed, sex, birth type and age.

Table 2. Numbers of Lambs Tested Assembled According to Year, Breed, Sex, Type of Birth, and Age in Weeks at Test.

Type of Birth	Age (Weeks)	MALES					FEMALES				
		1958		1959			1958		1959		
		KD	KC	S	SLC	KDS	KD	KC	S	SLC	KDS
Singles	2	2	3		3	2	3	2		3	2
	4			4	2	2			9	2	2
	6	2	4	5	2	1	4	4	9	2	1
	8			5	2				9	2	
	10	2	4	5			4	4	9		
	14	2	4	5			3	4	9		
	18	2	4	5			4	4	9		
Twins	2		3		1	2		1		1	2
	4				1	1			2	1	1
	6		5			1		1	2		1
	8								2		
	10		5					1	2		
	14		4					1	2		
	18		5					1	2		
Twins Raised As Singles	2						2				
	4										
	6	1					1				
	8										
	10	1					2				
	14	1					2				
18	1					2					

where: KD = Columbia by Dorset crosses
 KC = Columbia by Cheviot crosses
 S = Suffolk, inbred line "0"
 SLC = Suffolk inbred line crosses ($S_1 \times S_0$, $S_2 \times S_0$, $S_3 \times S_0$)
 KDS = Columbia-Dorset by Suffolk line "0" crosses

PARCHEMENT DEED

Data Collection

The detailed measurements can be divided into two major groups: physical records of growth as indicated by live weight, and size and weight of organs and glands; and by blood chemistry. Methods for obtaining these measures are described or referenced in the following.

Growth. Body weights of the lambs from birth through ten weeks of age were recorded to the nearest one-tenth pound by restraining the lamb in a sack or bucket and weighing on a dairy scale. At 14 and 18 weeks of age, most of the lambs exceeded the sixty pounds capacity of the dairy scale and had to be weighed on a large dial scale which recorded to the nearest one-half pound.

The 16 lambs that were slaughtered in 1959 for study of the growth and development of the stomach compartments were serially sacrificed in lots of 4 each at approximately two-week intervals between 14 and 57 days of age. Otherwise they were treated the same as the other lambs. Body weights were recorded and the blood samples collected and analyzed according to the same schedule as that used for those lambs which were not slaughtered. The lambs were sacrificed the following day, and the rumen, reticulum, omasum, and abomasum removed. Weights of other organs and endocrine glands were also recorded, but will be reported separately.

All masses of adipose tissue of any size, including that deposited along blood vessels, were removed from the exterior of the stomach

parts. After removal of the contents and a thorough flushing, the volume of the reticulum, rumen, and omasum-abomasum was measured by filling with distilled water to a constant manometer pressure of one inch of water. The stomach was then dissected into its respective parts, blotted to remove excess water and the wet tissue weights of each part obtained. Small representative sections of each organ were removed and prepared for histological studies. The individual parts were then placed in a drying oven for 48 hours after which they were removed and the dry tissue weights recorded. The dry weights reported include a correction for the small sections removed for histological studies.

Blood Chemistry. Blood samples from the lambs which were not slaughtered were collected at 2, 6, 10, 14, and 18 weeks of age in 1958, and at 4, 6, 8, 10, 14, and 18 weeks of age in 1959. All blood samples were collected between 7:30 and 8:30 A.M. The wool was clipped from a small area of the neck over the jugular vein, a number 16 gauge bleeding needle was inserted into the vein, and about 8 cc of blood was collected in oxalated tubes prepared according to Hawk, Oser, and Summerson (32, p. 540-542). The tubes were stoppered and taken directly to the laboratory where analytical procedures were started immediately.

Analytical procedures used to determine the concentration of urea nitrogen, amino acid nitrogen, uric acid, and creatinine in the blood have been used extensively in studies with beef cattle at Oregon

State University. These procedures have been described fully by Hawk, Oser, and Summerson (32, p. 543-567). Protein-free filtrates of whole blood were obtained by use of Haden's modification of the Folin-Wu precipitation method (29). Whole blood was laked with 8 volumes of N/12 sulfuric acid and precipitated with 1 volume of 10 o/o sodium tungstate. Filtering through dry paper gave a filtrate which represented a 1:10 dilution; i.e., 1 ml of filtrate corresponded to 0.1 ml of the whole blood.

Urea Nitrogen. The method chosen for the determination of blood urea nitrogen was that of Karr (37), as modified by Looney (40). In this procedure the urea in a protein-free filtrate is converted to ammonium carbonate by the action of urease. The ammonium carbonate is nesslerized in the presence of gum ghatti, a protective colloid. The interference of peptones and amino acids is considered to be so slight and uniform as not to influence the clinical value of the results (32, p. 549-554).

Uric Acid. The concentration of uric acid was determined by the direct method of Brown (9) which appears to be as satisfactory as any method and simpler than most. The tungstic acid filtrate was treated directly with a special uric acid reagent in the presence of optional amounts of cyanide-urea solution. The blue color developed is compared with that of a standard solution treated similarly (32, p. 559-565).

Amino Acid Nitrogen. The color developed by the reaction between amino acids and β -naphthoquinone-4-sulfonic acid in alkaline solution is the basis of this method. Originally prepared by Folin (23, p. 377-391) the method was considerably improved by Danielson (16). This procedure is essentially the one described here with heating to develop the color as suggested by Sahyun (54) and with photometric measurements according to Frane, Russell, and Wilhelmi (26, p. 255-270), as quoted in Hawk et al (32, p. 565-567).

Creatinine. The method of Folin and Wu (25, p. 81-110) was used wherein the protein-free filtrate was treated with an alkaline picrate solution. The color which developed was compared with that produced by a known amount of creatinine treated under the same conditions. Since the creatinine color reaction does not follow Beer's law exactly, a calibration curve relating optical density to concentration had to be prepared (32, p. 555-558). At the concentrations found, this curve was essentially linear.

IV. METHODS OF STATISTICAL ANALYSIS

The objectives of the statistical analyses of the data were three-fold: 1) To determine whether the concentrations of the non-protein nitrogenous (NPN) constituents of the blood varied significantly between lambs differing in age, weight, sex, breeding, and type of birth; 2) To determine whether the concentrations of the NPN constituents were related to growth and if so, to ascertain how they might be used as an aid to more objective evaluation of growth potential (i.e., as an aid in the selection procedure); and 3) To determine whether a relationship existed between the blood constituents and the growth and development of the rumen. Mathematically, all three objectives presented the same basic problem: the determination of the relative effects of several independent variables in predicting the response of the one dependent variable, or in successfully discriminating between two discrete groups of observations.

Principles of multivariate analysis involving multiple linear regression were utilized for the first objective; discriminant function analysis was employed for the solution to objective two; and product-moment correlations were used for the third objective.

Multiple Regression

A general linear model was postulated to describe the variation in each of the blood constituents due to genetic and environmental effects:

$$Y_f = \sum_{i=0}^r \beta_{fi} X_{fi} + e_f$$

where: Y_f = the concentration of the f-th blood constituent of an individual lamb expressed in milligrams per 100 milliliters of whole blood.

X_{fi} = the r fixed variates, breeds, sex, type of birth, age, and weight. These were the same for all blood constituents within any given year, but differed from year to year. The variables age and weight were continuous variables; the others took the values 1 or 0 according to the appropriate classification.

β_{fi} = The regression coefficients which are estimated by the b_{fi} , where b_{f0} is an effect common to all lambs and $b_{f1} \dots b_{fr}$ are the added effects due to the r variates.

e_f = Random error assumed normally and independently distributed with mean zero and variance σ^2 : $NID(0, \sigma^2)$.

Only the main effects due to each of the variables were considered in this model and although interaction effects between some of the variates were undoubtedly present, they were presumed to be non-existent or small for the purposes of this exploration. Thus, while the model was not a completely realistic one in the sense that it failed to consider all identifiable sources of variability, it was considered to be sufficient for a preliminary investigation of this nature where one of the primary objectives was the identification and selection of variables which had a recognizable effect on the blood constituents studied.

Discriminatory Analysis

The task was to find as early in lamb age as possible that weighted combination of the variables studied (body weight, urea nitrogen, uric acid, amino acid nitrogen, and creatinine) which most effectively discriminated between two groups of animals; i.e., the more desirable animals which were to be saved for breeding flock replacements and the less desirable ones which were to be culled. For the purpose of this study, a desirable lamb was defined as one which had a positive selection index while a culled animal was one which had zero or negative selection index. The selection index referred to is that one which has been used at the Oregon Experiment Station for a number of years:

$$I = 2 \left[\frac{W - \bar{W}}{SD(W)} \right] + \frac{C - \bar{C}}{SD(C)} + \frac{T - \bar{T}}{SD(T)}$$

where: I = selection index value

W = body weight in pounds

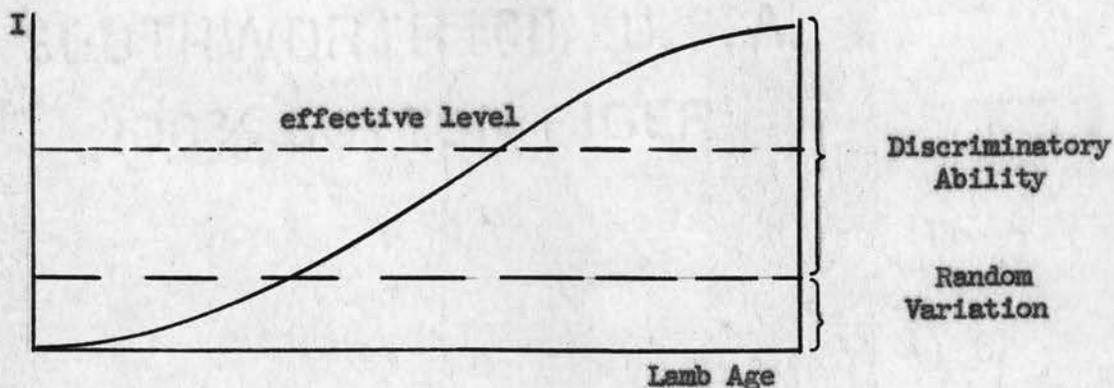
C = condition score

T = type or conformation score

SD = standard deviation

\bar{W} = mean of W

Perhaps the best way to visualize the problem mathematically is to see it as a suggested graph as shown below.



The need here was for a mathematical model which showed not only discriminating ability, but also indicated which factors were important in the discrimination process and at what point in time the function was deemed to be sufficiently effective.

One possible approach was to write each factor individually as a function of time as follows:

$$B_1 = f(t)$$

$$B_2 = g(t)$$

$$B_3 = h(t)$$

etc.

It would then be necessary to put these functions together to form an over-all discriminant at that point in time suggested by the various factors individually. However, it was not clear how these should be grouped or with what weights they should be endowed.

A second approach was to write a discriminant as follows:

$$D = f(B_1, B_2, B_3, \text{ etc})$$

Then by regressing D with time,

$$D = d(t)$$

it would be possible to select that point in time when the discriminant was deemed to be effective. This approach made use of the known technique of discriminant function analysis.

The general problem was to set up a weighted function

$$Z = \sum_{i=1}^k \lambda_i X_i$$

where the X_i are the variables measured and the λ_i are the corresponding weighting coefficients. If we let Z_s and Z_c represent such functions to be derived for the saved and culled groups of lambs respectively, it has been shown by Fisher (19) that the coefficients (λ) are obtained by maximizing the ratio

$$G = \frac{(\bar{Z}_s - \bar{Z}_c)^2}{\sum_1^{n_s} (z_i - \bar{Z}_s)^2 + \sum_1^{n_c} (z_j - \bar{Z}_c)^2}$$

where the numerator is the square of the difference between the means of Z for the saved and culled groups, and the denominator is the sum of squares within groups.

As a sub-problem in the above approach it was necessary to select those pertinent factors at each time period which maximized the discriminatory ability. The procedure followed was one that will be called stepwise discriminant function analysis which consists, as the name implies, of a review of the discriminating ability with each step of adding a factor.

In order to be absolutely certain of finding the most effective discriminating set of i variables out of k available, it would have been necessary to compute all possible sets of discriminants. The number of such sets is the number of combinations of k things taken i at a time, or

$$C \binom{k}{i} = \frac{k!}{i!(k-i)!}$$

But since i was rarely known in advance, the number of all possible discriminants became

$$\sum_{i=1}^k C \binom{k}{i}$$

With this approach it is readily apparent that if the number of variables is at all large, the number of combinations becomes prohibitive for many studies.

An alternative procedure requiring considerably less labor is described herein, which although not absolutely guaranteed to find the most effective set of variables, does have a high probability of finding such a set. This procedure makes one basic assumption: that the most effective two-factor set contains the most effective single variable, that the most effective three-factor set contains the most effective pair of variables, etc. In this sense a most effective set of variables is obtained, and it has been shown empirically that such a set will differ from the absolutely most effective set only infrequently when used analogously in multiple regression (57, p. 1-75).

The development is easily seen in the following. The first step was to look at each factor individually over all time periods by means of one-factor discriminants of the form

$$Z = \sum_{i=1}^{k=1} \lambda_i X_i$$

and by the usual procedures, to select that factor which gave the maximum discriminatory ability. The second step was to compute all two-factor discriminants

$$Z = \sum_{i=1}^{k=2} \lambda_{1i} X_{1i}$$

which included the variable selected in step one, and to choose that combination of two factors which gave maximum discriminating ability.

Thirdly, analysis of variance technique was used to determine whether the addition of the second variable significantly improved the discriminant function. If not, the procedure was stopped at this point. If so, the procedure was continued by computing in turn all three, four, etc. factor functions which contained those variables previously selected until the addition of another variable failed to significantly improve the function. Finally, the discriminant function at each step was followed through lamb age. Schultz and Goggans (57, p. 1-75) present detailed computing procedures.

Product-Moment Correlations

Product-moment correlations are well known and have been widely used for many years. Hence nothing further will be said here, except to state that they were utilized to assess the degree of relationship between the blood constituents and the measurements of the stomach compartments.

V. RESULTS AND DISCUSSION

Time Trends

Before delving into involved statistical procedures, the raw data were examined to determine whether they conformed in general to known ranges of values. Blood levels of all constituents except creatinine were in good agreement with the normal ranges of values for sheep previously reported in the literature. Four per cent of the urea values and two per cent of the amino acid nitrogen values fell slightly outside the ranges of 8 - 20 mg per cent for urea nitrogen and 5 - 8 mg o/o for amino acid nitrogen reported by Dukes (17, p. 49).

Urea nitrogen values fluctuated considerably over time, and no simple relationship between urea and age or weight was observed. In general there was a decrease in the mean urea levels between two and six weeks of age followed by increasing values which reached a maximum at ten to fourteen weeks. Between 14 and 18 weeks of age a moderate decrease in blood urea nitrogen levels was observed in nearly all sub-classes.

The uric acid content of whole sheep blood varied considerably between two and six weeks of age. After this period of apparent adjustment, there was a general rising monotonic trend over age and weight. Mean blood levels of amino acid nitrogen in lambs decreased markedly between two and four weeks of age, then increased gradually with age. Preformed creatinine was the only blood constituent

studied where an appreciable number of lambs differed from the normal range of 1.0 to 2.0 mg o/o reported by Dukes (17, p. 49). Fifty-seven per cent of the observed values fell between 0.5 and 1.0 mg o/o, while the remaining values were between 1.0 and 2.0 mg o/o. Considerable variation in the blood levels of preformed creatinine was observed between two and eight weeks of age, with some lambs showing increases and others decreases over this period of time, but from ten to eighteen weeks there was a steady linear increase in nearly all lambs.

Body weight from birth to eighteen weeks of age was linearly related to age, as is true for all lambs of this age. Linear regression coefficients varied from .36 pounds per day for Columbia by Dorset female twin lambs raised as singles to 0.63 pounds per day for Columbia by Dorset single, male lambs.

Table 3. Simple Linear Regressions of body weight on age (pounds per day)

<u>Breed</u>	<u>Sex</u>	<u>Type of Birth and Rearing</u>		
		<u>Single</u>	<u>Twin</u>	<u>Twin raised Single</u>
Columbia X Dorset	Male	.63		.62
	Female	.48		.36
Columbia X Cheviat	Male	.55	.46	
	Female	.51	.44	
Suffolk, line 0	Male	.61		
	Female	.49	.36	

The means and standard deviations for body weight and each blood constituent assembled according to age in weeks, years, breeds, sex, and type of birth are presented in appendix Tables A-1 through A-5. Figures 2 through 6 illustrate the variations in mean blood levels over age in single lambs by breed and sex. From these data it is apparent that the concentrations of the blood factors studied were subject to considerable variation in the very young lambs, but that by eight to ten weeks of age the lambs had become sufficiently adjusted so that trends could be detected in the levels of the blood constituents. There was also a suggestion that the stress induced by the act of weaning, either natural or man-induced, might have altered the previously established trend.

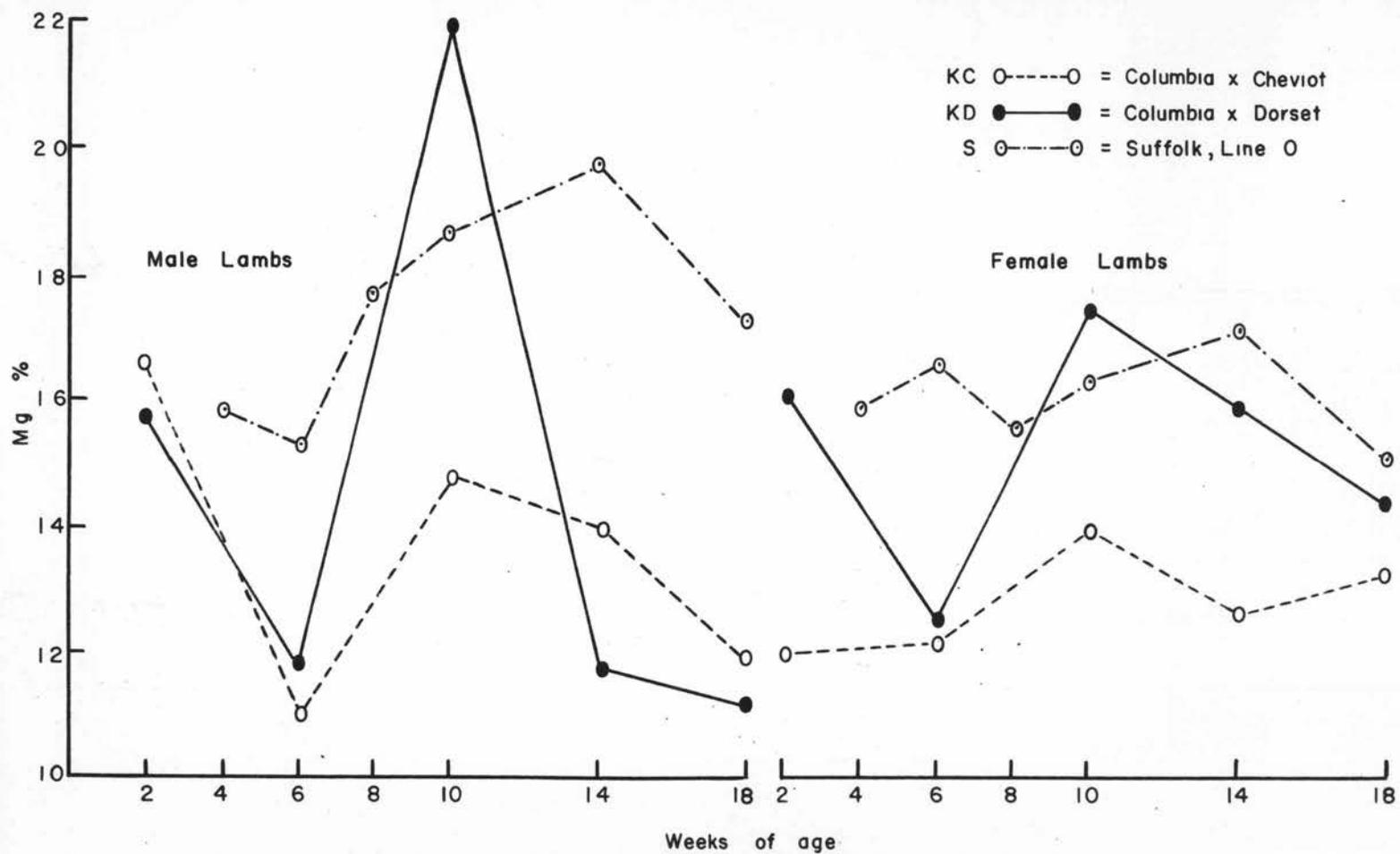


Figure 2. Urea Nitrogen Concentration (mg per 100 ml whole blood) in single lambs by breeds, sex, and age.

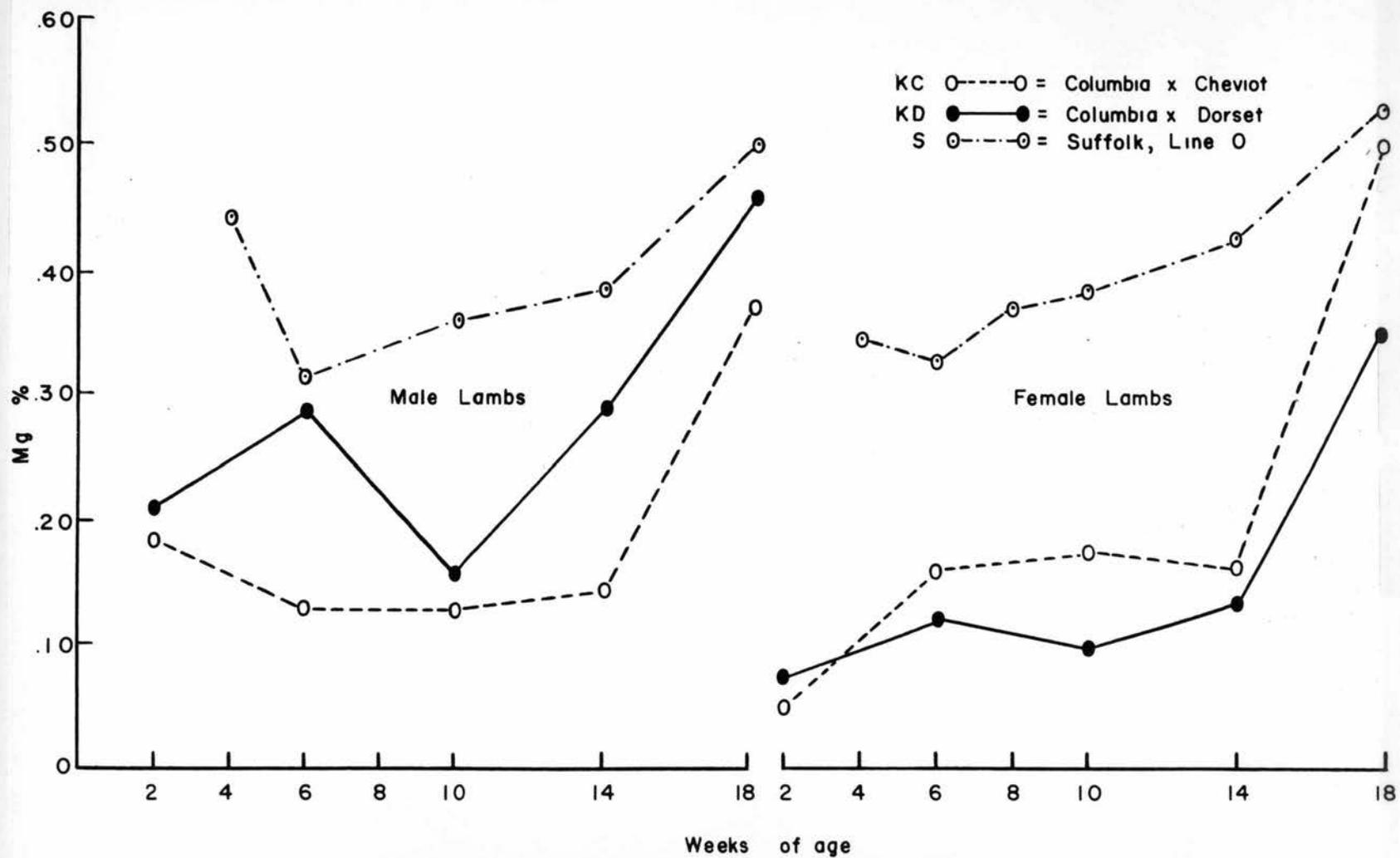


Figure 3. Uric Acid Concentration (mg per 100 ml whole blood) in single Lambs by breeds, sex, and age.

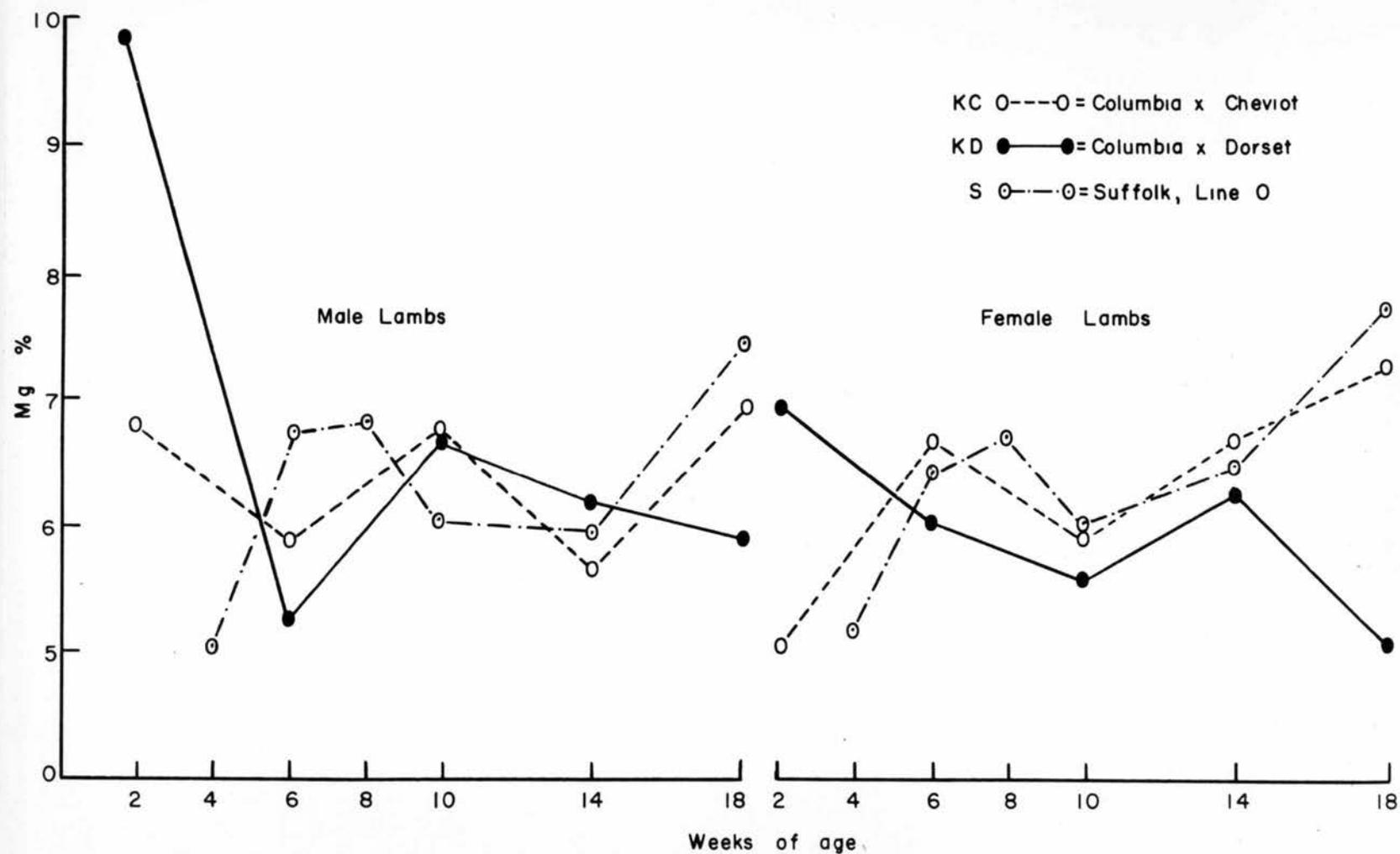


Figure 4. Amino Acid Nitrogen Concentration (mg per 100 ml whole blood) in single Lambs by breeds, sex, and age.

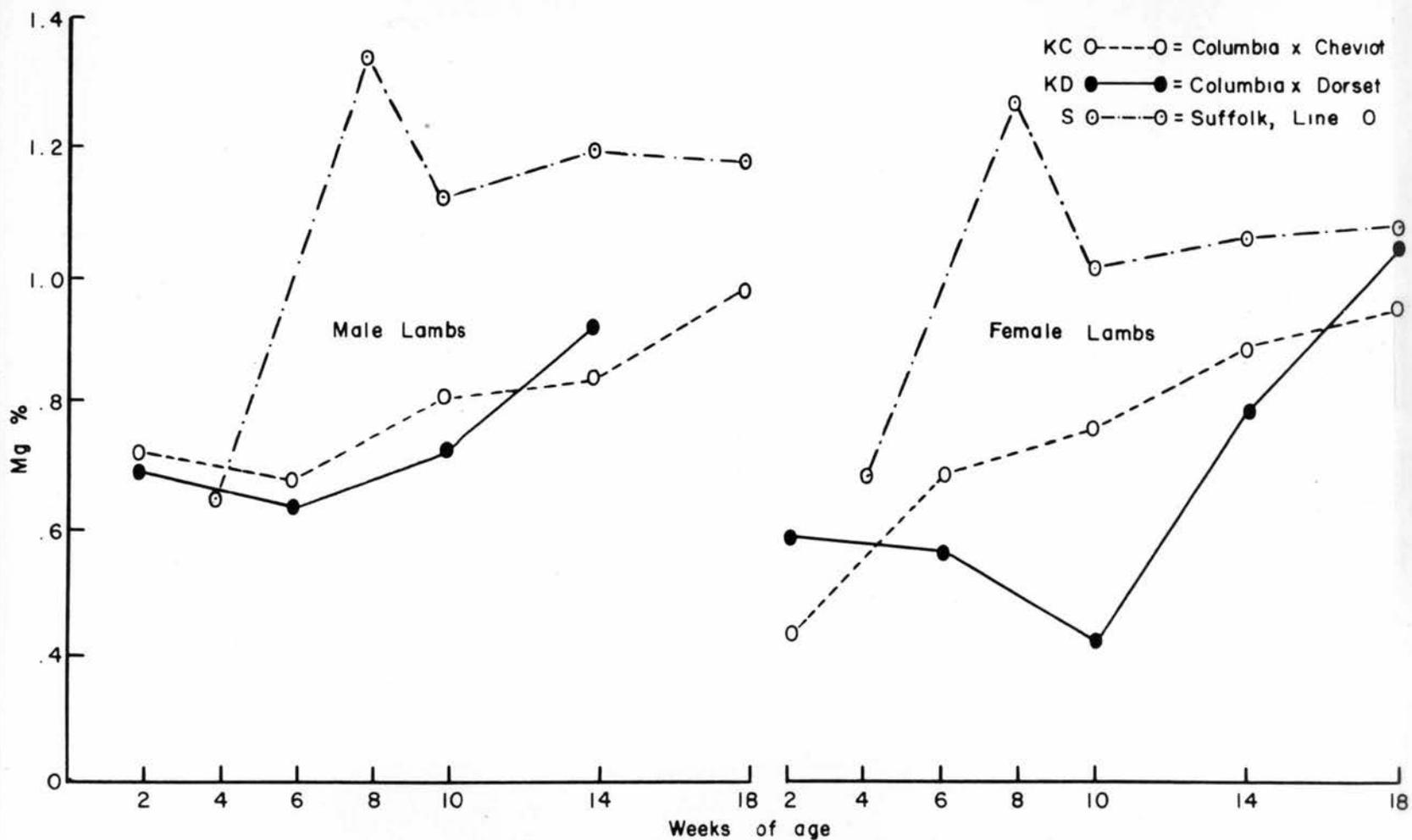


Figure 5. Creatinine Concentration (mg per 100ml whole blood) in single Lambs by breeds, sex, and age

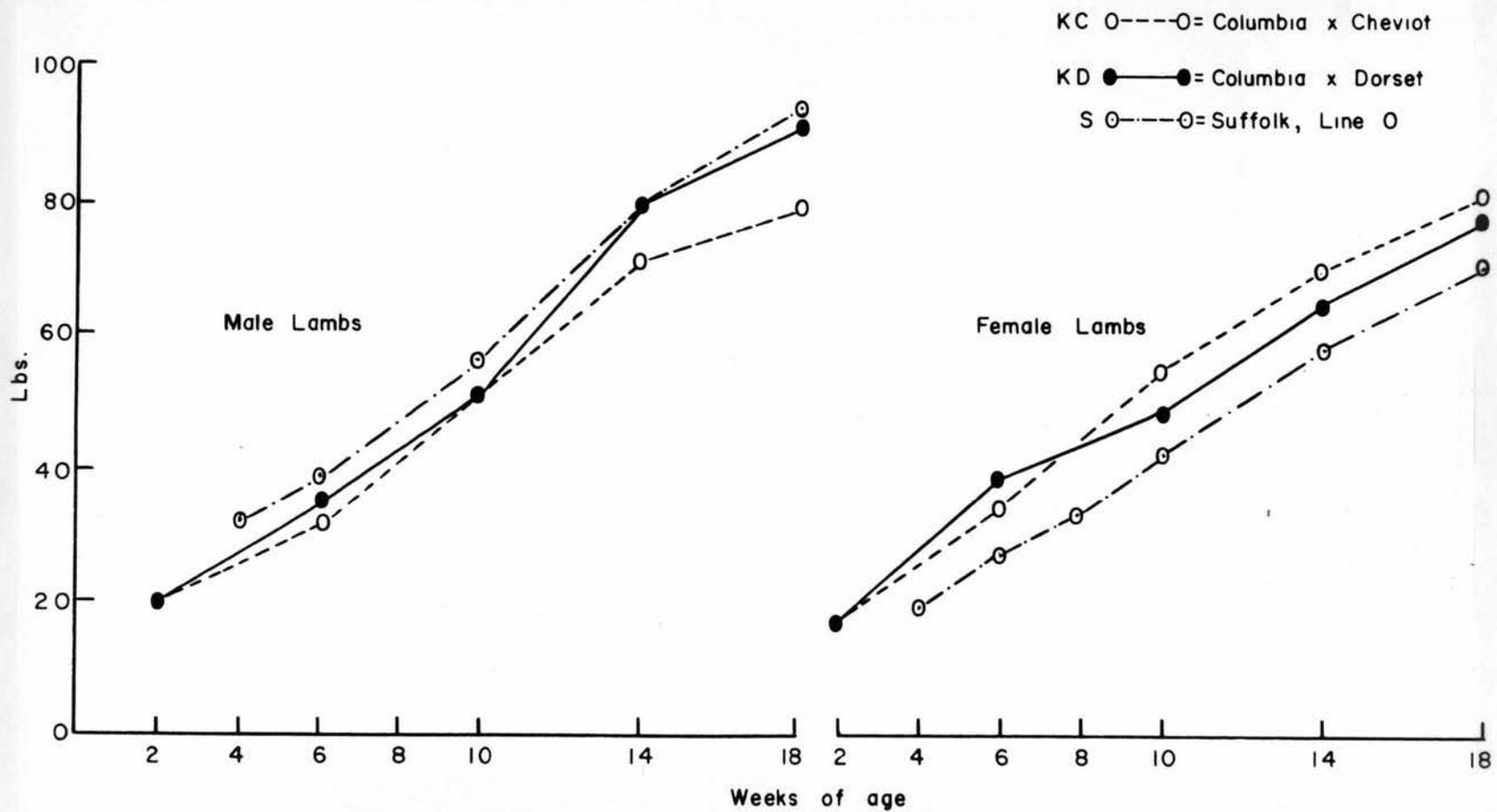


Figure 6. Body weight of single Lambs by breeds, sex, and age.

Genetic and Environmental Effects

The following multiple linear regression analysis was achieved in order to investigate the effects of sex, breed, type of birth and age upon the individual blood constituents measured. By means of the coefficients in the model shown below, the breeds have been contrasted according to the various effects given in the model. The regression equations for the three groups of lambs can be represented as follows:

$$1958: Y_{ijklmn} = M + B_i + S_j + T_k + A_1 + W_m + e_{ijklmn}$$

$$1959a: Y_{jklmn} = M + S_j + T_k + A_1 + W_m + e_{jklmn}$$

$$1959b: Y_{ijklmn} = M + B_i + S_j + T_k + A_1 + W_m + e_{ijklmn}$$

where: Y_{ijklmn} = Indicates the observation obtained for the nth lamb in the ijklmth subclass.

M = An effect constant for all lambs, the mean

B_i = the added effect due to the ith breed of lambs

S_j = the added effect due to the jth sex of lambs, male or female

T_k = the added effect due to the kth type of birth and rearing of the lambs, single, twin, or twin raised as a single

A_1 = A proportionality constant which measured the rate of change in concentration of a blood constituent per day of age

W_m = A proportionality constant which measured the rate of change in concentration of a blood constituent per pound of body weight gained

e_{ijklmn} = error, or the failure of the above constants to estimate the observed value

Breeds of lambs were different for each year, hence a comparison among all breeds would have been confounded with year differences; therefore the following contrasts have been restricted to each year. In 1958 the two breeds tested were Columbia by Dorset and Columbia by Cheviot crossbreeds. The group of lambs designated 1959a were all line 0 inbred Suffolks, while the 1959b lambs were intended as a comparison of the progeny of a Suffolk line 0 ram mated to Suffolk line 1, 2, and 3 ewes and the progeny of the same ram mated to Columbia by Dorset crossbred ewes. Types of birth and rearing included singles, twins and twins raised as singles in 1958, but was limited to singles and twins in both 1959 groups.

Urea Nitrogen: The comparison of the Dorset by Columbia with the Cheviot by Columbia crossbred lambs in 1958 showed that the Dorset cross lambs had higher concentrations of urea nitrogen ($P \leq .10$). With the Suffolk line 0 lambs in the 1959a group, it was shown that both age and weight affected the blood levels of urea nitrogen ($P \leq .001$). These results have been summarized in Table 4 which shows in analysis of variance format the factors involved and their statistical significance. An anomaly appears to exist in the 1959a analysis, as shown in Table 8, which reveals that the regression of urea nitrogen on age has a negative slope ($b = -.06$), while the regression of the urea nitrogen on body weight is positive. This seems to be inconsistent because one would not ordinarily expect, with two variables as highly related as

age and weight, to find one positively and the other negatively correlated with a third factor such as urea. However, it is a perfectly normal occurrence. Both regression coefficients specify changes in urea concentration independently of changes in the other independent variable. However, age and weight are positively associated. Therefore, when changing from one lamb to another, the dependent variable, urea, usually gets both a positive and a negative increment which tend to counterbalance each other. Snedecor (61, p. 342-346) explains this in considerable detail.

Uric Acid: Age of lamb was the only environmental factor which influenced the uric acid concentration in the blood of lambs to a degree sufficiently great to be considered statistically significant. ($P \leq .025$ in the 1958 and 1959b groups and $P \leq .005$ for the 1959a group). The analyses of variance summarizing these results are presented in Table 5. The relationship between age and uric acid concentration was relatively constant for all groups of lambs as evidenced by the regression constants of .0019 milligrams per cent uric acid increase per day of age for the 1958 and 1959a groups of lambs and the .0067 mg. percent per day increase for the 1959b lambs as shown in Table 8.

Amino Acid Nitrogen: The observed variation in amino acid nitrogen concentration in the blood was not readily attributable to the

environmental factors studied. In the Suffolk, line 0 lambs (1959a) there was a positive relation between amino acid concentration, and weight ($P \leq .10$), while in the line cross slaughter lambs (1959b) there was a negative regression of amino acid with age ($P \leq .025$). The data from the Dorset and Cheviot crossbred lambs tested in 1958 were not inconsistent with the null hypothesis of independence ($H_0: \beta = 0$). Table 6 summarizes these tests of significance in analysis of variance format, while Table 8 lists the estimated regression constants.

Preformed Creatinine: The effects of the environmental factors on preformed creatinine content of blood also differed by years. In the 1958 crossbred lambs there was a moderate probability ($P \leq .10$) of a positive effect due to age of the lamb, while in both of the 1959 groups only weight showed any significant probability of affecting creatinine level ($P \leq .025$ for 1959a and $P \leq .10$ for 1959b). Tables 7 and 8 show the analyses of variance and estimated regression constants.

If a probability level of 0.05 ($P \leq .05$) is selected as the criterion for rejection of the null hypothesis of independence, it is noted that of the variables studied, only age and weight were found to have any statistically significant effect on the levels of the non-protein nitrogenous constituents of the blood of lambs. From a review of the literature and from other experiences, it was expected

that differences would exist in blood levels due to breeds and sexes, and perhaps to type of birth and rearing. The purpose here is to discuss some of the reasons why these variables were not recognizable as factors affecting the blood levels and to show how the results could therefore be pooled over variables in order to obtain larger numbers for a more sensitive type of analysis. While it is believed that differences do exist in the blood levels of the various constituents which are due to breeds and sexes, it is recognized that these differences are probably small, a fact which has been borne out by the results. Any effects due to type of birth and rearing are largely nutritional in nature and consequently are reflected in the weights of the lambs. Therefore any effects which might have been attributable to type of birth will probably be shown in the analysis as an effect due to weight. The reason the data showed non-significance when it is believed that differences actually do exist is two-fold: (1) the use of an inappropriate mathematical model; and (2) the range of values over which the variables were studied was so limited that the differences were less than the relatively large normal variation present in the data.

The fact that the observed differences were small and were shown to be statistically non-significant was considered to be sufficient justification for the pooling of the data over breeds, sexes and types of birth and rearing. If actual differences due to these variables do

exist, however, the effect of the pooling will be to increase the standard errors of the estimates and to incorporate possible bias into the analysis. However, the size of any bias so introduced will probably be small in relation to the size of the bias which is due to the use of the improper mathematical model. Moreover, the increased size of the standard error due to the pooling will be more than offset by a corresponding decrease in the standard error due to the greater numbers of observations. The over-all effect therefore, will be a reduction in the size of the standard error.

As a result of the regression analysis, it is concluded that the effects of age and weight were manifested in the levels of the blood constituents, confirming previously known findings. However this analysis considers the effect of each factor independent of all other factors. Since age and weight are highly correlated, the effects due to each or both might possibly be greater than that indicated herein. When two factors are highly correlated, the procedure of adjusting for one reduces the effect due to the other. While the effects of breed, sex and type of birth were estimated to be small, it is believed that they are not zero. Differences between the crossbred lines tested in 1958 and between the inbred Suffolk lines tested in 1959 were expected to be small due to the common origin of the lines compared. On the other hand, the lack of a significant sex difference in lambs may seem surprising in view of some of the results reported with calves on feed test (48, p. 42-89). The young ages of the lambs and the fact

that metabolic differences due to proliferation of the sex hormones probably did not become manifest until the latter stages of this experiment are offered as explanation. It was not possible to use the device of pooling similar breeds and sexes to enhance the analysis in this section; rather such pooling was performed in anticipation of the same difficulty in the following analysis involving the discriminant function with the expectation of enhanced sensitivity.

TABLE 4. ANALYSES OF VARIANCE IN MULTIPLE REGRESSION:
EFFECTS OF ENVIRONMENTAL FACTORS ON UREA NITROGEN CONTENT
(mg per 100 ml) OF WHOLE BLOOD OF SUCKLING LAMBS

<u>SOURCE OF VARIATION</u>	<u>d.f.</u>	<u>SUM OF SQUARES</u>	<u>MEAN SQUARES</u>	<u>F</u>	<u>PROB.</u>
<u>1958 (Crossbred lambs)</u>					
TOTAL	105	21,289.48			
REGRESSION	7	20,094.59	2870.66		
MEAN (M)	1	3,510.96	3510.96		
BREED (B)	1	43.50	43.50	3.57	.10
SEX (S)	1	5.49	5.49	.45	NS
BIRTH TYPE (T)	2	.28	.14	.01	NS
AGE (A)	1	.42	.42	.03	NS
WEIGHT (W)	1	1.69	1.69	.14	NS
ERROR	98	1,194.89	12.19		
<u>1959a (Suffolks, line 0)</u>					
TOTAL	95	26,481.17			
REGRESSION	5	25,997.55	5199.51		
MEAN	1	2,787.86	2787.86		
SEX	1	2.96	2.96	.55	NS
BIRTH TYPE	1	11.78	11.78	2.19	.25
AGE	1	88.70	88.70	16.51	.001
WEIGHT	1	153.34	153.34	28.54	.001
ERROR	90	483.62	5.37		
<u>1959b (Slaughter lambs)</u>					
TOTAL	40	10,269.23			
REGRESSION	6	10,002.52	1667.09		
MEAN	1	724.50	724.50		
LINES	1	.00	.00	.00	NS
SEX	1	.33	.33	.04	NS
BIRTH TYPE	1	13.42	13.42	1.71	.25
AGE	1	.53	.53	.07	NS
WEIGHT	1	1.62	1.62	.21	NS
ERROR	34	266.71	7.84		

TABLE 5. ANALYSES OF VARIANCE IN MULTIPLE REGRESSION:
EFFECTS OF ENVIRONMENTAL FACTORS ON URIC ACID CONTENT
(mg per 100 ml) OF WHOLE BLOOD OF SUCKLING LAMBS

<u>SOURCE OF VARIATION</u>	<u>d.f.</u>	<u>SUM OF SQUARES</u>	<u>MEAN SQUARES</u>	<u>F</u>	<u>PROB.</u>
<u>1958</u>					
TOTAL	105	7.1861			
REGRESSION	7	5.9568	.8510		
MEAN	1	.0666	.0666		
BREED	1	.0006	.0006	.05	NS
SEX	1	.0204	.0204	1.63	.25
BIRTH TYPE	2	.0221	.0111	.89	NS
AGE	1	.0819	.0819	6.53	.025
WEIGHT	1	.0018	.0018	.14	NS
ERROR	98	1.2293	.0125		
<u>1959a (Suffolk, line 0)</u>					
TOTAL	95	15.7081			
REGRESSION	5	14.9162	2.9832		
MEAN	1	.8505	.8505		
SEX	1	.0012	.0012	.14	NS
BIRTH TYPE	1	.0195	.0195	2.22	.25
AGE	1	.1001	.1001	11.38	.005
WEIGHT	1	.0040	.0040	.45	NS
ERROR	90	.7919	.0088		
<u>1959b (slaughter lambs)</u>					
TOTAL	40	9.0247			
REGRESSION	6	8.4923	1.4154		
MEAN	1	1.4719	1.4719		
LINES	1	.0179	.0179	1.15	NS
SEX	1	.0094	.0094	.60	NS
BIRTH TYPE	1	.0007	.0007	.04	NS
AGE	1	.0908	.0908	5.80	.025
WEIGHT	1	.0017	.0017	.10	NS
ERROR	34	.5324	.0157		

TABLE 6. ANALYSES OF VARIANCE IN MULTIPLE REGRESSION:

EFFECTS OF ENVIRONMENTAL FACTORS ON AMINO ACID NITROGEN CONTENT
(mg per 100 ml) OF WHOLE BLOOD OF SUCKLING LAMBS

<u>SOURCE OF VARIATION</u>	<u>d.f.</u>	<u>SUM OF SQUARES</u>	<u>MEAN SQUARES</u>	<u>F</u>	<u>PROB.</u>
<u>1958</u>					
TOTAL	105	4455.3149			
REGRESSION	7	4281.3975	611.6282		
MEAN	1	816.6879	816.6879		
BREED	1	.2867	.2867	.16	NS
SEX	1	.7682	.7682	.43	NS
BIRTH TYPE	2	1.5142	.7571	.43	NS
AGE	1	.0150	.0150	.01	NS
WEIGHT	1	.4476	.4476	.25	NS
ERROR	98	173.9174	1.7747		
<u>1959a (Suffolks, line 0)</u>					
TOTAL	95	3915.7791			
REGRESSION	5	3848.2985	769.6597		
MEAN	1	324.4883	324.4883		
SEX	1	.6950	.6950	.93	NS
BIRTH TYPE	1	.0992	.0992	.13	NS
AGE	1	.7889	.7889	1.05	NS
WEIGHT	1	2.2868	2.2868	3.05	.10
ERROR	90	67.4806	.7498		
<u>1959b (Slaughter lambs)</u>					
TOTAL	40	1965.4167			
REGRESSION	6	1903.8693			
MEAN	1	184.1725	184.1725		
LINES	1	.1707	.1707	.09	NS
SEX	1	1.1663	1.1663	.64	NS
BIRTH TYPE	1	.2156	.2156	.12	NS
AGE	1	13.0289	13.0289	7.20	.025
WEIGHT	1	2.7522	2.7522	1.52	.25
ERROR	34	61.5474	1.8102		

TABLE 7. ANALYSES OF VARIANCE IN MULTIPLE REGRESSION:

EFFECTS OF ENVIRONMENTAL FACTORS ON PREFORMED CREATININE CONTENT
(mg/100 ml) OF WHOLE BLOOD OF SUCKLING LAMBS

<u>SOURCE OF VARIATION</u>	<u>d.f.</u>	<u>SUM OF SQUARES</u>	<u>MEAN SQUARES</u>	<u>F</u>	<u>PROB.</u>
<u>1958</u>					
TOTAL	105	64.7933			
REGRESSION	7	61.6775	8.8111		
MEAN	1	5.2192	5.2192		
BREED	1	.0262	.0262	.82	NS
SEX	1	.0178	.0178	.56	NS
BIRTH TYPE	2	.0332	.0166	.52	NS
AGE	1	.1068	.1068	3.36	.10
WEIGHT	1	.0035	.0035	.11	NS
ERROR	98	3.1157	.0318		
<u>1959a</u>					
TOTAL	95	102.1777			
REGRESSION	5	98.7396	19.7479		
MEAN	1	8.8772	8.8772		
SEX	1	.0007	.0007	.02	NS
BIRTH TYPE	1	.0016	.0016	.04	NS
AGE	1	.0102	.0102	.27	NS
WEIGHT	1	.2057	.2057	5.38	.025
ERROR	90	3.4381	.0382		
<u>1959b</u>					
TOTAL	40	47.9144			
REGRESSION	6	46.7132	7.7855		
MEAN	1	2.6759	2.6759		
BREED	1	.0004	.0004	.01	NS
SEX	1	.0019	.0019	.05	NS
BIRTH TYPE	1	.0932	.0932	2.64	.25
AGE	1	.0279	.0279	.79	NS
WEIGHT	1	.1057	.1057	2.99	.10
ERROR	34	1.2012	.0353		

TABLE 8. CONSTANTS ESTIMATED BY MULTIPLE REGRESSION

1958 (Crossbred Lambs)

		<u>Urea</u>	<u>Uric Acid</u>	<u>Amino Acid</u>	<u>Creatinine</u>
b ₀	Mean	14.269	.0621	6.8819	.5502
b ₁	Dorset x K	.833d	.0030	-.0677	-.0204
b ₂	Chev x K	-.833d	-.0030	.0677	.0204
b ₃	Males	-.260	.0158	.0972	.0148
b ₄	Females	.260	-.0158	-.0972	-.0148
b ₅	Singles	-.077	-.0113	-.0723	.0190
b ₆	Twins	-.036	-.0248	-.2226	-.0361
b ₇	T as S	.112	.0361	.2949	.0171
b ₈	Age/day	-.023	.0019c	-.0008	.0022d
b ₉	Weight/lb	.032	.0005	-.0084	.0007

1959a (Suffolks)

b ₀	Mean	15.217	.2658	5.1915	.8587
b ₁	Male	-.230	-.0046	-.1102	.0036
b ₂	Female	.230	.0046	.1102	-.0036
b ₃	Single	-.569	.0231	.0522	-.0066
b ₄	Twin	.569	-.0231	-.0522	.0066
b ₅	Age	-.057a	.0019b	.0054	-.0006
b ₆	Weight	.128a	-.0006	.0156d	.0047c

1959b (Slaughter lambs)

b ₀	Mean	14.609	.6585	7.3657	.8878
b ₁	S ₀ X S ₁	.003	.0226	.0698	-.0036
b ₂	S ₀ X DK	-.003	-.0226	-.0698	.0036
b ₃	Male	.096	-.0162	-.1809	-.0073
b ₄	Female	-.096	.0162	.1809	.0073
b ₅	Single	.870	.0061	-.1102	-.0725
b ₆	Twin	-.870	-.0061	.1102	.0725
b ₇	Age/day	-.016	-.0067c	-.0806c	-.0037
b ₈	Weight/lb	.050	-.0016	.0652	.0128d

a P < .001
 b P < .01
 c P < .05
 d P < .10

Selection by Discriminant Function Analysis

Since effects due to breeds, sexes, and type of birth and rearing could not be detected as being statistically significant, as was previously noted, the data were pooled over these classifications within years to obtain larger numbers for the discriminant functions. Means of the saved and culled groups are presented in Appendix table A-6. Analyses of variance and tests of significance of the eleven separate discriminant functions, one for each age period in each of the two years, are presented in Table 10. Variables were added to the function in decreasing order of effectiveness until the additional effect due to the last added variable was not statistically significant at the level ($F \geq 1.75$). In most instances the discriminating ability of the three most effective variables was included in the function. Exceptions occurred primarily at six weeks of age in both years where body weight was the only variable which was significantly effective. Also presented in Table 10 are the discriminatory functions with all five variables included. In all cases, the additional reduction in the residual sums of squares due to including the remaining two or three variables in the function was so slight as to be negligible. This would have been true even in the rather unlikely event that the entire reduction could have been attributable to one of the remaining variables.

Exact probabilities could not be associated with the "F" values of the stepwise analysis shown in table 10 because of the sequential nature of the stepwise process and because the tests were not

independent of the data. The combinations of variables tested were chosen after examination of the data rather than being predetermined in advance. However this criticism in no way interfered with the usefulness of the procedure for selecting potent variables in discriminant analysis. The primary objective here was the selection of variables rather than the assigning of probability statements, hence any logical criterion of selection could be utilized. With the degrees of freedom encountered in these data, an "F" level equal to or greater than 1.75 was considered reasonable.

1958: Body weight was the most effective discriminator at all age periods in 1958, and it was very highly significant ($P \leq .001$) at ten through eighteen weeks of age. In contrast to body weight, the discriminating ability of the blood constituents was relatively meager. Weight plus uric acid comprised the most effective pair of variables at two, ten, and fourteen weeks of age. If a third variable were to be included in the function, it would be creatinine at 14 weeks of age.

1959: The relative effectiveness of the various factors to discriminate between the saved and culled lambs was not as clear cut in 1959 as it was in the preceding year. Body weight had the greatest discriminatory ability at 4, 6, 10, and 18 weeks of age, while urea nitrogen was the most effective at 8 and 14 weeks. The most effective two variable functions were weight with urea nitrogen at 4 and 18 weeks, weight with creatinine at 10 weeks, urea with amino acid nitrogen at 8 weeks, and urea with creatinine at 14 weeks of age. The most effective

three variable functions were weight, creatinine, and amino acid nitrogen at 10 weeks, urea nitrogen, creatinine, and amino acid nitrogen at 14 weeks, and weight, urea nitrogen, and amino acid nitrogen at 18 weeks of age. When considered over all age periods, it would appear that the variables showing the greatest discriminating ability in 1959 were body weight and urea nitrogen. If a third variable were to be included in the function it would be either preformed creatinine or amino acid nitrogen, there apparently being little preference between the two.

The superior discriminating effectiveness of urea nitrogen over body weight at 8 and 14 weeks of age in 1959, while the reverse was true at all other age periods, might be attributed to several factors. First, the urea nitrogen values obtained for the saved and culled groups of lambs at these two age periods comprised relatively homogeneous groups which had comparatively small variability but proportionality large differences between means. That is, the $\sum (X_1 - \bar{X}_s)^2 + \sum (X_1 - \bar{X}_c)^2$, the sums of squares due to the variable urea nitrogen were small at these age periods, while the differences between the means of the saved and culled groups, $d = \bar{X}_s - \bar{X}_c$, were relatively large. Thus, in contrast to the results observed at other age periods, there was little or no overlapping of the ranges of values reported for the saved and culled groups at 8 and 14 weeks of age in 1959. Second, since the single variable discriminant, D , is obtained by

$$D = d^2 / \sum (X - \bar{X})^2$$

the relatively large d and small $\sum (X - \bar{X})^2$ led to large D 's for urea nitrogen at 8 and 14 weeks of age. Third, the size of the D 's for weight remained relatively constant at all age periods in 1959.

The most effective discriminating equations at each age period, as determined by the analysis of variance, are presented in Table 9. Included in the same table are the percentages of the total variability, R^2 , which have been accounted for by each function ($R^2 = \text{SS due to the function} / \text{Total SS}$). Two equations are given at each age period. The first of these shows the discriminatory equations exactly as computed ($Z = \lambda_1 X_1 + \lambda_2 X_2 + \dots + \lambda_r X_r$). However, the second equation of each pair is a modification which is perhaps somewhat more useful and meaningful. These equations were obtained by dividing all coefficients of the original equation (λ_i) by the coefficient of the most potent variable (λ_1), thereby obtaining a coefficient of unity for the most potent variable.

TABLE 9. Discriminant equations by years and weeks of age with the relative amount of the total variability (R^2) accounted for by the function.

<u>Age in Weeks</u>	<u>Equation</u>	<u>R^2</u>
<u>1958 lambs</u>		
2	$Z = .057W - 1.625Ua + 0.738C$ $= W - 28.730Ua + 13.043C$.546
6	$Z = .012W$ $= W$.273
10	$Z = .015W + 0.671Ua$ $= W + 44.886Ua$.533
14	$Z = .014W + 1.648Ua + 0.799C$ $= W + 119.082Ua + 57.717C$.735
18	$Z = .015W + 0.031Aa$ $= W + 2.054Aa$.634
<u>1959 lambs</u>		
4	$Z = .057W + 0.081UN + 1.705Ua$ $= W + 1.406UN + 29.768Ua$.772
6	$Z = .028W$ $= W$.588
8	$Z = .197UN + 0.348Aa$ $= UN + 1.768Aa$.845
10	$Z = .070W - 4.345C + 0.403Aa$ $= W - 62.476C + 5.800Aa$.801
14	$Z = .227UN + 2.152C - 0.338Aa$ $= UN + 9.466C - 1.488Aa$.882
18	$Z = .016W + 0.069UN - 0.214Aa$ $= W + 4.279UN - 1.324Aa$.752

where: W = weight
 UN = Urea Nitrogen
 Ua = Uric Acid
 Aa = Amino Acid
 C = Creatinine

Since year and breed effects are confounded, the possibility exists that part of the observed differences between years in the discriminant functions is in fact due to differences between breeds. The so-called "wool breeds" such as the Columbia, from which the 1958 crossbred lambs received one-half of their inheritance, differ in their growth patterns from the strictly "mutton type" sheep such as the Suffolks which were used in 1959. "Mutton type" lambs are more heavily muscled, generally mature earlier, and tend to fatten readily, but are poor wool producers. "Wool type" lambs on the other hand produce an abundance of wool but tend to be slower maturing and deposit much of their fat internally rather than as a covering over the muscle tissues. It is noted (11, p. 195) that ribonucleic acid (RNA) is present in greatest abundance in actively growing cells and in those cells engaged in the production and excretion of proteins, and that uric acid is a waste product of the metabolism of purines, a component of RNA. Creatine on the other hand is associated with muscle tissue, and creatinine is the waste product of its metabolism. It is postulated that these facts may in part account for the difference in the discriminant functions computed for the two years. It is possible that in the Columbia crossbred lambs with their greater propensity for wool production, the faster growing lambs will also be producing wool at a faster rate. It is therefore suggested that in these lambs uric acid will be more effective as a predictor than creatinine. Among the Suffolk lambs however, with their highly developed musculature it would seem probable that creatinine would become the more dominant predictor.

Comparison of the R^2 's shows that the greatest percentage of the total variability accounted for by the discriminant function was obtained at 14 weeks of age in both years, or approximately three to four weeks prior to the generally accepted weaning age of 120 days. However, in 1959 at least, it is noted that in spite of the different equations obtained, there was little difference in the relative amount of the total variability accounted for by the discriminant functions at eight, ten, and fourteen weeks of age. Why the discriminant functions were singularly ineffective at six weeks of age in both years is not readily explainable. In examining the graphs of the blood constituents over time (Figures 2 through 5), it is noted that several of these constituents exhibit minimum values or a change in trend or both at six weeks of age. While this is not suggested as a cause-and-effect relationship, it is observed that rumination in the lamb becomes effective at approximately this age. It is conceivable that a temporary period of adjustment in the nutritional and metabolic status of the lamb could have occurred at this age as a consequence of the rather rapid transition from a single-stomach to ruminal system of digestion. If this was so, it would appear rather unlikely, in retrospect, that a discriminant function or any other predictive system based on blood constituents collected at this period of development could have been highly effective.

Having selected the best prediction equation for each time period, and recognizing that the percentage of the total variability accounted

for by the selected function (R^2) varies with time, an additional problem immediately became apparent. This is the choice of the optimum time period such that the cost of errors in selection will be minimized. Choice here refers to the decision of the breeder or geneticist to keep or cull a particular animal. Obviously, the longer the decision is delayed, the more expensive a cull animal becomes if it should be rejected. Conversely, an excessively early decision is more likely to lead to the inadvertent rejection of an ultimately superior animal whose potential has yet to be recognized. It is not the intention, however, to answer this problem here, but merely to recognize that it exists. The purpose of this section will be to identify some of the salient factors and to outline in general procedures for solutions.

As a first step, it would be necessary to recognize how the quantity, $(1 - R^2)$, could be utilized as a measure of the extent of potential management error, that is, to what extent will the discriminant function fail to give the right answer in keeping or culling an animal. If $(1 - R^2)$ is large, then the percentage of wrong decisions will be large.

Secondly, it is necessary to define and evaluate the costs of such wrong decisions. These costs can be of two types: (a) that of keeping an animal which should have been culled (e_1), or (b) that of culling a desirable animal which should have been saved (e_2).

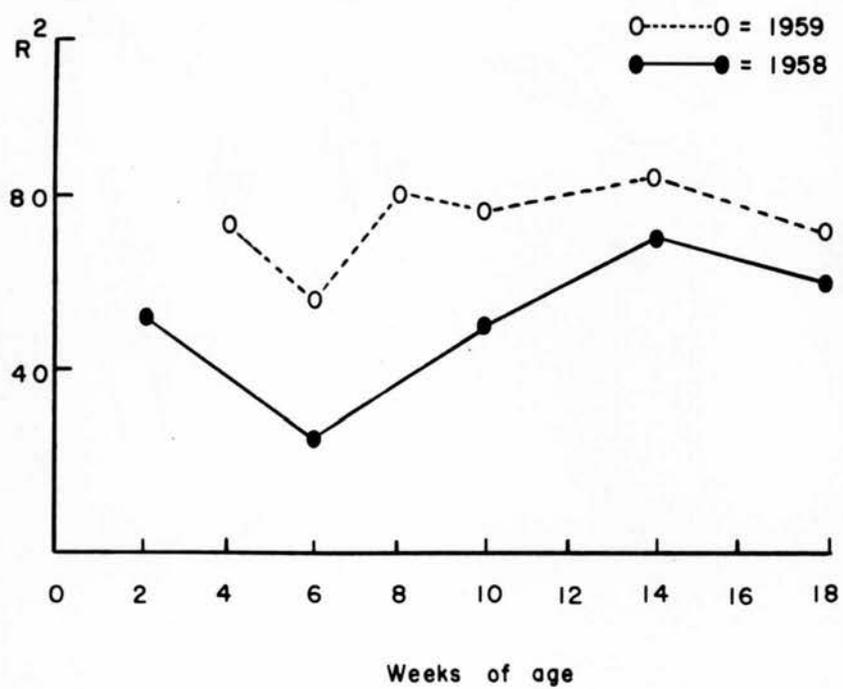


Figure 7. Coefficients of Determination (R^2) of the most effective Discriminant functions at each age period by years.

Other cost factors which might be considered are those of adequate management (m) to prevent errors and intangible factors (i) such as the reputation of the producer to his potential buyers. Cost factors which quite likely would have to be ignored in such a study include fluctuations in the market prices, changes over time in production costs, and the size of the operation. Moreover, it appears that it would be difficult to avoid the multivariate assumptions of equal variances between saved and culled groups and a multivariate normal distribution even though such assumptions may not always be strictly applicable in practice.

Having considered all of the above factors, it would then be necessary to develop a cost function of the form $c = f(e_1, e_2, m, i, t)$ where t refers to time period of the test. Of these factors, e_2 , the cost of culling a good animal which should have been retained in the breeding herd, would be the most difficult to estimate. When a mathematical expression has been devised and solved for the above function, it would be straightforward to select that time period which maximizes the precision of the selection index subject to the minimized total cost due to errors in selection.

It is concluded from the discriminant function analysis that the non-protein nitrogenous constituents of the blood are related to growth as determined by size and condition of the lamb at weaning age. However, the relative importance of the four constituents in predicting the acceptability of a lamb at weaning age varied according to years

and age at which the measurements were recorded. Nevertheless, the additional information contributed by the assays of one or more of the blood constituents over that supplied by body weight alone was statistically significant at all time periods except six weeks of age. Compared to the current selection index¹ which includes body weight plus condition and conformation scores, the discriminant function method of selection utilizing blood assays would be more expensive and time consuming, but it has the advantage that it could be performed at an earlier age. On the other hand, condition and conformation scores are both subjective measurements which are highly correlated with each other and with weaning weight.² Consequently there may be reason to question just how much, if any, additional information is contributed by the condition and conformation scores. However, the ultimate test of any selection procedure depends not on the ratings assigned at the time of selection, but rather on the relative performance of the saved and culled animals at mature, productive ages. Where facilities permit the maintenance of an unselected group, a "paper culling" would afford such a comparison. In large flocks where selection has been practiced, a second "paper culling" of the selected animals would provide a similar test. Unfortunately neither of these conditions were available to the present study.

¹See text, p. 36.

²Among the Suffolk, line 0 lambs measured in 1959, the correlation coefficients were .97 between condition and conformation scores and .82 and .85 between meaning weight with condition and conformation scores respectively.

TABLE 10. STEPWISE DISCRIMINANT FUNCTION ANALYSIS

Tests of Significance of Additional Variables in the
Discriminant Function for Selection of Desirable Lambs

<u>SOURCE OF VARIATION</u>	<u>d.f.</u>	<u>SUMS OF SQUARES*</u>	<u>MEAN SQUARES</u>	<u>F</u>
1958, AGE TWO WEEKS				
Total	15	.1617		
Most Effective Variable, X_1^{**}	1	.0483	.0483	5.96
Residual	14	.1134	.0081	
Most Effective 2 Variables				
X_1, X_3	2	.0751	.0375	5.63
X_3 Independent of X_1	1	.0268	.0268	4.02
Residual	13	.0866	.0067	
Most Effective 3 Variables				
X_1, X_3, X_5	3	.0883	.0294	4.81
X_5 Independent of X_1, X_3	1	.0132	.0132	2.16
Residual	12	.0734	.0061	
All 5 Variables				
Residual	5	.1000	.0200	3.24
	10	.0617	.0062	
1958, AGE SIX WEEKS				
Total	21	.1035		
Most Effective Variable, X_1	1	.0283	.0283	7.54
Residual	20	.0752	.0038	
Most Effective 2 Variables				
X_1, X_5	2	.0319	.0159	4.51
X_5 Independent of X_1	1	.0035	.0035	.94
Residual	19	.0717	.0038	
All 5 Variables				
Residual	5	.0342	.0068	1.58
	16	.0693	.0043	

* All sums of squares were adjusted so that every discriminant would have the same total sum of squares as the most potent single discriminants

** NOTE: X_1 = Body Weight X_4 = Amino Acid Nitrogen
 X_2 = Urea Nitrogen X_5 = Creatinine
 X_3 = Uric Acid

TABLE 10. (Continued)

<u>SOURCE OF VARIATION</u>	<u>d.f.</u>	<u>SUMS OF SQUARES*</u>	<u>MEAN SQUARES</u>	<u>F</u>
1958, AGE 10 WEEKS				
Total	22	.2953		
Most Effective Variable, X_1	1	.1371	.1371	18.21
Residual	21	.1582	.0075	
Most Effective 2 Variables				
X_1, X_3	2	.1575	.0787	11.43
X_3 Independent of X_1	1	.0204	.0204	2.96
Residual	20	.1378	.0069	
Most Effective 3 Variables				
X_1, X_3, X_4	3	.1582	.0527	7.31
X_4 Independent of X_1, X_3	1	.0007	.0007	.10
Residual	19	.1371	.0072	
All 5 Variables				
Residual	5	.1584	.0317	3.93
	17	.1369	.0081	
1958, AGE 14 WEEKS				
Total	20	.4727		
Most Effective Variable, X_1	1	.2515	.2515	21.61
Residual	19	.2212	.0116	
Most Effective 2 Variables				
X_1, X_3	2	.3111	.1555	17.32
X_3 Independent of X_1	1	.0595	.0595	6.63
Residual	18	.1617	.0090	
Most Effective 3 Variables				
X_1, X_3, X_5	3	.3476	.1159	15.74
X_5 Independent of X_1, X_3	1	.0365	.0365	4.96
Residual	17	.1252	.0074	
All 5 Variables				
Residual	5	.3540	.0708	8.95
	15	.0087	.0079	

TABLE 10. (Continued)

<u>SOURCE OF VARIATION</u>	<u>d.f.</u>	<u>SUMS OF SQUARES*</u>	<u>MEAN SQUARES</u>	<u>F</u>
1958, AGE 18 WEEKS				
Total	22	.6805		
Most Effective Variable, X_1	1	.4077	.4077	31.38
Residual	21	.2728	.0130	
Most Effective 2 Variables				
X_1, X_4	2	.4315	.2157	17.33
X_4 Independent of X_1	1	.0238	.0238	1.91
Residual	20	.2490	.0125	
Most Effective 3 Variables				
X_1, X_4, X_5	3	.4379	.1456	11.43
X_5 Independent of X_1, X_4	1	.0064	.0064	.51
Residual	19	.2426	.0128	
All 5 Variables				
Residual	5	.4452	.0891	6.44
	17	.2352	.0138	
1959, AGE 4 WEEKS				
Total	14	.9556		
Most Effective Variable, X_1	1	.5609	.5609	18.47
Residual	13	.3947	.0304	
Most Effective 2 Variables				
X_1, X_2	2	.6929	.3465	15.28
X_2 Independent of X_1	1	.1320	.1320	5.82
Residual	12	.2627	.0227	
Most Effective 3 Variables				
X_1, X_2, X_3	3	.7382	.2461	12.45
X_3 Independent of X_1, X_2	1	.0453	.0453	2.29
Residual	11	.2174	.0198	
All 5 Variables				
Residual	5	.7416	.1483	6.24
	9	.2140	.0238	

TABLE 10. (Continued)

<u>SOURCE OF VARIATION</u>	<u>d.f.</u>	<u>SUMS OF SQUARES*</u>	<u>MEAN SQUARES</u>	<u>F</u>
1959, AGE 6 WEEKS				
Total	15	.8788		
Most Effective Variable, X_1	1	.5167	.5167	19.97
Residual	14	.3621	.0259	
Most Effective 2 Variables				
X_1, X_4	2	.5460	.2730	10.67
X_4 Independent of X_1	1	.0294	.0294	1.15
Residual	13	.3327	.0256	
All 5 Variables				
Residual	5	.6058	.1211	4.43
	10	.2731	.0273	
1959, AGE 8 WEEKS				
Total	15	1.7127		
Most Effective Variable, X_2	1	1.1681	1.1681	30.03
Residual	14	.5446	.0389	
Most Effective 2 Variables				
X_2, X_4	2	1.4480	.7240	35.55
X_4 Independent of X_2	1	.2799	.2799	13.74
Residual	13	.2647	.0204	
Most Effective 3 Variables				
X_2, X_4, X_1	3	1.4673	.4891	23.91
X_1 Independent of X_2, X_4	1	.0193	.0193	.94
Residual	12	.2454	.0205	
All 5 Variables				
Residual	5	1.4800	.2960	12.72
	10	.2327	.0233	

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TABLE 10. (Continued)

<u>SOURCE OF VARIATION</u>	<u>d.f.</u>	<u>SUMS OF SQUARES*</u>	<u>MEAN SQUARES</u>	<u>F</u>
1959, AGE 10 WEEKS				
Total	15	.7962		
Most Effective Variable, X_1	1	.4559	.4559	18.76
Residual	14	.3403	.0243	
Most Effective 2 Variables				
X_1, X_5	2	.5487	.2743	14.41
X_5 Independent of X_1	1	.0928	.0928	4.87
Residual	13	.2475	.0190	
Most Effective 3 Variables				
X_1, X_5, X_4	3	.6374	.2125	16.05
X_4 Independent of X_1, X_5	1	.0887	.0887	6.70
Residual	12	.1588	.0132	
All 5 Variables				
Residual	5	.6609	.1322	9.77
	10	.1353	.0135	
1959, AGE 14 WEEKS				
Total	15	2.2918		
Most Effective Variable, X_2	1	1.6454	1.6454	35.64
Residual	14	.6464	.0462	
Most Effective 2 Variables				
X_2, X_5	2	1.9183	.9592	33.39
X_5 Independent of X_2	1	.2730	.2730	9.50
Residual	13	.3734	.0287	
Most Effective 3 Variables				
X_2, X_5, X_4	3	2.0207	.6736	29.82
X_4 Independent of X_2, X_5	1	.1024	.1024	4.53
Residual	12	.2710	.0226	
All 5 Variables				
Residual	5	2.0631	.4126	18.04
	10	.2287	.0229	

TABLE 10. (Continued)

<u>SOURCE OF VARIATION</u>	<u>d.f.</u>	<u>SUMS OF SQUARES*</u>	<u>MEAN SQUARES</u>	<u>F</u>
1959, AGE 18 WEEKS				
Total	15	.9664		
Most Effective Variable, X_1	1	.5820	.5820	21.19
Residual	14	.3844	.0275	
Most Effective 2 Variables				
X_1, X_2	2	.6879	.3439	16.05
X_2 Independent of X_1	1	.1059	.1059	4.94
Residual	13	.2785	.0214	
Most Effective 3 Variables				
X_1, X_2, X_4	3	.7270	.2423	12.15
X_4 Independent of X_1, X_2	1	.0391	.0391	1.96
Residual	12	.2394	.0199	
All 5 Variables				
Residual	10	.2337	.0234	6.27

* NOTE X_1 = Body Weight
 X_2 = Urea Nitrogen
 X_3 = Uric Acid
 X_4 = Amino Acid Nitrogen
 X_5 = Creatinine

Rumen Development

One of the things noted in the results from the first year's assays of the blood constituents (1958) particularly during the first six weeks of life, was the rather abrupt changes in the levels of some of those constituents. Consequently sixteen lambs were made available the following year to investigate the possibility that metabolic changes stemming from the rapidly expanding size and function of the rumen might in some way have been at least partly responsible for those abrupt changes in concentrations of the blood constituents. The purpose of this section is: 1) to present the results of the measurements of the several compartments of the ruminant stomach, and 2) to relate stomach growth, as revealed by said measurements, to the concentrations of the blood constituents.

Measurements of the fluid volume capacity of the stomach compartments, as explained in the chapter on materials and methods, were obtained by filling the excised organs with water to a constant manometric pressure of one inch of water. Such measurements obviously can only be considered as relative estimates of physiological capacity. Not only would the rumen and other stomach compartments never be completely filled with solids and liquids in the living animal, but in addition the capacity of a relaxed, dead organ is probably somewhat greater than that of the living, active tissue where considerable muscle tension is present. Nevertheless, in spite of these objections, the mean values presented in Table 11 reveal that the relative size of the stomach compartments changes rapidly during the first few weeks of

life. The mean rumen volume expressed as a percentage of mean total volume increased rapidly from 27.3 per cent at two weeks of age to 68.4 percent at eight weeks. The greatest proportion of this increase occurred between two and six weeks of age.

Mean wet and oven-dry weights of the four stomach compartments are shown in Table 12. The rumen and reticulum wet weights were highly correlated with the volume measurements ($r = .97$ and $r = .91$ respectively). Moreover the amount of water removed from the tissues by the drying process was relatively constant and averaged approximately 87.5 percent. Consequently the growth picture as shown by the volume determinations and the wet and oven-dry weights of the tissues was quite similar. Although all four compartments increased in size in an approximately linear manner, the rumen grew at the fastest rate while the reticulum and omasum grew at the slowest rates. On an oven-dry weight basis, the rumen increased from 35.9 percent of the total weight of the four stomach parts at two weeks of age to 63.8 percent at eight weeks. Conversely, the abomasum decreased at the same time and at approximately the same rate from 47.8 to 19.5 percent of the total oven-dry weight. Slightly more than half of the change in both organs occurred between two and four weeks of age. Over the same time span, the reticulum and omasum appeared to maintain their relative proportion of the total dry weight of the four stomach compartments.

Table 11. Mean volume in milliliters of the stomach compartments of suckling lambs.

	<u>Age of lamb (weeks)</u>			
	2	4	6	8
Rumen	325	969	1461	2746
Reticulum	40	138	224	339
Rumen plus Reticulum	365	1107	1685	3085
Omasum plus Abomasum	824	899	652*	929
Total Vol	1189	2006	2337	4014
(Rumen/Total)100	27.3	48.3	62.5	68.4

*Two small (apparently stunted) lambs accounted for decrease in volume.

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Table 12. Mean wet and oven-dry weights of the stomach compartments of suckling lambs.

	<u>Weeks of age</u>			
	2	4	6	8
Age in Days	14	26	39	54
Body weight (pounds)	17.2	28.0	26.9	41.1
Rumen Measurements				
Wet weight (grams)	26.9	91.1	142.8	284.9
Dry weight (grams)	3.3	10.8	17.2	32.4
Percent of total dry weight	35.9	50.0	56.2	63.8
Reticulum				
Wet weight (grams)	6.0	17.9	25.5	46.0
Dry weight (grams)	.9	2.4	3.4	5.6
Percent of total dry weight	9.8	11.1	11.1	11.0
Omasum				
Wet weight (grams)	5.0	7.9	9.4	23.8
Dry weight (grams)	.6	1.4	1.3	2.9
Percent of total dry weight	6.5	6.5	4.2	5.7
Abomasum				
Wet weight (grams)	53.2	56.1	71.4	88.1
Dry weight (grams)	4.4	7.0	8.7	9.9
Percent of total dry weight	47.8	32.4	28.4	19.5

Since the relative sizes of the rumen and abomasum were changing rapidly during the early weeks of life, and since the concentrations of the non-protein nitrogenous constituents of the blood were fluctuating considerably during this same period, simple product-moment correlations were computed between each blood constituent and each measurement of the stomach compartments to determine whether relationships existed.

The simple correlation coefficients based on 16 lambs slaughtered in groups of four each at 2, 4, 6 and 8 weeks of age which are presented in Table 13, indicate that the levels of the concentration of uric acid and creatinine are correlated ($P < .05$) with age of lamb, body weight of lamb, and wet weight, oven-dry weight and volume of the rumen and reticulum. However, the coefficients of determination (r^2) are relatively small, varying from 0.25 for uric acid concentration with rumen wet weight to 0.48 for creatinine concentration with rumen volume. Creatinine is also shown to be related to weight of the omasum and volume of the omasum-abomasum. While the interpretation of the relationship between creatinine level and omasum size is not immediately apparent, it can be accounted for by the fact that both variables made most of their increase between six and eight weeks of age after having remained relatively constant between two and six weeks of age.

Table 13. Simple correlations coefficients (r) between stomach compartments measurements and non-protein nitrogenous constituents of the blood.

<u>Measurement</u>	<u>Blood Constituents</u>			
	<u>Urea Nitrogen</u>	<u>Uric Acid</u>	<u>Amino Acid Nitrogen</u>	<u>Preformed Creatinine</u>
Age	.47	.52	.11	.57
Body weight	.31	.59	.01	.54
Wet weights				
Rumen	.45	.50	.05	.68
Reticulum	.40	.55	.001	.63
Omasum	.46	.37	.18	.70
Abomasum	.09	.33	.04	.29
Dry weights				
Rumen	.42	.52	.02	.66
Reticulum	.36	.59	.03	.60
Omasum	.26	.44	.001	.54
Abomasum	.22	.53	.29	.27
Volume				
Rumen	.44	.54	.04	.69
Reticulum	.38	.61	.05	.57
Omasum-Abomasum	.32	.18	.68	.52

Percentage points for the distribution of the correlation coefficient, r, with $n-2=14$ degrees of freedom; i.e., significance level.

P = .10	.426
P = .05	.497
P = .01	.623

The data are not inconsistent with the hypothesis that blood urea levels are not related to size of stomach compartments ($H_0: \rho = 0$), unless one is willing to increase to 0.10 the probability of finding significant relationships when, in fact, none exist (type 1 error). At that probability level, urea nitrogen concentration is related to rumen weight and volume, but the coefficient of determination is quite small ($r^2 = 0.2$). Amino acid nitrogen concentrations, on the other hand, appear to be completely independent of size and weight of stomach compartments. The only exception to this is the correlation between amino acid nitrogen and volume of omasum-abomasum ($r = 0.68$). In view of the very low correlations between amino acid nitrogen and weights of stomach compartments, this value should be regarded with suspicion even though there was a low correlation ($r = .36$) between wet weight and volume of the combined omasum-abomasum.

These correlations do not necessarily imply cause and effect relationships, since the possibility can not be eliminated that two variables are correlated due to their mutual dependence upon a third, unspecified variable. It can be concluded, however, that the concentrations of creatinine, uric acid, and to a lesser extent urea, vary directly with rumen size. The concentration of amino acid nitrogen, on the other hand, is independent of rumen size. The problem remaining is to ascertain what physiological significance can be attached to these observed relationships. The following discussion

reviews the statistical evidence obtained in this study against the backdrop of known and postulated physiological effects of the development of the rumen.

It has been shown (2, p. 92-93) that the main contribution of the rumen to nitrogen metabolism is that it can modify or supplement the amino acids of the ingested protein and alter the amount of nitrogen available to the animal. Thus the increased amounts of amino acids available to the lamb as the rumen becomes functional are probably greatly in excess of actual requirements. However the excess amino acid is rapidly deaminized by the liver to urea and ammonia (11, p. 132-149), a fact which may help explain the rapid increase in urea concentration between six and ten weeks of age as shown in Figure 2. It also accounts for the essentially zero correlation between amino acid nitrogen concentration and rumen size, since the deamination process tends to maintain the amino acid concentration of the blood at some relatively constant level. In addition to protein anabolism, amino acids are utilized in special metabolic pathways for the synthesis of other nitrogen-containing compounds including, among others, creatine.

The increase in the concentration of creatinine, a waste product of creatine metabolism, with increase in size of the rumen as reflected in the generally significant positive correlation coefficients is an expected result according to the role accorded creatine in the physiological development of the young lambs. Identified as a measure of the

development of muscular tissue, creatinine concentration over time during the early growth period increased as a result of the production of muscular tissue, and at a roughly linear rate. The delayed appearance of creatinine as a significant predictor in the discriminant function for the selection of lambs to be kept in the flock can also be explained on a physiological basis. While creatinine concentration was increasing slowly during the early weeks, it did not make appreciable increases nor attain to a sufficiently high degree to be manifested physically in the lambs so as to be noticed by the observer. This is to say that the difference in musculature of the very young lambs had not as yet become a decisive factor in the discriminant function, but with the full development of the rumen, the state of development of muscular tissue and creatinine concentration became sufficient to serve as a predictor in the discriminant function. Specifically, these observations imply that muscular development is dependent on, or is associated with the structural and functional development of the rumen. It is postulated that a contributing factor is the increased level of nitrogen metabolism supplied by the functional rumen. Additional support for this line of reasoning is supplied by the findings of significant correlations between rate of gain and rumen characteristics (58, p. 57-61) and that stunted or slowly growing calves have under developed rumens (10, p. 369-375).

Uric acid, the concentration of which was positively and significantly correlated with rumen size, is found in the blood primarily as a waste product of purine metabolism. However purines and pyrimidines, which are synthesized from ammonia, are utilized in the formation of nucleotides and nucleic acids. Therefore uric acid concentration in the blood may be considered as a measure of nucleic acid and nucleoprotein metabolism, hence as a measure of cellular activity and proliferation. The ammonia utilized in purine synthesis is furnished primarily by the rumen, and is obtained either by direct absorption into the blood or indirectly through deamination of absorbed amino acids. In early weeks of life the uric acid concentration was of a low magnitude and varied considerably, but following rumen development it increased rapidly and in quantities sufficient to be detected as a decisive factor in the discriminant function. This was particularly true among the crossbred lambs studied in 1958.

It could therefore be concluded that both protein synthesis for muscle growth and nucleic acid synthesis for the formation of essential cellular components, as indicated by blood concentrations of creatinine and uric acid, respectively, were dependent upon, or associated with a functionally complete rumen.

The inference suggested in the foregoing paragraphs concerning the role of the rumen in the physiological development of the lambs during the early periods on the basis of the observations is, of course, over-simplified. An attempt to follow the details of organ growth

through body chemistry would require a completely different approach than the problem covered in this thesis. Nevertheless, this section is given as an accounting of basic processes as suggested by a study of the blood measurements taken.

VI. SUMMARY AND CONCLUSIONS

It has long been recognized that individual animals of a given breed and species differ in their rate of growth or production, that the more rapid producers are also the more efficient producers, and that to some degree these differences are heritable in a quantitative manner. Since genic effects are exerted biochemically through the control of the production of specific proteins, the inherited portion of the differences in rate and efficiency of production must be due to genetic control of the various metabolic processes. Four physiological processes involved in the production of differential growth rates are: absorption of nutrients from the digestive tract, transfer of these nutrients from the blood into the cells, cellular metabolism, and excretion of waste products of cellular metabolism into the blood. Thus an animal which is superior in rate and efficiency of production should also be superior in one or more of these four processes. Therefore differences in growth rate should be reflected by corresponding differences in the concentrations of the various blood constituents. Since growth in the young animal is characterized largely by deposition of protein and nucleo-protein, the blood constituents investigated herein have been confined to those nitrogenous ones involved in those types of metabolism. Specifically, these were urea nitrogen, amino acid nitrogen, uric acid, and creatinine.

At birth the digestive system of the ruminant may be considered equivalent to that of the monogastric animals, but the rumen grows much more quickly than the other stomach parts and rapidly assumes a

major share of the digestive functions. This change in type of digestive system no doubt is associated with metabolic changes, and conceivably results in changes in quantity of quality of nutrients absorbed which affect the growth rate of the animal.

The objectives of this research were: 1) to describe the blood picture of the non-protein nitrogenous (NPN) constituents in suckling lambs; 2) to determine whether the concentration of these constituents was significantly affected by age, weight, sex, breeding, and type of birth; 3) to determine whether the concentrations of the blood constituents were related to growth and if so, to ascertain how these relationships could be incorporated into an objective selection procedure; and 4) to determine whether relationships existed among rumen development, the concentration of the NPN constituents in the blood, and growth rate in suckling lambs.

Data for this research were collected from lambs born in 1958 and 1959 in the experimental breeding flock maintained by the Oregon Agricultural Experiment Station, Corvallis, Oregon. Management of the breeding flock was in accordance with recommended commercial practices with supplemental feeding for the ewes during the breeding and lambing seasons. Body weights of the lambs were recorded at birth and 2, 6, 10, 14 and 18 weeks of age in 1958 and at 2, 4, 6, 8, 10, 14 and 18 weeks in 1959. Blood samples were collected and analyzed in accordance with recognized procedures (32, p. 540-567). Lambs utilized in the rumen study were serially sacrificed in groups of four each at 2, 4, 6

and 8 weeks of age, but otherwise were treated the same as all other lambs. The rumen, reticulum, omasum and abomasum were removed immediately after slaughter, washed, and all exterior adipose tissue removed. After removal of the contents and thorough flushing of the organs, the volume and wet and dry weights were determined.

Multiple linear regression analysis was used to determine the effects of breed, sex, birth type, age, and weight upon the individual blood constituents measured. The mathematical model took the form: $Y_{ijklmn} = M + B_i + S_j + T_k + A_l + W_m + e_{ijklmn}$, where Y_{ijklmn} indicates the observation obtained on the n th lamb of the m th weight, l th age, k th type of birth, j th sex and i th breed; M indicates an effect common for all lambs, the mean; and e_{ijklmn} indicates the experimental error. This model was limited in the sense that the relationships studied were probably not strictly linear, that only main effects due to each of the variables were considered, and that the effect due to each factor was considered as being independent of all other factors. Since age and weight are highly correlated, it is possible that part of the effect due to one of these variables was removed by the process of holding the other constant. This was not true for the other variables which are relatively independent. Although interaction effects between some of the variables were undoubtedly present, they were presumed to be near-zero or negligible in the scope of this exploration. Thus while the model was not completely realistic, it was considered to be adequate for a preliminary investigation of this nature where one of the primary objectives was the identification of those variables which

had a recognizable effect on the blood constituents studied. Since breeds of lambs were different each year, separate analyses were performed on each year's data. In 1958 the breeds were Columbia by Dorset and Columbia by Cheviot crossbreeds, while the lambs tested in 1959 were all line "O" inbred Suffolks.

Results of the 1958 analysis showed that the Columbia-Dorset crossbred lambs had higher concentrations of urea nitrogen ($P < .10$) and that the older lambs had greater concentrations of uric acid ($P < .025$) and creatinine ($P < .10$). Among the inbred line "O" Suffolk lambs studied in 1959, it was shown that urea nitrogen concentration increased with both age and weight ($P < .001$), uric acid concentration increased with age ($P < .005$), and that amino acid nitrogen and creatinine concentration increased with weight ($P < .025$ and $P < .10$, respectively). Contrary to expectation, and with the one exception noted above, effects due to breeds, sex, and birth type did not significantly influence the concentrations of the blood constituents. It is believed that differences due to these factors did exist, but that they were small. Breed differences were expected to be small due to the common origins of the lines compared, and although lack of significant sex differences may seem surprising, they can be explained on the basis that proliferation of the sex hormones responsible for metabolic differences between sexes probably did not become manifest until the latter stages of this experiment. Since the effects due to birth type are largely nutritional in nature, any effects which might have been attributable to birth type was probably shown in the analysis as an effect due to weight. Other

reasons for the lack of statistical significance when it is believed that differences actually do exist include the use of a not completely appropriate mathematical model and the fact that the range of values over which the variables were studied was so limited that the differences were no greater than the normal variation present in the data.

Stepwise discriminant functions were computed at each time period to find as early in lamb age as possible that combination of the variables weight, urea nitrogen, uric acid, amino acid nitrogen and creatinine which most effectively distinguished between the saved and culled lambs. For the purposes of this study, lambs which had a positive selection index value were saved, while lambs which had a zero or negative selection index value were culled. The selection index referred to is the one in use at the Oregon Experiment Station,

$$I = 2 \frac{W - \bar{W}}{SD(W)} + \frac{C - \bar{C}}{SD(C)} + \frac{T - \bar{T}}{SD(T)} \quad \text{where } I = \text{selection index value,}$$

W = weight in pounds, C = condition score, T = type score, SD = standard deviation, and \bar{W} = mean of W . The mathematical model for the discriminant functions took the general form $Z = \sum_{i=1}^k \lambda_i X_i$ where the X_i are the variables measured and the λ_i are the corresponding weighting coefficients. Since the effects due to breeds, sexes, and type of birth could not be shown to be statistically significant by the regression analysis, the data were pooled over these classifications within years to obtain larger numbers for the discriminant functions. Variables were added to the functions stepwise in decreasing order of effectiveness until the additional effect due to the last added variable

was not statistically significant at the level ($F \geq 1.75$). Exact probabilities were not assignable to these "F" values because of the sequential nature of the stepwise process and because the combinations of variables tested were suggested by the data rather than being predetermined in advance. However this did not interfere with the usefulness of the procedure. Since the primary objective was the selection of variables rather than the assigning of probability statements, any reasonable criterion of selection could be used.

Results of these analyses revealed that among the crossbred lambs studied in 1958 the most effective discriminator at all age periods was body weight, but that the discriminating ability of the blood constituents in general was not large. Weight and uric acid comprised the most effective pair of variables at two, ten and fourteen weeks of age, while creatinine in addition to the above variables comprised an effective three-factor discriminant at fourteen weeks of age. Among the inbred Suffolk lambs studied in 1959 the results were not so clear cut. Body weight was the most effective discriminator at 4, 6, 10 and 18 weeks of age while urea nitrogen concentration was most effective at 8 and 14 weeks. The most effective two-factor functions included weight with urea nitrogen at 4 and 18 weeks, weight with creatinine at 10 weeks, urea with amino acid nitrogen at 8 weeks and urea with creatinine at 14 weeks. Amino acid nitrogen added to the above functions comprised the most effective three-factor functions at 10, 14 and 18 weeks. The additional effect due to the amino acid was greatest at 10 weeks and least at 18 weeks.

In both years the coefficients of determination, R^2 , reached a minimum value at six weeks of age when only weight was an effective predictor, then climbed to a maximum value at fourteen weeks. The problem of choice of the optimum time period for making selections by means of the discriminant function method such that costs of errors in selection would be minimized was discussed but not answered here inasmuch as such a problem in itself would comprise a major area of investigation.

Simple product-moment correlations based on sixteen lambs serially slaughtered in groups of four each at 2, 4, 6 and 8 weeks of age were computed to determine the relationships between the concentrations of the NPN blood constituents and the volume and weight of the four stomach compartments. Although volume determinations as made in the laboratory were considered as the theoretical rather than the physiological capacity, the rumen volume and weight were highly correlated ($r = .97$), while wet and oven-dry weights were also highly correlated ($r = .99$). On a dry weight basis, the rumen comprised 35.9 per cent of the total weight of the four stomach compartments at two weeks of age, and increased linearly to 63.9 per cent at eight weeks. At the same time the abomasum was decreasing from 47.8 to 19.5 per cent of the total dry weight. In these lambs the uric acid and creatinine concentrations were significantly correlated with size of the rumen ($P < .05$), while the relationship between urea nitrogen concentration and rumen size had a lower probability ($P < .10$) of statistical significance. Amino acid

nitrogen concentration however appeared to be completely independent of rumen size as indicated by the essentially zero correlation. The physiological significance of these relationships were discussed in light of the fact that the main contribution of the rumen to nitrogen metabolism is that it can modify or supplement the dietary amino acids and thus augment the amount of nitrogen available to the animal. From the foregoing observations it was postulated that protein synthesis for muscle growth and nucleic acid synthesis for the formation of essential cellular components, as indicated by blood concentrations of creatinine and uric acid, respectively, were dependent upon, or associated with a functionally complete rumen.

Consideration of the foregoing observations led to the following conclusions:

1. The concentration of the NPN constituents in the blood of lambs fluctuated considerably at the very young ages, but became less variable with increasing age. This is in general agreement with the findings of other authors using other species of ruminants (42, 70).
2. The effects on the concentration of the blood constituents due to sex differences cannot be detected in young lambs, a result which is no doubt due to insufficient production of sex hormones at such ages.
3. Due to natural variability, as discussed in conclusion number one above, it may only be possible to detect significant effects due to breed differences between rather widely differing populations.

When comparing closely related breeds, the number of observations required for statistical significance may be excessive. With older lambs which have less variability however, the differentiation between breeds of similar type may be more successful.

4. Although results differed by years, the concentrations of all blood constituents studied were found to be significantly affected by age, and all but uric acid concentration were significantly affected by weight.

5. Although the confounding effects of years must not be discounted, it is postulated that the difference between the discriminant functions derived for the lambs studied in 1958 and 1959 may be due in part to the difference in metabolism between "wool type" and "mutton type" breeds.

6. It was further postulated that the protein synthesis for muscle growth and nucleic acid synthesis for the formation of essential cellular components, as indicated by the concentrations of the blood constituents studied, were dependent upon, or associated with the functional development of the rumen.

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APPENDIX

Tables of Means and Standard Deviations

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TABLE A-1. BODY WEIGHT. MEANS AND STANDARD DEVIATIONS BY YEARS, BREEDS, SEX, TYPE OF BIRTH, AND AGE IN WEEKS AT TEST.

Type of Birth	Breed	Sex	N	Statistic	Age in Weeks							
					Birth	2	4	6	8	10	14	18
Single	K x D	Male	2	\bar{y} s	10.30 1.84	20.50 1.82		32.60 6.92		50.05 7.28	74.50 12.02	88.00 7.07
		Female	4	\bar{y} s	10.3 1.53	18.63 1.53		36.68 2.26		46.92 2.86	60.50 8.23	73.88 5.89
	K x C	Male	4	\bar{y} s	10.2 3.51	19.67 3.51		34.28 6.94		49.85 9.69	68.25 16.29	79.00 14.85
		Female	4	\bar{y} s	11.4 .99	19.10 .99		34.42 3.57		50.58 5.84	64.88 6.83	76.25 7.14
Twins	K x C	Male	5	\bar{y} s	9.68 1.75	16.33 1.75		26.44 4.14		37.96 7.28	48.75 8.13	65.60 12.03
		Female	1	y	8.8	13.00		26.70		37.30	47.50	63.00
Tw/Single	K x D	Male	1	y	9.8			34.50		53.80	75.00	85.00
		Female	2	\bar{y} s	6.6 2.46	11.75 2.46		21.60		28.65 5.71	41.00 5.66	54.50 7.78
Single	S ₀	Male	5	\bar{y} s	10.26		30.00 3.10	36.82 7.17	44.16 8.30	50.84 8.36	73.80 16.19	89.00 21.30
		Female	9	\bar{y} s	9.38 1.59		21.42 5.72	27.80 7.37	33.36 8.22	39.97 8.19	57.00 9.38	68.00 11.67
	S ₁ x S ₀	Male	3	\bar{y} s	11.73	18.50 3.69	26.15 6.71	33.90 9.04	40.30 8.62			
		Female	3	\bar{y} s	10.73	20.77 4.56	26.85 7.14	33.70 6.78	41.90 10.46			
	DK x S ₀	Male	2	\bar{y} s	13.0	22.95 2.45	31.10 3.81	35.50				
		Female	2	\bar{y} s	10.55	19.25 3.17	27.05 5.29	28.90				
Twins	S ₀	Female	2	\bar{y} s	9.15		18.00 2.97	20.65 4.87	26.95 7.98	32.85 9.54	44.00 16.97	52.00 24.04

TABLE A-1. (Continued)

Type of Birth	Breed	Sex	N	Statistic	Age in Weeks							
					Birth	2	4	6	8	10	14	18
$S_1 \times S_0$		Male	1	y	11.7	17.50	25.40					
		Female	1	y	9.0	14.00	22.10					
DK x S_0		Male	2	\bar{y} s	9.35	14.10 1.09	19.50	26.80				
		Female	2	\bar{y} s	7.2	10.55 2.18	12.20	16.50				

TABLE A-2. UREA NITROGEN IN MILLIGRAMS PER ONE HUNDRED MILLILITERS WHOLE BLOOD.
MEANS AND STANDARD DEVIATIONS BY YEARS, BREEDS, SEX, TYPE
OF BIRTH, AND AGE IN WEEKS AT TEST.

Year	Type of Birth	Breed	Sex	N	Statistic	Age in Weeks							
						2	4	6	8	10	14	18	
1958	Single	D x K	Male	2	\bar{y} s	15.90 1.15		11.66 1.11		22.10 1.44	11.67 1.06	11.24 2.27	
			Female	4	\bar{y} s	16.08 3.20		12.69 2.12		17.46 7.40	15.96 2.06	14.49 2.47	
		Ch x K	Male	4	\bar{y} s	16.38 1.47		10.71 2.61		15.00 5.89	14.04 3.40	11.68 .87	
			Female	4	\bar{y} s	12.19 1.21		12.34 1.38		14.04 4.05	12.94 2.15	13.42 1.81	
	Twins	Ch x K	Male	5	\bar{y} s	15.51 6.79		12.54 2.54		12.08 1.43	11.18 2.37	12.79 1.27	
			Female	1	y	15.99		11.89		14.25	12.00	13.04	
	Tw/S	D x K	Male	1	y			11.47		14.50	11.93	18.64	
			Female	2	\bar{y} s	13.87 .56		11.45		19.10 8.83	13.73 4.68	15.85 5.05	
	1959	Single	S ₀	Male	5	\bar{y} s		15.80 3.06	15.19 1.58	17.48 2.11	18.61 1.76	19.27 1.62	17.30 3.32
				Female	9	\bar{y} s		15.91 2.29	16.53 3.07	15.62 2.52	16.35 2.41	16.90 2.89	15.34 2.76
S ₀ x S ₁			Male	3	\bar{y} s	16.61 2.89	14.90 3.93	15.45 .50	19.63 4.16				
			Female	3	\bar{y} s	16.86 2.37	15.20 1.73	15.61 2.99	16.43 1.10				
S ₀ x DK			Male	2	\bar{y} s	19.55 2.26	13.70 .58	13.74					
			Female	2	\bar{y} s	18.42 3.43	16.01 4.78	16.23					

TABLE A-2. (Continued)

Year	Type of Birth	Breed	Sex	N	Statistic	Age in Weeks						
						2	4	6	8	10	14	18
	Twins	S ₀	Female	2	\bar{y} s		16.08 2.04	12.86 .40	16.66 6.10	17.68 3.63	18.51 2.14	14.98 1.15
Male			1	y	11.66	17.04						
		S ₀ x S ₁	Female	1	y	12.38	17.66					
		S ₀ x DK	Male	2	\bar{y} s	14.27 2.57	15.86	15.65				
			Female	2	\bar{y} s	12.76 2.58	10.26	16.51				

TABLE A-3. URIC ACID IN MILLIGRAMS PER ONE HUNDRED MILLILITERS WHOLE BLOOD. MEANS AND STANDARD DEVIATIONS BY YEARS, BREEDS, SEX, TYPE OF BIRTH, AND AGE IN WEEKS AT TEST.

Year	Type of Birth	Breed	Sex	N	Statistic	Age in Weeks							
						2	4	6	8	10	14	18	
1958	Single	D x K	Male	2	\bar{y} s	.216 .068		.297 .039		.177 .059	.295 .020	.451 .078	
			Female	4	\bar{y} s	.083 .077		.136 .054		.121 .075	.157 .119	.357 .124	
		C x K	Male	4	\bar{y} s	.195 .032		.151 .103		.151 .065	.169 .093	.370 .126	
			Female	4	\bar{y} s	.058 .082		.168 .095		.184 .085	.172 .122	.508 .098	
	Twin	C x K	Male	5	\bar{y} s	.099 .124		.193 .040		.193 .871	.074 .039	.412 .151	
			Female	1	y	.072		.092		.226	.290	.336	
	Tw/S	D x K	Male	1	y			.259		.285	.362	.442	
			Female	2	\bar{y} s	.154 .056		.067		.258 .028	.193 .059	.411 .137	
	1959	Single	S ₀	Male	5	\bar{y} s		.431 .074	.318 .141	.343 .064	.366 .047	.381 .057	.487 .047
				Female	9	\bar{y} s		.339 .112	.321 .116	.384 .091	.392 .039	.441 .113	.538 .114
S ₀ x S ₁			Male	3	\bar{y} s	.546 .020	.420 .055	.312 .022	.398 .085				
			Female	3	\bar{y} s	.551 .030	.380 .022	.318 .156	.328 .020				
S ₀ x DK		Male	2	\bar{y} s	.613 .157	.346 .010	.331						
		Female	2	\bar{y} s	.674 .151	.318 .010	.423						

TABLE A-3. (Continued)

Year	Type of Birth	Breed	Sex	N	Statistic	Age in Weeks						
						2	4	6	8	10	14	18
	Twin	S ₀	Female	2	\bar{y} s	.269	.269	.423	.321	.390	.358	.407
.040						.045	.120	.032	.020	.024		
		S ₀ x S ₁	Male	1	y	.666	.304					
			Female	1	y	.946	.311					
		S ₀ x DK	Male	2	\bar{y} s	.536	.268	.261				
			Female	2	\bar{y} s	.545	.318	.476				
						.066						

TABLE A-4. AMINO ACID NITROGEN IN MILLIGRAMS PER ONE HUNDRED MILLILITERS WHOLE BLOOD.
MEANS AND STANDARD DEVIATIONS BY YEARS, BREEDS, SEX,
TYPE OF BIRTH, AND AGE AT TEST.

Year	Type of Birth	Breed	Sex	N	Statistic	Age in Weeks							
						2	4	6	8	10	14	18	
1958	Single	D x K	Male	2	\bar{y} s	9.82 2.23		5.22 .67		6.73 .60	6.18 1.20	5.90 .81	
			Female	4	\bar{y} s	6.93 1.90		6.04 .69		5.68 1.05	6.30 .95	5.08 1.23	
		Ch x K	Male	4	\bar{y} s	6.66 2.72		5.92 .69		6.69 .84	5.69 1.55	6.92 1.86	
			Female	4	\bar{y} s	5.02 .81		6.62 .69		5.93 1.11	6.60 .75	7.16 2.09	
	Twins	Ch x K	Male	5	\bar{y} s	5.76 1.94		6.69 .51		6.37 .97	6.56 1.34	6.40 1.31	
			Female	1	y	8.09		5.76		5.71	6.90	5.35	
	Tw/S	D x K	Male	1	y			6.00		7.20	5.17	6.55	
			Female	2	\bar{y} s	8.79 1.36		5.66		6.94 .35	6.15 1.13	6.12 1.82	
	1959	Single	S ₀	Male	5	\bar{y} s		5.06 .70	6.77 .26	6.84 1.40	6.02 1.09	5.97 .66	7.34 .50
				Female	9	\bar{y} s		5.32 .60	6.37 .67	6.60 .93	6.01 .79	6.42 .98	7.54 .50
S ₀ x S ₁			Male	3	\bar{y} s	7.55 1.18	5.99 1.69	6.01 1.55	6.85 .49				
			Female	3	\bar{y} s	7.54 1.21	5.06 .28	6.69 .01	7.44 .43				
S ₀ x DK			Male	2	\bar{y} s	9.50 .26	5.52 .70	5.30					
			Female	2	\bar{y} s	8.91 .17	6.55 .07	4.54					

TABLE A-4. (Continued)

Year	Type of Birth	Breed	Sex	N	Statistic	Age in Weeks					
						2	4	6	8	10	14
	Twins	S ₀	Female	2	\bar{y} s	6.18 .59	6.21 .38	5.65 .32	5.28 .13	6.99 .36	6.49 .39
			S ₀ x S ₁	Male	1	y	7.24	5.54			
				Female	1	y	8.00	8.08			
		S ₀ x DK	Male	2	\bar{y} s	8.02 1.41	6.57	5.05			
				Female	2	\bar{y} s	7.72 1.75	5.25	5.16		

TABLE A-5. PREFORMED CREATININE IN MILLIGRAMS PER ONE HUNDRED MILLILITERS WHOLE BLOOD. MEANS AND STANDARD DEVIATIONS BY YEARS, BREEDS, SEX, TYPE OF BIRTH, AND AGE AT TEST.

Year	Type of Birth	Breed	Sex	N	Statistic	Age in Weeks							
						2	4	6	8	10	14	18	
1958	Single	D x K	Male	2	\bar{y} s	.699 .088		.654 .024		.717 .063	.878 .010	.931 .088	
			Female	4	\bar{y} s	.649 .092		.632 .092		.512 .384	.809 .084	1.021 .308	
		C x K	Male	4	\bar{y} s	.720 .074		.681 .117		.789 .045	.811 .178	.931 .075	
			Female	4	\bar{y} s	.520 .010		.726 .085		.793 .039	.891 .107	.940 .024	
	Twin	C x K	Male	5	\bar{y} s	.678 .119		.708 .110		.676 .303	.860 .236	.865 .184	
			Female	1	y	.654		.619		.208	.887	.797	
	Tw/S	D x K	Male	1	y			.583		.744	.780	.869	
			Female	2	\bar{y} s	.878 .215		.637		.395 .366	.762 .010	.985 .063	
	1959	Single	S ₀	Male	5	\bar{y} s		.662 .077	1.041 .042	1.346 .140	1.046 .124	1.024 .169	1.111 .074
				Female	9	\bar{y} s		.732 .252	.956 .156	1.215 .179	1.000 .117	1.037 .133	1.049 .113
S ₀ x S ₁			Male	3	\bar{y} s	1.099 .105	.796 .544	1.064 .026	1.334 .069				
			Female	3	\bar{y} s	1.038 .088	.721 .062	1.003 .060	1.374 .038				
S ₀ x DK		Male	2	\bar{y} s	1.139 .107	1.036 .068	.911						
		Female	2	\bar{y} s	1.196 .134	1.142 .055	1.025						

TABLE A-5. (Continued)

Year	Type of Birth	Breed	Sex	N	Statistic	Age in Weeks						
						2	4	6	8	10	14	18
	Twin	S ₀	Female	2	\bar{y} s		.929 .024	1.142	1.016	.838	.860	1.036
								.014	.070	.079	.103	.103
		S ₀ x S ₁	Male	1	y		1.177	1.123				
			Female	1	y		1.158	1.316				
		S ₀ x DK	Male	2	\bar{y} s		1.142 .050	1.045	1.177			
			Female	2	\bar{y} s		1.038 .009	.987	.854			

TABLE A-6. OBSERVED MEANS FOR THE SAVED AND CULLED GROUPS OF LAMBS, THEIR DIFFERENCES, AND THE STANDARD ERRORS OF THOSE DIFFERENCES.

Weeks of Age	Item	n	Weight X_1	Urea X_2	Uric Acid X_3	Amino Acid X_4	Creatinine X_5
Crossbred Lambs (1958)							
2	Saved	10	18.89	14.92	.101	7.02	.681
	Culled	6	15.15	15.76	.175	7.21	.696
	S - C		3.74	-.84	-.074	-.19	-.015
	SE(S-C)		1.61	1.78	.037	1.06	.079
6	Saved	14	34.21	12.05	.166	6.18	.686
	Culled	8	27.85	11.87	.184	6.15	.654
	S - C		6.36	.18	-.018	.03	.032
	SE(S-C)		2.49	.83	.037	.29	.039
10	Saved	14	49.65	16.10	.192	6.28	.707
	Culled	9	36.94	14.67	.164	6.36	.559
	S - C		12.71	1.43	.028	-.08	.148
	SE(S-C)		3.18	2.49	.032	.42	.121
14	Saved	12	68.66	13.41	.239	6.20	.857
	Culled	9	48.72	12.70	.099	6.30	.815
	S - C		19.94	.71	.140	-.10	.042
	SE(S-C)		4.19	1.21	.034	.46	.057
18	Saved	14	80.53	13.43	.417	6.60	.967
	Culled	9	61.11	13.35	.406	5.80	.873
	S - C		19.42	.08	.011	.80	.094
	SE(S-C)		3.52	1.10	.057	.62	.061

TABLE A-6. (Continued)

Weeks of Age	Item	n	Weight X ₁	Urea X ₂	Uric Acid X ₃	Amino Acid X ₄	Creatinine X ₅
Inbred Suffolk Lambs (1959a)							
4	Saved	9	27.10	16.55	.416	5.49	.618
	Culled	6	17.48	14.93	.262	5.19	.922
	S - C		9.62	1.62	.154	.30	-.304
	SE(S-C)		2.30	1.06	.033	.36	.076
6	Saved	9	35.37	15.85	.293	6.70	.977
	Culled	7	22.47	15.39	.384	6.18	1.042
	S - C		12.90	.46	-.091	.52	-.065
	SE(S-C)		2.88	1.49	.056	.27	.072
8	Saved	9	42.41	18.30	.326	7.18	1.354
	Culled	7	27.60	13.79	.410	5.74	1.072
	S - C		14.81	4.51	-.084	1.44	.282
	SE(S-C)		3.27	.85	.040	.40	.058
10	Saved	9	48.93	18.10	.366	6.39	1.038
	Culled	7	34.17	16.10	.406	5.32	.937
	S - C		14.76	2.00	-.040	1.07	.101
	SE(S-C)		3.48	1.18	.018	.35	.192
14	Saved	9	70.89	19.72	.370	5.84	1.148
	Culled	7	47.43	15.43	.467	7.01	.905
	S - C		23.46	4.29	-.097	-1.17	.243
	SE(S-C)		5.00	.78	.046	.33	.194
18	Saved	9	85.66	17.81	.503	7.38	1.117
	Culled	7	55.71	13.46	.509	7.31	1.003
	S - C		29.95	4.35	-.006	.07	.114
	SE(S-C)		6.47	.93	.056	.32	.198