# COMPARISON OF CHROMOSOME AND BLOOD CONSTITUENTS OF ROCKY MOUNTAIN AND CALIFORNIA BIGHORN AND DALL AND STONE THINHORN SHEEP

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## ABSTRACT

Karyotypes and banding patterns are described for captive Rocky Mountain and California bighorn, Dall and Stone thinhorn, and European mouflon sheep. One abnormality is noted. Data on 13 serum parameters of nine bighorn and six thinhorn sheep are presented and analyzed, and show some between- and within-species differences. Hematological values for bighorn and thinhorn sheep are also presented and briefly discussed.

## INTRODUCTION

With regard to the genus Ovis, in recent years chromosome analysis has become recognized as a practical laboratory technique for research. This method has allowed reevaluation of taxonomic relationships between existing sheep into four genetic groups based on their diploid chromosome number - 52, 54, 56 and 58 (Nadler et al. 1973a, Korobitsyna et al. 1974). Further, the technique has aided in the construction of ovine evolutionary theories (Nadler et al. 1973b, Korobitsyna et al. 1974, Valdez et al. 1977), and has shown similarities to divisions based upon social behaviour and social morphology (Geist 1971).

Blood analysis is now recognized as an essential tool in the study of wildlife populations. Generally, such investigations are aimed at

establishing average or basal values for a species. Once these have been ascertained, they can serve as standards against which researchers may equate existing values in order to determine the health and nutritional status of a population. Utilization of this method in the assessment of wild sheep populations is presently being investigated (Hebert, D.M. 1978 pers. comm.). Moreover, established serological and hematological profiles should provide an additional means of taxonomic classification. For example, it is reasonable to assume that sheep in a single diploid group could be further differentiated into existing genotypes (subgroups or subspecies, races or breeds) by comparing serum constituent levels. Levels of various constituents which are shown to be genetically controlled may be useful as polymorphic or polygenic markers for inbreeding studies and in determining the effective number of breeding males in a population. This latter point has obvious implications for development of harvest strategies where only males are hunted.

However, due in part to the difficulty in obtaining samples, neither chromosomal nor blood analyses methods have been extensively employed in research on wild sheep. The number of wild sheep studied by chromosomal procedures is extremely limited, with entire populations as yet unexamined. Although hematological data and serum constituent levels for bighorn and thinhorn sheep have been reported (Franzmann and Thorne 1970, Franzmann 1971a, 1971b, Peterson and Bottrell 1978) work in this field is far from complete.

Certainly a more complete interpretation of sheep evolution and taxonomy requires a larger and more representative sample to determine the range of variation. Similarly, the development of blood profiles,

as a means of determining the nutritional status of wild sheep populations or their indices of inbreeding, demands much additional information . It is hoped that the chromosome data, hematological values and serological analysis described here will assist workers whose interests are in these areas.

## MATERIALS AND METHODS

Four Rocky Mountain bighorn sheep (O. canadensis canadensis)from the Canadian Wildlife Service boarding pens at the University of British Columbia were sampled for preliminary chromosome analyses trials. All subsequent samplings for chromosomal, hematological and serological tests were done using lambs born and raised on the Okanagan Game Farm, Penticton, British Columbia. These animals included: 5 Rocky Mountain bighorns, 4 California bighorns (O. canadensis californiana), 3 Dall thinhorns (O. dalli dalli), 3 Stone thinhorns (O. dalli stonei) and 2 Europena mouflons (O. musimon).[1]

Sterile blood samples were taken by jugular venepuncture using heparinized vacutainer tubes (Becton-Dickson Company). Chromosome spreads (Bottrell 1977) were prepared from 72 hour whole blood cultures. Generally the numerical and karyotyping methods adopted were those described in "Cytogenetics" (Priest 1969) and in "Laboratory Procedures in Human Genetics" (Sarma and Talukder 1974). Karyotypes were established from photomicrographs taken of several good metaphase spreads. The banding technique employed was that used routinely in the University of British Columbia Medical Genetics laboratory. This method is referred

[1]Hematological and serological tests were not done for mouflon sheep.

to as the trypsin-giemsa banding techniques (Masui, pers. comm. 1977).

Serum was obtained from clotted blood, decanted, and frozen. Serological analyses were done by the British Columbia Biomedical Laboratories, Burnaby, British Columbia. Eleven of the variables analyzed (excluding sodium and potassium) were included in a standard package. Considerations of economics, speed, and convenience governed our choice of this analysis package. Results of these analyses were then statistically analyzed to determine differences between and within the species of the sheep examined.

Hematological determinations were performed on whole blood containing ethylenediaminetetracetic acid (EDTA) by B. McCoy, Central Veterinary Hospital, Victoria, B.C. However, not all samples were analyzed for hematology values. With the resultant small sample size and missing data, a statistical evaluation could not be employed.

## RESULTS AND DISCUSSION

Qualitative evaluation of cultures revealed that the medium mixture chosen - RPMI 1640 plus additions - was inadequate for Stone sheep leukocyte culture, although it did support good cell growth for the other 4 races of wild sheep.

With one exception all animals displayed typical 2N=54 diploid values and karyotypes (Figure 1). Stone sheep, which have not been previously described, exhibited the expected 2N=54 karyotype. However, one californiana - Cal #6-74(F) - exhibited an abnormal 2N=54 chromosome number for 141 of 200 examined metaphase spreads. Figure 2 shows the abnormal karyotype of this sheep as determined by banding

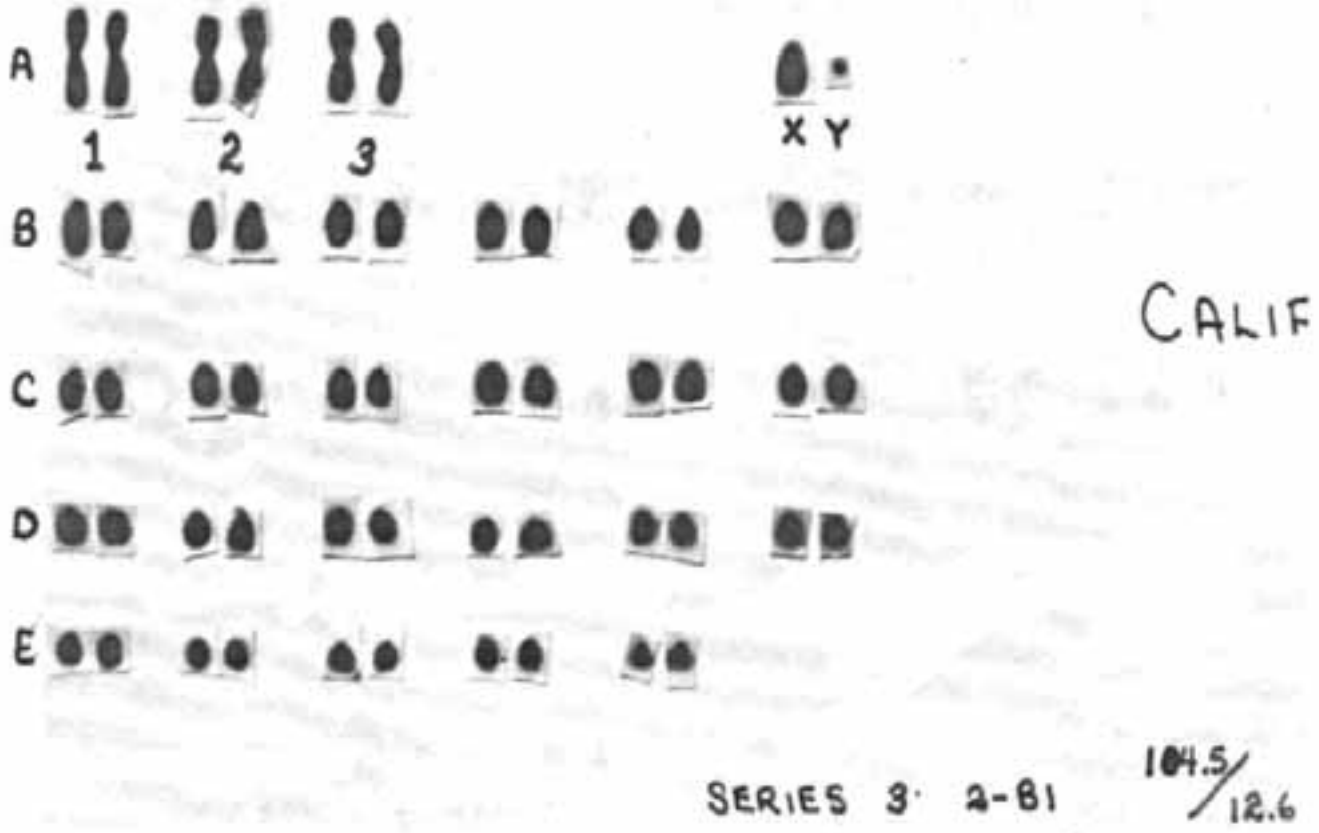


Figure 1. Karyotype of a Californian bighorn sheep (male) 2N=54

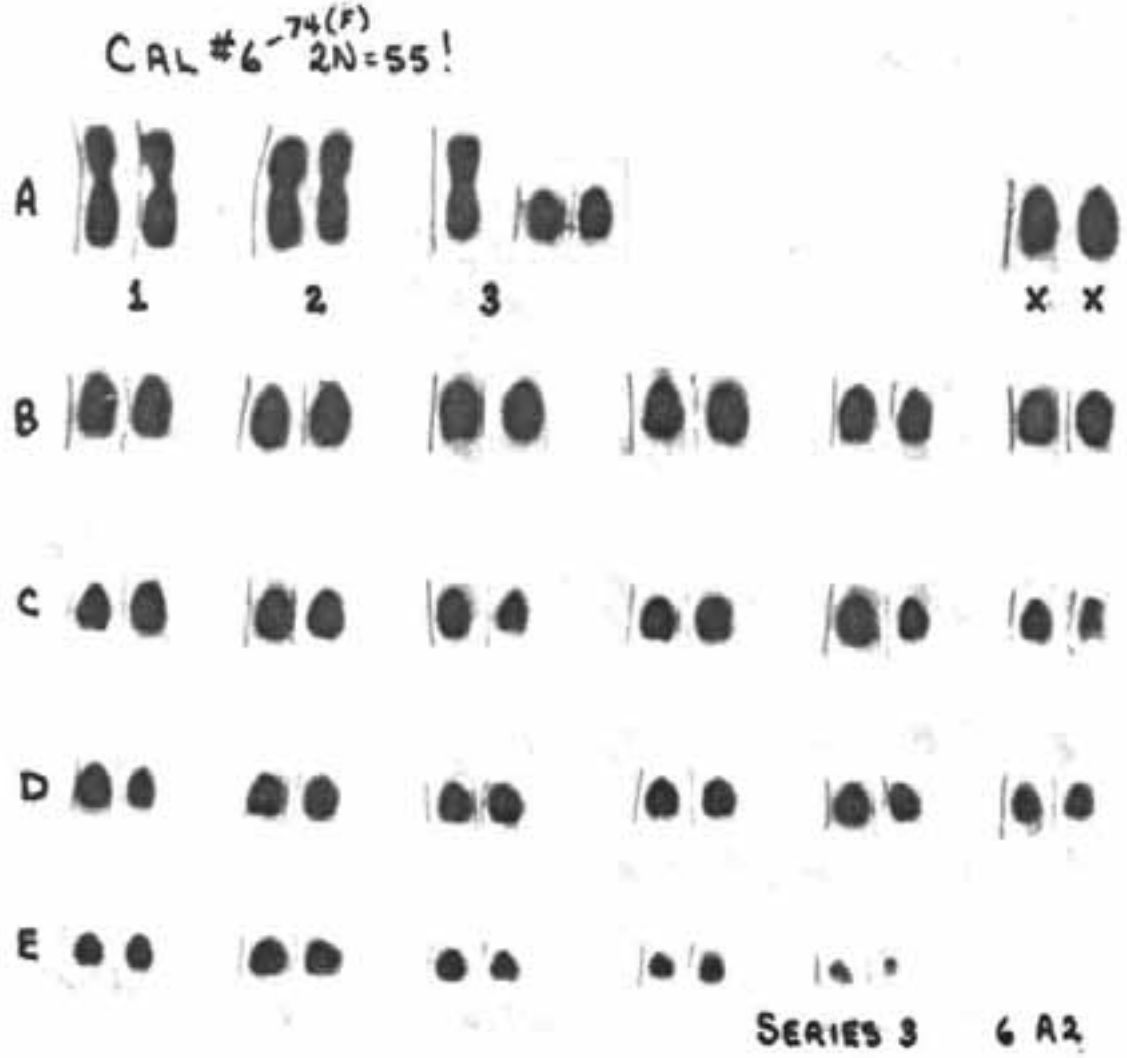


Figure 2. Abnormal karyotype of California bighorn sheep (female): Cal #6-74 (F) with 2N=55

analysis. Figure 1 is representative of the normal 2N=54 California bighorn karyotype. Unfortunately before a more careful inspection and follow up sampling of the animal could be done, the sheep died and was disposed of. Since this is the only reported instance of an abnormal diploid value being found in a North American sheep, an adequate discussion of 2N=55 bighorn sheep must undoubtedly await a more thorough investigation.[1]

The submetacentric nature of the X chromosome was evidenced in several stained preparations (Figure 2). Figure 3 is a banded karyotype thought to be representative of all the 2N=54 wild sheep studied. G - banding patterns of the three large metacentric autosomes (Group A) from each of the 5 sheep types were found to be identical. Figure 4 is a schematic illustration of these Group A metacentric autosomes. The reported Group A ideogram for the five project categories - canadensis, californiana, dalli, stonei and musimon, may be regarded as identical to that presented and discussed by Nadler (1973b) for mexicana, musimon, orientalis and, $F_1$ and $F_2$ musimon x candensis. Slight differences between Nadler's banding patterns and those of the current study were assumed to be due to interpretations and methods. Thus the Group A metacentric chromosomes of 2N=54 European, Asiatic and North American wild sheep appear to be structurally homologous. Hybridization and meiotic bivalent studies by Nadler (1973b) further substantiate these results.

Means and standard deviations for serum constituent levels of canadensis, californiana, dalli and stonei are given in Table 1. As

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[1] A. Bottrell has recently analyzed 32 California bighorn sheep. All have 2N=54 karyotypes.

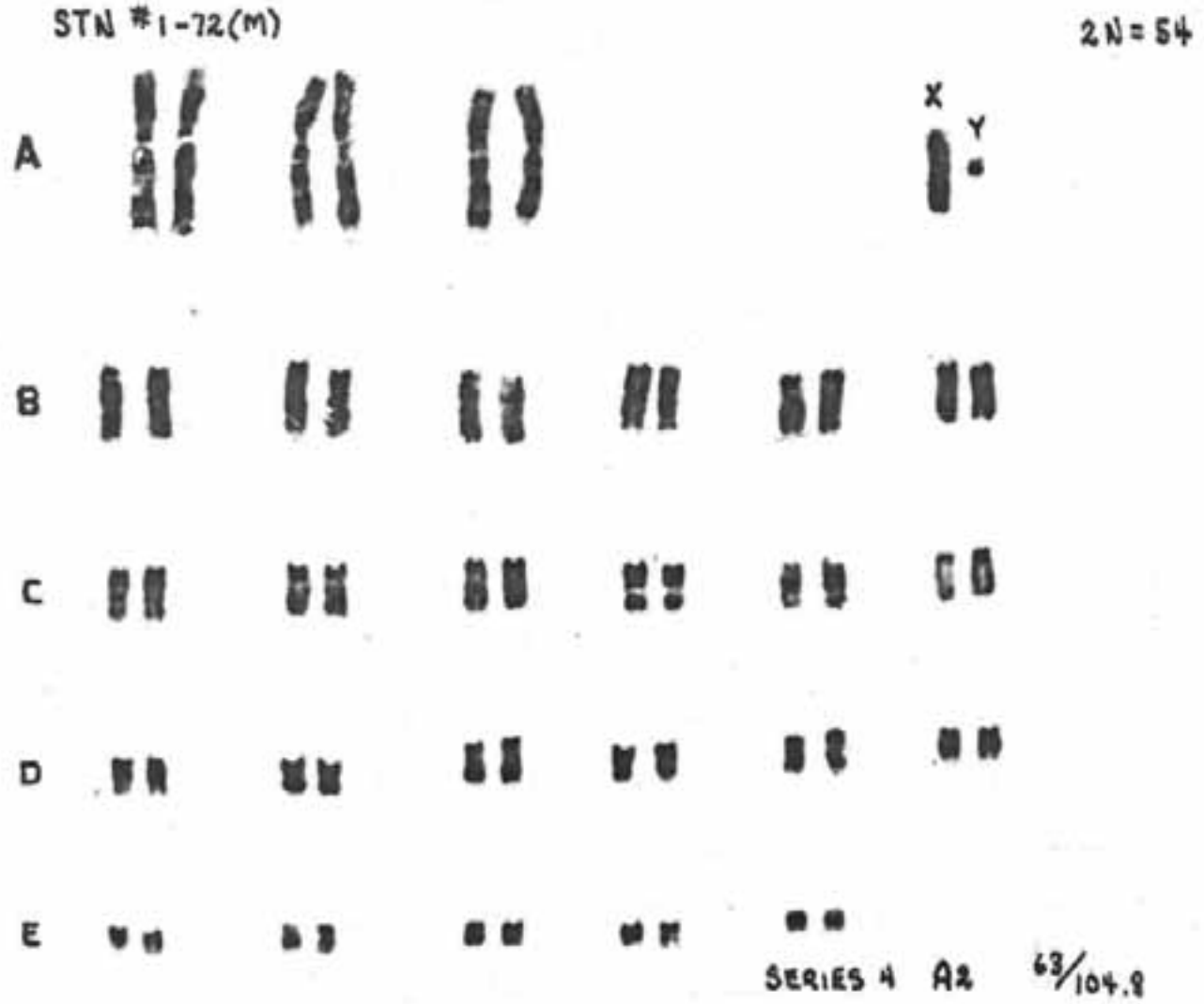


Figure 3. Giemsa-banded karyotype of a Stone thinhorn sheep (male) 2N=54. (Banding pattern presented is representative of all 2N=54 sheep studied).

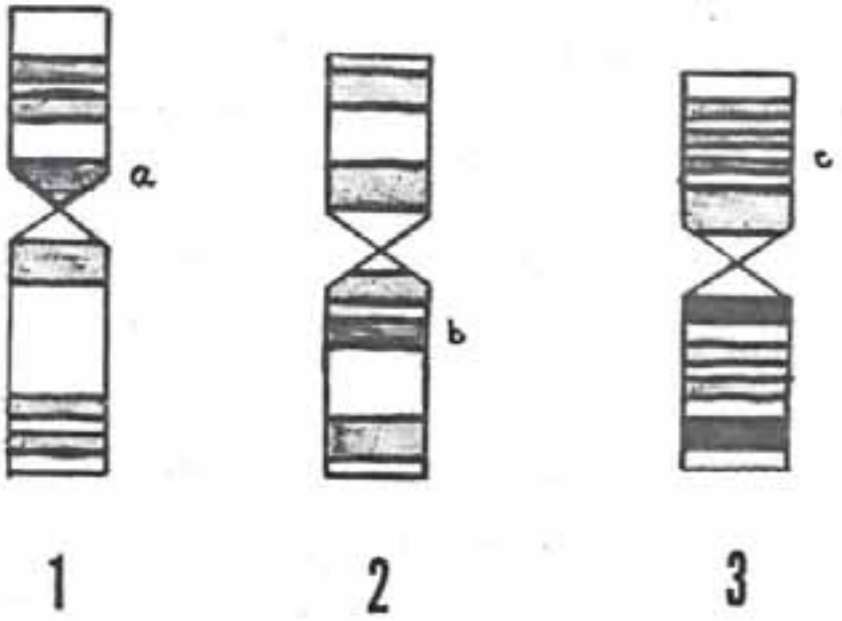


Figure 4. Schematic representation of Group A metacentric chromosomes. The letters a, b and c denote bands not appearing in Nadler's (1973b) ideogram.

mentioned above some investigations of wild sheep blood parameters have been done by Franzmann and Thorne (1970), Franzmann (1971a, 1971b) and Peterson and Bottrell (1978). Mean values for glucose, total protein, albumin, globulin, and sodium obtained here, were in agreement with the findings of these authors. The calcium, phosphorous, blood urea nitrogen (BUN),uric acid, and potassium levels found in this study were higher than those previously reported, while thyroxine and cholesterol values were lower. Many of these differences can probably be accounted for, by differences in age and diet of animals of these studies. The sheep in this study were lambs born into captivity and fed alfalfa hay with supplements; the lambs of the study reported by Peterson were range fed; while in the determinations of Franzmann lambs were often not separated from adults.

Differences in serum constituents were found between and within species of bighorn and thinhorn sheep (Table 2). Glucose albumin and alkaline phosphotase levels were unique for species. Total serum protein and albumin differed for bighorns, and cholesterol levels were higher for Dall than Stone sheep. Noting that animals sampled for this study had been born, and fed and cared for in a similar manner, which would reduce environmental influence, the differences reported here may be indicative of genetic variation between species and subspecies.

Ranges of hematological values for some bighorn and thinhorn sheep are presented in Table 3. Although hemoglobin and packed cell volume (PCV) values appear to be slightly lower than those given by Franzmann and Thorne (1970) and Franzmann (1971a, 1971b), it must be remembered that the wild sheep described in this study were lambs, while the

TABLE 1. Means and standard deviations for serum constituent levels of bighorn and thinhorn sheep

| | | | BIGHORN SHEEP | | THINHORN SHEEP | |
|---|---|---|---|---|---|---|
| | | | Rocky Mountain | California | Dall | Stone |
| Number of Observations | | | 5 | 3 | 3 | 3 |
| Calcium | mg % | Mean | 10.10 | 9.78 | 9.53 | 9.33 |
| | | SD | (0.84) | (0.44) | (0.61) | (1.56) |
| Phosphorus | mg % | Mean | 7.75 | 7.40 | 9.20 | 7.40 |
| | | SD | (3.66) | (1.73) | (1.59) | (3.47) |
| Glucose | mg % | Mean | 140.5 | 129.2 | 92.67 | 90.00 |
| | | SD | (37.19) | (16.75) | (16.17) | (11.3) |
| BUN[1] | mg % | Mean | 37.00 | 33.80 | 38.00 | 35.00 |
| | | SD | (5.29) | (3.18) | (2.00) | (7.94) |
| Uric Acid | mg % | Mean | 0.38 | 0.46 | 0.33 | 0.40 |
| | | SD | (0.17) | (0.05) | (0.12) | (0.10) |
| Cholesterol | mg % | Mean | 51.75 | 38.00 | 53.67 | 29.33 |
| | | SD | (8.73) | (5.14) | (28.04) | (4.93) |
| Total Protein | gm % | Mean | 7.13 | 6.22 | 7.13 | 6.40 |
| | | SD | (0.15) | (0.44) | (1.22) | (0.17) |
| Albumin | gm % | Mean | 3.80 | 3.48 | 3.27 | 3.10 |
| | | SD | (0.28) | (0.33) | (0.58) | (0.45) |
| Globulin | gm % | Mean | 3.32 | 2.74 | 3.86 | 3.30 |
| | | SD | (0.23) | (0.16) | (0.79) | (0.52) |
| Alkaline Phosphatase | u/ml | Mean | 275.7 | 356.2 | 215.3 | 273.7 |
| | | SD | (40.3) | (156.6) | (167.5) | (21.1) |
| Sodium | meq/l | Mean | 152.5 | 150.6 | 151.7 | 148.3 |
| | | SD | (1.9) | (2.1) | (1.5) | (2.9) |
| Potassium | meq/l | Mean | 5.60 | 5.34 | 6.13 | 5.53 |
| | | SD | (0.71) | (0.87) | (0.15) | (1.14) |
| Thyroxine ($T_4$) | mg % | Mean | 4.15 | 4.38 | 5.07 | 2.93 |
| | | SD | (1.36) | (0.66) | (0.91) | (0.25) |

[1] Blood urea nitrogen.

TABLE 2. Means for serum constituent level differences between and within species of bighorn and thinhorn sheep.+

| | | Bighorn (9)@ vs Thinhorn (6) | Rocky Mountain (5) vs California (4) | Dall (3) vs Stone (3) |
|---|---|---|---|---|
| Calcium | mg % | 0.51 | 0.32 | 0.20 |
| Phosphorus | mg % | -0.73 | 0.35 | 1.80 |
| Glucose | mg % | 43.52* | 11.30 | 2.67 |
| BUN | mg % | -1.10 | 3.20 | 3.00 |
| Uric Acid | mg % | 0.06 | -0.08 | -0.07 |
| Cholesterol | mg % | 3.38 | 13.75 | 24.34* |
| Total Protein | gm % | -0.09 | 0.91* | 0.73 |
| Albumin | gm % | 0.46* | 0.32 | 0.17 |
| Globulin | gm % | -0.55* | 0.58* | 0.56 |
| Alkaline phosphatase | u/ml | 121.45* | -80.5 | 41.60 |
| Sodium | meq/l | 1.55 | 1.90 | 3.40 |
| Potassium | meq/l | -0.36 | 0.26 | 0.60 |
| Thyroxin ($T_4$) | mcg/l | 0.27 | -0.23 | 2.14 |

\+ Determined by analysis of variance using orthogonal contrasts from Table 1.
@ Bracketed values designate the number of observations.
* Significant $P \leq 0.05$

TABLE 3. Hematological values of bighorn and thinhorn sheep

| | | BIGHORN SHEEP | | THINHORN SHEEP | |
|---|---|---|---|---|---|
| | | Rocky Mountain | California | Dall | Stone |
| Number of observations | | 3 | 1 | 1 | 3 |
| Hemoglobin | g/100 ml | 14.7 - 14.9 | 16.4 | 16.0 | 12.7 - 17.6 |
| Packed Cell Volume (PCV) | % | 43 - 44 | 47 | 44 | 39 - 49 |
| Total Leukocytes | 1000/ml | 11.0 - 12.9 | 9.4 | 6.0 | 6.6 - 10.8 |
| Lymphocytes | 1000/ml | 28 - 32 | 29 | 28 | 12 - 38 |
| Neutrophils | 1000/ml | 59.0 - 61.7 | 70.0 | 66.0 | 74.0 |
| Basophils | 1000/ml | 0 | 0 | 0 | 0 |
| Monocytes | 1000/ml | 1 - 6 | 0 | 4 | 0 - 2 |
| Eosinophils | 1000/ml | 0 - 2 | 1 | 2 | 0 |

animals of their studies included both adults and lambs. Since lower hemoglobin and PCV values have been noted in very young domestic lambs (Blunt 1975), the values disclosed are probably in accord with those of prior research.

The leukocyte counts obtained in this study were in keeping with those obtained by other workers. However, the count of neutrophils is higher than that published by other authors (Woolf and Kradel 1970, Franzmann 1971a, 1971b), and the number of lymphocytes is lower. Once again it must be emphasized that this project dealt solely with lambs while the previous studies did not. Blunt (1975) has stated that in domestic sheep shortly after birth neutrophils are the dominant white blood cell type and their relative frequency decreases with age. If this is the case for wild sheep the values reported here would appear correct.

Chromosome studies in this report were complimentary to those reported in the literature. The karyotype for Stone sheep (2N=54) is reported here for the first time. One adult California ewe had an atypical karyotype of 2N=55. Species differences in seriological and hematological values between and within bighorn and thinhorn sheep are presented. It should be noted that no attempt is made here to evaluate the utility of these results in determining ovine species differences, or their nutritional status, as this will require a more extensive data base. Rather the hope at this stage is that this may stimulate other workers to collect comparable data from populations and races of sheep inhabiting many different environments.

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