A STARCH-GEL ELECTROPHORETIC STUDY OF SOME OF THE SOURCES OF VARIATION IN THE BLOOD SERA OF DEER OF THE GENUS ODOCOILEUS

by

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ABSTRACT

After standardizing a starch-gel electrophoretic technique, variations in the serum proteins of the genus Odocoileus due to the condition of the sample and the condition of the animal were studied.

Significant changes in the serum sample were brought about by hemolysis, cloudiness, and decomposition. Cold storage for two years of adult deer serum, the addition of a bacteriostat to the sample, and the use of a muscle relaxant to procure samples from captive deer produced no significant changes in either the mobility or the percent composition of the protein fractions.

A large individual variation was found in both the mobility and the percent composition of the protein fractions.

The percent composition of the protein fractions was affected by sex, age, and season. The mobility of the protein fractions was affected by sex, but not by age or season.

Captive deer at the University of British Columbia exhibited an additional negatively migrating protein

fraction when compared to their wild counterparts.

Comparisons of the mobilities in three groups of adult females of the genus Odocoileus indicate greater intra-subspecific differences than inter-specific differences.

The technique of starch-gel electrophoresis, therefore, may be useful in individual and herd recognition but it is not useful in the recognition of subspecies or species of deer of the genus Odocoileus.

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INTRODUCTION

Morphological characteristics have been used since the days of Aristotle to classify organisms and since Darwin's "Origin of Species" to postulate evolutionary relationships. More recently, other factors such as the behavioural, physiological, and biochemical attributes of organisms are also being considered. The present study tries to evaluate whether serum proteins can help to elucidate the phylogeny of the genus Odocoileus.

An excellent review of the underlying philosophy of the application of protein studies to phylogeny has been presented by Sibley (1960). Investigations of the proteins of amphibians and reptiles (McCabe & Deutsch, 1952), birds (Sibley, 1960), and small mammals (Johnson et al, 1958, Johnson & Wicks, 1959) to a large extent substantiate classifications based on morphology.

A variety of techniques for the study of the blood proteins of animals have been elaborated and their applications to taxonomy are outlined below.

Serology

Kraus in 1897 discovered the precipitin reaction

and understood it to be absolutely specific (Boyden, 1943). This reaction was first extensively applied to the problems of animal systematics by Nuttall (1901) and has since been widely employed by several workers. Decapod crustacea have been studied in this respect by Leone (1954). Stallcup's (1954) study of the family Fringillidae is the first application of serology to actual problems in avian taxonomy. Mammals involved in serological investigations include the Orders Rodentia and Lagomorpha (Moody et al, 1949, Moody & Doniger, 1956), Carnivora (Leone & Wiens, 1956, Pauly & Wolfe, 1957), and Cetacea and Artiodactyla (Boyden & Gemeroy, 1950).

Serological studies distinguishing the cervid genera Odocoileus, Axis, and Cervus, were carried out by Wolfe (1939) and Baier & Wolfe (1942). Wolfe (1939) reports that Nuttall (1904) also noted interspecific differences in deer.

Paper Chromatography

Aside from the use of paper chromatography as an analytical tool, its application to taxonomy was not suggested until relatively recent years. Micks (1956) and Micks & Gibson (1957) showed that the basic amino acid

patterns of various insects and ticks were species specific and differed quantitatively. Paper chromatography was used by Buzzati-Traverso & Rechnitzer (1953) to detect specific differences in several morphologically identical species of fish, and by Mainardi (1958) to clarify the phylogeny of some Gallinaceous birds.

Electrophoresis

Tiselius (1937) designed the basic electrophoretic apparatus and called his method "free" electrophoresis. Using horse serum, he compared the protein fractions obtained by salt precipitation with those obtained by electrophoresis and was the first to apply the terms "albumin, alpha-, beta-, and gamma-globulin" respectively to fractions of decreasing electrophoretic mobility. He was also the first to notice quantitative differences in the protein fractions of human, horse, and rabbit. The Tiselius apparatus has been used to investigate a wide variety of animals: invertebrates, fish, amphibians, and reptiles (Deutsch & McShan, 1949); birds (Bain & Deutsch, 1947, Landsteiner et al, 1938); and mammals (Deutsch & Goodloe, 1945, Moore, 1945).

Modifications of the Tiselius apparatus have mainly involved the addition of a supporting medium. In the late

1930's, filter paper provided one of the first supporting media (Wunderly, 1959) and one of its advantages over free electrophoresis is that small samples can be analysed. Paper electrophoresis has been used to investigate the blood proteins of the following taxa: insecta (Stephen, 1961), various small vertebrates (Gleason & Friedberg, 1953), amphibia (Dessauer & Fox, 1956), reptilia (Dessauer & Fox, 1956, Zweig & Crenshaw, 1957), and mammalia (Auernheimer et al, 1960, Bangham, 1957, Johnson & Wicks, 1959, Welling & van Bekkum, 1958). The lens proteins of fish (Smith, 1962) and the egg white proteins of birds (Forsythe & Foster, 1950, McCabe & Deutsch, 1952) have also been studied by this method.

Starch has largely replaced filter paper as a supporting medium because of its greater resolution. Starch-gel electrophoresis has been used to investigate the egg white proteins of birds (Sibley & Johnsgard, 1959b, Sibley, 1960); whole serum of invertebrates (Woods et al, 1958), alligators (Baril et al, 1961), and domestic mammals (Latner & Zaki, 1957); and fractions of whole blood (haemoglobins, haptoglobins, and transferrins) of amphibians (Dessauer et al, 1962, Fox et al, 1961), reptiles (Dessauer et al, 1962), domestic mammals (Ashton, 1957a, 1957b, 1958, 1958a-e, 1959, 1960, Ashton & Fallon, 1962, Ashton & McDougall, 1958, Hickman & Smithies,

1957), mice (Mainardi, 1958, Rosa et al, 1958, Thompson et al, 1954), and humans (Allison et al, 1958, Harris et al, 1958a, 1958b, Parker & Bearn, 1961, Smithies, 1955a, 1955b, 1957, 1958, 1959b, Smithies & Hiller, 1959, Smithies & Walker, 1955b, 1956, Sutton et al, 1956, 1959, 1960).

Lowe & McDougall (1961) used starch-gel electrophoresis to study the serum beta-globulin types in Red Deer (Cervus elaphus).

Other supporting media include agar-gel (Graber & Williams, 1955, Ressler & Jackson, 1955), cellulose acetate (Kohn, 1957), and polyacrylamide gel (Raymond & Weintraub, 1959). Besides this development and utilization of new media, combinations of tried techniques have also been shown to yield useful information. Immunoelectrophoresis which is a combination of serology and starch-gel electrophoresis (Williams & Graber, 1956) has proven to be a valuable tool for the study and characterization of various antigen-antibody systems (Kunkel & Trautman, 1959).

In the present investigation the separation of serum proteins was obtained by utilizing the technique of starch-gel electrophoresis.

MATERIALS AND METHODS

Source and collection date of samples

Odocoileus hemionus columbianus (Richardson) 1829

- captive in Vancouver, British Columbia, Canada.
  Originally from Wolf Lake, Vancouver Island, British Columbia, Canada.
  Collected June, 1961 - October, 1962.
- wild - Wolf Lake, Vancouver Island, British Columbia, Canada.
  Collected December, 1961.
- wild - Chemainus, Vancouver Island, British Columbia, Canada.
  Collected December, 1961.
- wild - Mendocino County, California, U.S.A.
  Collected October, 1962, November, 1962, and January, 1963.

Odocoileus hemionus crooki (Mearns) 1897

- wild - Southern Arizona, U.S.A.
  Collected December, 1962.

Odocoileus hemionus sitkensis Merriam, 1898

- captive in Vancouver, British Columbia, Canada.
  Originally from Petersburg, Alaska, U.S.A.
  Collected February, 1961 - March, 1962.
- wild - Mitkof Island and Kupreanof Island,
  Alaska, U.S.A.
  Collected April, 1963.

Odocoileus virginianus leucurus (Douglas) 1829

- wild - Clatskanie County, Oregon, U.S.A.
  Collected December, 1962.

Odocoileus virginianus texanus (Mearns) 1898

- wild - Sinton, Texas, U.S.A.
  Collected April - May, 1963.

Preparation of the sample

Captive stock at the U.B.C. deer unit was immobilized with succinylcholine chloride (Anectine - Burroughs Wellcome - Canada) as described by Cowan et al (1962). Blood was taken in vacutainer tubes from the recurrent tarsal vein and was allowed to clot for 24 hours at 5°C. After centrifugation for 10 minutes at 3,500 r.p.m., the serum was removed and stored at - 18°C until used for analysis.

Workers at the University of California and Associated Hopland Field Station, Mendocino County, California, are carrying out a large experimental programme involving tagging, taking body measurements, and collecting blood samples from O. h. columbianus (Leopold et al, 1951, Leopold, personal communication, 1964). Sheppard's Keidel Vacuum Tubes were used to draw the blood samples, the sera of which were frozen after removal of the clot and were then sent airmail to U.B.C.

The sample of O. v. leucurus was obtained from the jugular vein within 5 minutes of shooting. The serum separated from the clot after 12 hours at room temperature. It was then pipetted into a clean container containing preservative and 24 hours later it was stored at - 18°C at U.B.C.

For those samples obtained elsewhere the deer were either shot or restrained physically. For these samples an instruction sheet was prepared which appears on the following

page. The equipment sent with these instructions included labelled glass test tubes, glass pipettes, corks wrapped in aluminum foil, labelled cardboard mailing containers, and vermiculite for packing.

All samples were stored at - 18°C upon arrival at U.B.C. For those samples which could not be frozen during shipment to U.B.C., the bacteriostat, propyl p-hydroxy-benzoate, was put in the collecting vials to prevent decomposition.

DIRECTIONS FOR OBTAINING BLOOD SAMPLES FROM DEER

A. If deer killed:

1. slit jugular vein or carotid artery or use any available blood vessels immediately.
2. allow blood to flow into a clean container.
3. let sit at room temperature for 12 - 15 hours.
   a. if separation has occurred, that is if yellowish-coloured fluid is seen distinct from a red semi-solid mass (the clot), then pour this fluid only into another clean container and discard the clot.
   b. if no separation has occurred after 12 - 15 hours, that is if no fluid is seen to be distinct from the clot, then centrifuge at 3,000 r.p.m. (or highest speed on a hand centrifuge) for about 10 minutes. Then pour the fluid into another clean container and discard the clot.
4. complete the label on the container.
5. freeze or refrigerate until mailing.
6. use cardboard mailing tube provided.
7. tape corks into place.
8. air mail.

B. If deer is not to be killed:

1. if possible, immobilize the deer with succinylcholine

chloride before bleeding.

2. bleed from recurrent tarsal vein (most prominent vein in the leg) or from the jugular vein or from an ear vein.

3. use vacutainer needles and tubes if available.

More information or materials regarding the above may be obtained if required.

4. follow directions of A. no. 3 - no. 8.

Powder in tubes is a preservative.

Electrophoretic procedure

Smithies gives the original description of zone electrophoresis in starch-gel in 1955b, an improved procedure in 1959a, and a detailed diagram of the apparatus in 1959b. The procedure used in this study was only slightly modified from his by placing the gel in a horizontal rather than a vertical position during the run and by substituting the suggested buffer trays and electrodes by those designed by the E-C Apparatus Company (538 Walnut Lane, Swarthmore, Pennsylvania, U.S.A.). Wooden presses were designed with which constant pressure could be applied to the setting gels in molds supplied by Otto Hiller (P.O. Box 1294, Madison 1, Wisconsin, U.S.A.).

Briefly the procedure was as follows: All mold pieces except the slot sections were very lightly greased with mineral oil. One litre of starch solution, the concentration depending on lot specifications (Connaught Medical Research Laboratories, University of Toronto, Toronto, Ontario), was continuously heated (to 95°C, Smithies, 1959b) and stirred for a constant 20 minutes (the peak of viscosity occurring at 10 minutes), evacuated for about 5 minutes to remove all air bubbles, and poured into the trays (500 ml. starch solution / tray). The cover was carefully lowered into position and even pressure was applied. The setting gels were then kept at room temperature for about 1 hour and at 10°C until used - usually about 8 hours later.

At the beginning of a run the press and cover were removed and 50 µl of serum were injected into each slot with a Hamilton microsyringe. The slots were sealed with mineral oil and "Saran Wrap" was placed over the gel surface so as to eliminate air bubbles. Number 17 filter papers (6" square) which had just previously been wetted with buffer were placed at each end of the tray which was then placed between the two cooling plates of the E-C Apparatus so as to bring the filter paper into contact with its buffer trays. The cooling system was activated and the current applied.

At the end of the run, the current and the cooling system were disconnected. The filter paper wicks, the "Saran Wrap", and the trough-ends of the gel were discarded. The remaining rectangle of starch-gel was placed upside down into a slicing tray. A flat weight the same size as the starch-gel was placed on top and by slicing in a horizontal direction with a microtome blade two equal halves of the starch-gel were obtained.

Staining followed immediately. The lower half (inner surface uppermost) was stained for protein with Amido Black (500 mg. Amido Black / 90 ml. Methanol / 10 ml. Water) for 5 minutes and was then rinsed with 8 litres of 50 ml. Methanol / 50 ml. Water / 10 ml. Glacial Acetic Acid in a gel washer (Pert et al, 1959) for 13 hours. The inner surface of the upper half was stained for lipid. The results of this will appear in a later publication.

The stained starch-gel was then wrapped in "Saran Wrap" and stored at $5^{o}C$.

In order to standardize the technique, yellow serum from captive, male, adult, O. h. columbianus was run under various conditions as elaborated below. The final method adopted was based on the number of protein bands visible to the naked eye and the distance the furthest band had migrated.

Buffer

Since proteins are amphoteric, the pH of the buffer solution will exert a great influence on their mobility. For maximum migration to occur a pH should be used which is greater than the isoelectric points of the majority of the proteins in the solution to be examined. As suggested by Smithies (1959b), a borate buffer of pH 8.8 was used.

The velocity of migration also depends on the ionic strength of the solution, the velocity decreasing as the ionic strength increases for any given field strength. This decrease is due to the effective charge of the migrating molecule being reduced by ions of opposite charge surrounding it.

Using constant time (12½ hours) and voltage (800 volts) various concentrations of borate buffer of pH 8.8 were used to test the effect of ionic strength ranging from 0.0610 - 0.0745. Among these there was little difference noted in the migration distance of the furthest fraction. However, the buffer concentration, 0.0092 M sodium hydroxide / 0.0230 M boric acid, of ionic strength 0.0667 and pH 8.8 produced the greatest separation of the protein fractions and was therefore selected as the constant buffer concentration.

Attempts made using Tris (hydroxymethyl) aminomethane standard buffer as suggested by Poulik (1957) were not successful.

The electrodes were reversed after each run. It was found that the buffer could be used 4 times before the pH began to alter. Consequently, no more than 4 runs were made before the apparatus was cleaned and new buffer supplied.

A humidity of 45 - 50% was found to prevent condensation on the apparatus and evaporation of the buffer during the run.

Time

Because each protein fraction in the solution will differ in the net number of negative and positive charges per unit area of molecular surface, it will have a specific mobility and will separate from the others. Enough time must be allowed for the fractions to achieve their maximum separation. However, if allowed to run indefinitely, the fastest moving fractions at least, would migrate into the trough region of the gel tray making their analysis impossible.

With the standard borate buffer of ionic strength 0.0667 and pH 8.8, and 900 volts, times from 8 - 18 hours were tested. At 13 hours and longer, a yellow line

appeared on the filter paper wick at the negative electrode suggesting some change occurring in the buffer solution. This line did not appear at 12½ hours or less. Because a greater number of fractions separated with progressively increasing lengths of time, 12½ hours running time was made standard.

Voltage

One advantage in using an extremely high voltage is that maximum separation of the protein fractions will be obtained more quickly than at a lower voltage. In addition to saving time, the application of higher field intensities (20 - 100 volts / cm.) has the advantage of producing sharper separations of the protein fractions since the interfering diffusion of the substances in the supporting medium (starch-gel) is of less significance (Wieland, 1959). A disadvantage, however, lies in the fact that an increase in voltage means an increase in the amount of heat generated during the run. Even though this is partially negated by the low ionic strength of the buffer (Smithies, 1955b), the maintenance of an efficient cooling system becomes necessary.

Using the standard borate buffer of ionic strength 0.0667 and pH 8.8, and a constant time of 12½ hours, voltages from 130 - 900 volts were tested. 900 volts produced

the greatest separation and gave a voltage gradient of approximately 28 volts / cm.

At 900 volts the initial amperage was 20 ma. After 10 minutes this decreased and remained constant at 16 ma.

Temperature

As proteins are thermolabile they will be denatured at high temperatures and migration will cease. However, even before this critical point of denaturation is reached, heat may distort the pattern of electromigration by causing diffusion within the gel. At the high voltage used (900 volts) it was necessary, therefore, to keep the apparatus in a constant temperature room at 10°C. The cooling plates on either side of the gel were constant at 3°C.

Serum aliquot

The volume of each slot in the starch-gel is about 55 µl. Samples of 30, 35, 40, 45, and 50 µl were tested and it was found that with increasing serum volume the protein pattern became more distinct. Therefore, a 50 µl aliquot was taken as the standard volume.

The number of aliquots required per serum sample

Each gel contained eight slots into which the serum

samples were injected. When all the slots of one gel were filled with aliquots from one sample, slots 2 - 7 inclusive produced patterns whose protein fractions were extremely similar in mobility and percent composition. Runs in slots 1 and 8 were obviously inconsistent and were discarded.

This similarity of runs from slots 2 - 7 justifies the use of the minimum of two aliquots (100 µl) from each sample required for statistical analysis. If only two aliquots are available, however, it is preferable to put them into separate gels to allow for slight gel variations (McIntyre, personal communication).

Dialysis of sample

Dialysis serves to remove from the serum virtually all diffusible solutes (that might interfere with migration) other than buffer electrolytes (Longsworth, 1959).

50 µl aliquots were taken from samples dialysed at 10^{o}C from 0 - 86 hours. A few samples were left for longer periods with aliquots taken at various intervals. The buffer was changed every day on each sample.

With no dialysis few protein bands separated and the run was shorter than with samples dialysed 23 - 53 hours. There were no visible differences between samples dialysed

20 - 69 hours. At 86 hours there was some loss in clarity and with successive days of dialysis the runs became progressively poorer.

Therefore, dialysis could be no less than 20 hours and no more than 69. For convenience the samples used for the previously mentioned tests and for those discussed below were dialysed for 27 - 28 hours.

Time between completion of run and staining

After the current was disconnected, 4 minutes were required to remove the two gels from the apparatus and the constant temperature room, slice them, and place them in staining dishes. If the interval between disconnecting the current and staining the gels were extended to 14 minutes, the protein bands were still distinct. If there were a 20 minute interval, however, some diffusion could be observed, i.e. the borders of the bands were indistinct. An interval of one hour caused diffusion great enough to destroy the pattern.

Because diffusion of the protein bands was undesirable, the gels were stained immediately after placing them in the staining dishes - no more than 5 minutes after the current was disconnected.

Time of staining

The time of staining must be short enough to prevent the background gel from becoming permanently impregnated with dye thereby lessening the contrast between the protein bands and yet long enough to stain those fractions present in the least amount.

When the gels were stained for 10 minutes with Amido Black the background did not become white with rinsing and therefore did not provide the greatest contrast between the protein bands. Because 5 minutes staining produced distinct bands and the background rinsed white, this time was made constant.

Time of rinsing

Using a gel washer (Pert et al, 1959) the background rinsed white at 12 hours and there was no visible change in the protein pattern colour if rinsing continued for 18 hours. After this time, however, the pattern appeared lighter. For convenience the rinsing time was made constant at 13 hours.

Analysis of results

Photography

A view camera containing fast Pan film and with a

red filter was used to photograph the gels. The exposure rate was f16 at 1 second by reflected and transmitted light. 8" x 10" film was used. Replicates photographed on the same day and at a later date were found to be identical to the first. Duplicates made on 5" x 7" film later had to be discarded as the densitometer could not resolve the resulting shorter distances between protein bands on the smaller negative. A centimeter ruler was placed alongside each gel to give the scale.

Densitometry

The negative of the whole gel was cut so that the band pattern emanating from each slot could be run individually through a Zeiss densitometer. Black photography paper was cut so as to leave a rectangular space 1 cm. wide x 17 cm. long. The negative was then placed between a piece of glass and this paper. Another sheet of glass was placed on top of this and the resulting sandwich was placed in the densitometer. The light slot in the Zeiss densitometer is 1 mm. wide. With the photography paper a constant area of 1 x 10 mm. was scanned for a distance of 17 cm. at an approximate speed of 1.5 mm. / second.

Thus the starch-gels, by means of photographic negatives and the densitometer were finally resolved into

curves showing the amount of dye taken up by the protein in the gel (Wunderly, 1959). Four identical curves were obtained for each aliquot, three to be used in the percent composition determinations and the fourth to be used for the measurement of migration distances.

Calculations

In order to minimize any background dye a baseline was drawn touching the lowest trough of the curve. To find the relative proportions of the area under each peak, i.e. the relative amounts of protein present, each of the three curves was weighed and then the area under each peak was weighed separately. Averages were calculated and each peak was expressed as a percentage of the average total. Because of the very small amounts present in some cases all of the percentages were then converted to arcsin $\sqrt{\text{percentage}}$ degrees (Snedecor, 1956, pages 318 - 319). Calculations of the means, standard deviations, and standard errors were on the transformed numbers. Student's t-tests were performed in the appropriate circumstances.

To calculate the mobility of each protein fraction a line was drawn perpendicular to the baseline through the centre of each peak of the curve. The distance to each line was then measured along the baseline from the origin.

Because of slight photographic reduction the actual measurement as occurred in the starch-gel was obtained by a proportional conversion of the centimeter scale on the negative to the measured distance on the curve. The following is the calculation of the mobility (Raymond, 1955).

E = field strength

V = voltage

D = length of medium in cm.

d = distance protein has moved in cm.

t = time in seconds

μ = mobility

$E = \frac{V}{D}$, $d = \mu E t$, therefore, $\mu = d \times \frac{D}{V\ t}$ cm^2 / volt seconds.

Mean values, standard deviations, and standard errors were calculated for each mobility and Student's t-tests were performed where required as follows.

$$\text{Standard Deviation (S.D.)} = \sqrt{\frac{\Sigma(x^2) - \frac{(\Sigma x)^2}{N}}{N - 1}}$$

$$\text{Standard Error (S.E.)} = \frac{\text{S.D.}}{\sqrt{N}}$$

$$\text{Student's t-test (t)} = \frac{\bar{x}_A - \bar{x}_B}{\sqrt{(S.E.)_A^2 + (S.E.)_B^2}}$$

The method of statistical analysis was checked by Mr. G. McIntyre (see Acknowledgments) who found that the variation within gels is numerically less than that between gels. The latter variation, in turn, is numerically less than that between individuals. He also found no significant change in the method during the course of the study.

RESULTS AND DISCUSSION

CONDITION OF SAMPLE

Hemolysis and cloudiness

Deer serum is normally a dark yellow colour. As Haugen (1960) found no carotenoids in the blood serum of nineteen O. virginianus any reddish colouration visible to the naked eye was assumed to be an indication of hemolysis. The appearance of cloudiness in some samples is probably due to lipids (Cantarow & Schepartz, 1957).

All samples were arbitrarily classified as yellow, yellow but cloudy, slightly hemolysed, hemolysed, and very hemolysed. Those samples used to test the effect of hemolysis and cloudiness were run within one year of sampling.

The term "hemolysis" implies that haemoglobin has been released from the blood cells into the serum. This additional protein in the serum would be expected to have some effect on the mobility and/or the percent composition of the protein fractions as separated by electrophoresis.

Very hemolysed samples usually varied the electrophoretic pattern by either an apparent increase or decrease in the number of protein fractions as compared to the

number resolved in yellow, slightly hemolysed, and hemolysed samples. The t-test comparisons of slightly hemolysed and hemolysed vs. yellow samples (Tables 2 & 4) show no significant differences in either the mobility or the percent composition of the protein fractions. Closer examination of Tables 1 & 3, however, reveals that over 50% of the standard errors of the slightly hemolysed and hemolysed samples are greater than the corresponding standard errors of the yellow samples.

This indication of greater variability with hemolysis and the change in fraction number in very hemolysed samples compels the elimination of all categories of hemolysed samples from further comparisons.

Lipids are carried by the alpha- and beta-globulins (Moore, 1959). If cloudiness is an indication of excess lipid in the serum then some effect on the electrophoretic protein pattern might be expected. A few cloudy samples did show an apparent decrease in the number of protein fractions as compared to those resolved in yellow samples. In O. v. texanus (Table 2) cloudiness caused a significant increase in the mobility of fraction +7 in the comparison of yellow vs. yellow but cloudy samples. In the same

comparison (Table 4) the percent composition of fraction +1 increased significantly.

Examination of Tables 1 & 3 reveals that over 50% of the standard errors for the mobility and that 100% of the standard errors for the percent composition of the protein fractions of cloudy samples are greater than those of corresponding yellow samples.

The decrease in fraction number in some cloudy samples, the significant differences in mobility and percent composition, and the greater variability in the standard errors of cloudy samples warrant eliminating them from further comparisons.

TABLE 1

EFFECT OF HEMOLYSIS - mobilities in 10^{-5} cm^2 / volt seconds

FRACTION		10	9	8	7	6	5	4	3	2	+ 1	- 1	2
wild, female, adult, *O. v. texanus*, Texas													
yellow	$\bar{X}$	.829	.464	.406	.371	.327	.254	.190	.158	.079	.015	.011	.143
N = 8	± S.E.	.008	.016	.016	.013	.011	.013	.013	.003	.005	.002	.001	.015
yellow + cloudy	$\bar{X}$	.772	.515	.444	.416	.380	.277	.210	.185	.105	.010	.017	.111
N = 2	± S.E.	.124	.018	.000	.000	.013	.003	.006	.018	.018	.001	.007	.044
slightly hemolysed	$\bar{X}$	.813	.437	.374	.364	.329	.258	.187	.165	.065	.010	.013	.117
N = 2	± S.E.	.013	.011	.006	.002	.013	.003	.000	.004	.016	.000	.002	.008
wild, male, adult, *O. h. columbianus*, Chemainus, B.C.													
yellow	$\bar{X}$		.829	.494	.423	.383	.313	.260	.165	.133	.022	.012	
N = 2	± S.E.		.013	.008	.008	.005	.011	.012	.005	.011	.012	.003	
hemolysed	$\bar{X}$		.815	.480	.400	.370	.296	.231	.142	.071	.027	.011	
N = 3	± S.E.		.018	.014	.011	.012	.011	.019	.012	.020	.010	.002	

N = number of animals

TABLE 2

EFFECT OF HEMOLYSIS - Student's t-test comparisons of the mobilities of the serum protein fractions.

FRACTION	10	9	8	7	6	5	4	3	2	+1	-1	2
wild, female, adult, *O. v. texanus*, Texas												
yellow vs. slightly hemolysed	=	=	=	=	=	=	=	=	=	=	=	=
yellow vs. yellow + cloudy	=	=	=	++	=	=	=	=	=	=	=	=
wild, male, adult, *O. h. columbianus*, Chemainus, B.C.												
yellow vs. hemolysed		=	=	=	=	=	=	=	=	=		

= no significant difference between the two samples.
++ second item significantly faster than the first item to the .01 level.

TABLE 3

EFFECT OF HEMOLYSIS - percent composition in arcs $\widehat{\text{in}}\sqrt{\text{percentage}}$ degrees.

FRACTION		10	9	8	7	6	5	4	3	2	+1	-1	2
wild, female, adult, *O. v. texanus*, Texas													
yellow N = 8	$\bar{X}$	38.5	16.2	13.1	12.9	10.3	7.3	9.8	11.6	13.7	13.1	15.1	21.1
	± S.E.	0.9	1.0	0.6	0.9	1.5	0.6	0.8	0.5	1.1	0.5	1.2	1.2
yellow + cloudy N = 2	$\bar{X}$	33.1	18.4	14.7	12.5	6.9	9.5	9.5	12.6	16.6	17.6	16.7	17.4
	± S.E.	3.4	3.4	3.9	1.7	2.6	1.5	1.5	1.4	2.4	1.0	5.0	5.2
slightly hemolysed N = 2	$\bar{X}$	35.6	14.0	15.0	13.7	12.5	9.4	8.0	8.4	9.4	11.4	13.9	22.7
	± S.E.	2.2	4.0	0.4	0.3	0.7	1.0	0.6	1.1	2.0	0.6	1.5	8.7
wild, male, adult, *O. h. Columbianus*, Chemainus, B.C.													
yellow N = 2	$\bar{X}$		44.6	19.2	14.2	7.4	6.9	7.2	10.4	11.3	18.0	19.9	
	± S.E.		0.1	2.4	0.5	1.4	0.8	1.8	0.1	1.8	3.2	0.6	
hemolysed N = 3	$\bar{X}$		41.0	18.2	13.8	12.2	11.3	8.8	10.1	13.9	15.9	20.4	
	± S.E.		2.4	1.6	2.1	1.2	1.7	2.1	0.9	0.4	3.6	4.1	

N = number of animals

TABLE 4

EFFECT OF HEMOLYSIS - Student's t-test comparisons of the percentage composition (in arcs $\sqrt{\text{percentage}}$ degrees) of the serum protein fractions.

FRACTION	10	9	8	7	6	5	4	3	2	+1	-1	2
wild, female, adult, *O. v. texanus*, Texas												
yellow vs. slightly hemolysed	=	=	=	=	=	=	=	=	=	=	=	=
yellow vs. yellow + cloudy	=	=	=	=	=	=	=	=	=	++	=	=
wild, male, adult, *O. h. columbianus*, Chemainus, B.C.												
yellow vs. hemolysed		=	=	=	=	=	=	=	=	=	=	

= no significant difference between the two samples.
++ second item significantly greater than the first item to the .01 level.

Preservation

Serum samples were arriving at U.B.C. that were obviously decomposing. A NIPA ester (propyl p-hydroxybenzoate), because of its bacteriostatic action (Merck, 1960), was tried and found to be successful in preventing decomposition. It became important, therefore, to determine whether this ester had any effect on the mobility or the percent composition of the serum proteins as separated by electrophoresis.

A serum sample from a wild, female, adult, O. h. columbianus - California, was divided into two samples with and two samples without the NIPA ester upon arrival at U.B.C. They were then all kept at room temperature. The addition of the preservative on the day of arrival did not significantly alter either the mobility or the percent composition of the protein fractions and after 16 days at room temperature there were still no changes in the sample containing the preservative. However, after 16 days at room temperature without the preservative, fraction +9 was lost and fraction +10 exhibited a significantly faster mobility (Table 10). There were no significant changes in the mobilities of the remaining fractions. The percent composition of all comparable fractions also did not change (Table 12).

Bacterial decomposition of proteins can also be prevented by freezing. Previous work on deer serum utilizing paper electrophoresis (Johnston, B.Sc. Thesis, 1961) had shown that serum from a captive adult, O. v. ochrourus, could be stored at - 18°C for at least 5½ months with no significant changes in the percent composition of the serum protein fractions, while serum from a captive fawn, O. h. columbianus - Vancouver Island, changed significantly after three weeks storage.

In order to test the effect of storage at - 18°C in the present study, aliquots were taken at various time intervals from a captive, female, adult, O. h. columbianus - Wolf Lake (Vancouver Island), B.C. Storage times begin with the arrival of the serum at U.B.C. and its subsequent storage at - 18°C. It was found that these samples could be stored as long as 761 days with no significant changes occurring in either the mobility (Tables 5 & 6) or the percent composition (Tables 7 & 8) of the protein fractions. As fawn serum was not examined for cold storage effects, only those fawn samples stored less than three weeks were used in further analyses.

The effect of freezing on the bacteriostat was tested

by dividing a serum sample from a wild, female, fawn, O. h. columbianus - California, into one sample with and one without the NIPA ester. These samples were stored for 58 days at - 18°C. No significant differences in either mobility (Tables 9 & 10) or the percent composition (Tables 11 & 12) of the protein fractions were found.

In summary, the bacteriostat, propyl p-hydroxybenzoate, was found necessary to prevent decomposition during storage at room temperature. Neither the NIPA ester nor freezing had any effect on the mobility or the percent composition of the protein fractions.

TABLE 5

EFFECT OF COLD STORAGE - mobilities in 10^{-5} cm^2 / volt seconds.

FRACTION		9	8	7	6	5	4	3	2	+1	-1	2
captive, female, adult, *O. h. columbianus*, Wolf Lake, B.C.												
2 days	$\bar{X}$	.913	.519	.471	.442	.403	.300	.240	.164	.019	.019	.087
N = 2	± S.E.	.023	.023	.023	.000	.033	.023	.002	.000	.001	.005	.020
221 days	$\bar{X}$	.908	.512	.454	.438	.375	.296	.245	.169	.016	.010	.095
N = 2	± S.E.	.073	.005	.021	.026	.016	.005	.005	.005	.002	.005	.001
304 days	$\bar{X}$	.947	.515	.471	.442	.409	.305	.260	.164	.019	.019	.087
N = 2	± S.E.	.033	.004	.000	.000	.005	.000	.020	.011	.000	.000	.005
761 days	$\bar{X}$	.924	.518	.464	.432	.394	.295	.238	.160	.018	.013	.086
N = 5	± S.E.	.029	.010	.008	.010	.009	.018	.018	.022	.004	.005	.005

N = number of aliquots

TABLE 6

EFFECT OF COLD STORAGE - Student's t-test comparisons of the mobilities of the serum protein fractions.

FRACTION	9	8	7	6	5	4	3	2	+1	-1	2
captive, female, adult, *O. h. columbianus*, Wolf Lake, B.C.											
2 days vs. 221 days	=	=	=	=	=	=	=	=	=	=	=
vs. 304 days	=	=	=	=	=	=	=	=	=	=	=
vs. 761 days	=	=	=	=	=	=	=	=	=	=	=
221 days vs. 304 days	=	=	=	=	=	=	=	=	=	=	=
vs. 761 days	=	=	=	=	=	=	=	=	=	=	=
304 days vs. 761 days	=	=	=	=	=	=	=	=	=	=	=

= no significant difference between the two samples.

TABLE 7

EFFECT OF COLD STORAGE - percent composition in arcs$\widehat{\ }$in $\sqrt{\text{percentage}}$ degrees.

FRACTION		9	8	7	6	5	4	3	2	+1	-1	2
captive, female, adult, *O. h. columbianus*, Wolf Lake, B.C.												
2 days	$\bar{X}$	31.5	11.5	14.4	9.5	6.5	9.8	10.9	20.5	21.7	18.5	20.4
N = 2	± S.E.	1.3	0.6	1.5	0.7	0.5	1.3	1.1	0.8	1.0	1.0	0.8
221 days	$\bar{X}$	31.9	13.2	15.9	7.9	8.5	10.4	10.8	18.0	19.9	18.6	22.6
N = 2	± S.E.	0.2	2.9	0.5	0.2	0.2	0.9	0.6	0.3	1.4	0.9	2.0
304 days	$\bar{X}$	30.1	12.3	12.2	10.6	8.1	10.5	12.3	21.8	22.3	19.6	21.3
N = 2	± S.E.	1.2	0.9	2.6	2.4	2.0	0.2	0.2	1.7	1.4	1.4	0.2
761 days	$\bar{X}$	32.4	12.7	14.3	9.4	7.3	9.9	11.0	19.7	20.6	18.8	22.3
N = 5	± S.E.	1.4	1.0	1.2	1.0	1.0	0.6	0.6	0.9	1.3	0.5	0.8

N = number of aliquots

TABLE 8

EFFECT OF COLD STORAGE - Student's t-test comparisons of the percent composition (in arcs $\sqrt{\text{percentage}}$ degrees) of the serum protein fractions.

FRACTION		9	8	7	6	5	4	3	2	+1	-1	2
captive, female, adult, *O. h. columbianus*, Wolf Lake, B.C.												
2 days	vs. 221 days	=	=	=	=	=	=	=	=	=	=	=
	vs. 304 days	=	=	=	=	=	=	=	=	=	=	=
	vs. 761 days	=	=	=	=	=	=	=	=	=	=	=
221 days	vs. 304 days	=	=	=	=	=	=	=	=	=	=	=
	vs. 761 days	=	=	=	=	=	=	=	=	=	=	=
304 days	vs. 761 days	=	=	=	=	=	=	=	=	=	=	=

= no significant difference between the two samples.

TABLE 9

EFFECT OF A BLOOD PRESERVATIVE - mobilities in 10^{-5} cm^2 / volt seconds.

FRACTION		10	9	8	7	6	5	4	3	2	+1	-1
wild, female, adult, *O. h. columbianus*, California												
room temperature, 1 day storage												
no preservative	$\bar{X}$	1.060	.557	.461	.457	.433	.347	.300	.203	.130	.017	.012
N = 2	±S.E.	.018	.023	.023	.013	.018	.005	.005	.023	.000	.005	.003
plus preservative	$\bar{X}$	1.062	.570	.480	.457	.423	.345	.294	.208	.130	.009	.009
N = 2	±S.E.	.011	.003	.007	.001	.018	.001	.018	.005	.003	.003	.001
room temperature, 16 days storage												
no preservative	$\bar{X}$	.913		.466	.423	.401	.331	.234	.214	.145	.036	.010
N = 4	±S.E.	.028		.016	.016	.036	.048	.036	.036	.032	.023	.000
plus preservative	$\bar{X}$	1.063	.569	.489	.459	.433	.345	.305	.208	.120	.010	.011
N = 3	±S.E.	.007	.013	.005	.003	.008	.011	.003	.005	.018	.001	.001
wild, female, fawn, *O. h. columbianus*, California												
- 18° C., 58 days storage												
no preservative	$\bar{X}$	.866	.491	.419	.390	.347	.258	.184	.170	.097	.014	.010
N = 2	±S.E.	.018	.004	.015	.018	.013	.001	.004	.001	.028	.009	.000
plus preservative	$\bar{X}$	.871	.496	.418	.394	.347	.260	.183	.173	.068	.014	.010
N = 2	±S.E.	.004	.009	.005	.000	.000	.001	.004	.004	.009	.005	.000

N = number of aliquots

TABLE 10

EFFECT OF A BLOOD PRESERVATIVE - Student's t-test comparisons of the mobilities of the serum protein fractions.

FRACTION	10	9	8	7	6	5	4	3	2	+1	-1
wild, female, adult, *O. h. columbianus*, California											
room temperature, 1 day storage no preservative vs. plus preservative	=	=	=	=	=	=	=	=	=	=	=
room temperature, 16 days storage no preservative vs. plus preservative	++	0	=	=	=	=	=	=	=	=	=
room temperature, no preservative 1 day vs. 16 days storage	--	0	=	=	=	=	=	=	=	=	=
room temperature, plus preservative 1 day vs. 16 days storage	=	=	=	=	=	=	=	=	=	=	=
wild, female, fawn, *O. h. columbianus*, California											
- 18°C., 58 days storage, no preservative vs. plus preservative	=	=	=	=	=	=	=	=	=	=	=

= no significant difference between the two samples.
++ second item significantly faster than the first item to the .01 level.
-- second item significantly slower than the first item to the .01 level.
0 protein fraction present in one sample but not in the other.

TABLE 11

EFFECT OF A BLOOD PRESERVATIVE - percent composition in arcs⌒in $\sqrt{\text{percentage}}$ degrees.

FRACTION		10	9	8	7	6	5	4	3	2	+ 1	- 1
wild, female, adult, O. h. columbianus, California												
room temperature, 1 day storage												
no preservative	$\bar{X}$	38.7	17.2	10.9	11.5	10.0	14.4	13.2	13.0	18.6	12.6	19.5
N = 2	± S.E.	1.1	0.9	0.6	0.0	0.9	0.6	1.4	0.0	1.1	1.1	1.4
plus preservative	$\bar{X}$	35.9	17.6	8.7	12.0	11.0	12.2	12.1	12.0	18.0	10.2	19.0
N = 2	± S.E.	0.5	1.9	0.3	0.6	0.0	0.6	0.4	0.9	0.9	0.6	0.0
room temperature, 16 days storage												
no preservative	$\bar{X}$	36.2		10.3	11.7	10.0	15.0	12.4	10.4	23.6	15.1	23.9
N = 3	± S.E.	1.5		0.8	1.9	0.2	3.6	0.6	0.5	1.4	0.4	0.9
plus preservative	$\bar{X}$	36.9	18.3	9.5	12.3	10.2	13.0	13.0	14.5	19.0	11.9	20.7
N = 2	± S.E.	0.1	0.0	0.1	1.2	1.1	0.1	0.6	2.1	0.0	1.9	1.5
wild, female, fawn, O. h. columbianus, California												
- 18°C., 58 days storage												
no preservative	$\bar{X}$	41.5	17.9	9.2	15.7	6.8	7.1	9.9	8.1	19.5	12.2	22.6
N = 2	± S.E.	1.4	0.0	0.6	1.2	3.4	1.5	0.0	0.6	2.0	1.6	0.6
plus preservative	$\bar{X}$	40.9	18.6	10.5	12.5	7.9	5.6	8.1	8.9	20.7	10.7	20.8
N = 2	± S.E.	2.4	0.6	0.0	0.0	0.2	0.0	1.1	0.9	0.7	2.1	0.0

N = number of aliquots

TABLE 12

EFFECT OF A BLOOD PRESERVATIVE - Student's t-test comparisons of the percent composition (in arcs-in$\sqrt{\text{percentage}}$ degrees) of the serum protein fractions.

FRACTION	10	9	8	7	6	5	4	3	2	+ 1	- 1
wild, female, adult, *O. h. columbianus*, California											
room temperature, 1 day storage no preservative vs. plus preservative	=	=	=	=	=	=	=	=	=	=	=
room temperature, 16 days storage no preservative vs. plus preservative	=	0	=	=	=	=	=	=	=	=	=
room temperature, no preservative 1 day vs. 16 days storage	=	0	=	=	=	=	=	=	=	=	=
room temperature, plus preservative 1 day vs. 16 days storage	=	=	=	=	=	=	=	=	=	=	=
wild, female, fawn, *O. h. columbianus*, California											
- 18° C., 58 days storage no preservative, vs. plus preservative	=	=	=	=	=	=	=	=	=	=	=

= no significant difference between the two samples.
0 protein fraction present in one sample but not in the other.

Succinylcholine chloride

According to Craighead et al (1960), succinylcholine chloride is a skeletal muscle relaxant that acts by replacing acetylcholine at the motor end plates thereby blocking nervous transmission at the myoneural junction. Muscular paralysis persists until the diacetylcholine is hydrolysed by cholinesterase to succinic acid and choline. Effects on the cardiovascular system are minimal (Goodman & Gelman, 1956).

In the deer unit at the University of British Columbia, it was possible to obtain blood samples from the deer by throwing and holding them. However, the struggle was undesirable and the technique of immobilizing the deer with succinylcholine chloride (Anectine - Burroughs Wellcome - Canada) was used (Cowan et al, 1962, Pearson et al, 1963).

Serum samples 7 days apart were taken from a captive, adult, male, O. h. columbianus - Wolf Lake, Vancouver Island, B.C., with and without the help of succinylcholine chloride. These samples when compared showed no significant differences in either the mobility (Tables 13 & 15) or the percent composition (Tables 14 & 16) of the protein fractions.

Therefore, serum samples obtained from U.B.C. captive deer with the help of succinylcholine chloride can be used in further comparisons.

TABLE--13

EFFECT OF SUCCINYLCHOLINE CHLORIDE - mobilities in 10^{-5} cm^2 / volt seconds.

FRACTION	9	8	7	6	5	4	3	2	+1	-1	-2
captive, male, adult, *O. h. columbianus*, Wolf Lake, B.C.											
without aid of succinylcholine chloride N = 2											
$\bar{X}$	.854	.654	.582	.458	.425	.372	.315	.134	.043	.015	.083
± S.E.	.005	.004	.000	.000	.004	.000	.000	.000	.005	.004	.003
with aid of succinylcholine chloride N = 2											
$\bar{X}$	.856	.659	.568	.472	.439	.377	.310	.123	.053	.019	.086
± S.E.	.005	.009	.016	.005	.009	.005	.005	.004	.004	.000	.000

N = number of aliquots

TABLE 14

EFFECT OF SUCCINYLCHOLINE CHLORIDE - Student's t-test comparisons of the mobilities of the serum protein fractions.

FRACTION	9	8	7	6	5	4	3	2	+1	-1	2
captive, male, adult, *O. h. columbianus*, Wolf Lake, B.C.											
with vs. without aid of succinylcholine chloride	=	=	=	=	=	=	=	=	=	=	=

= no significant difference between the two samples.

TABLE 15

EFFECT OF SUCCINYLCHOLINE CHLORIDE - percent composition in arcs $\widehat{\sqrt{\text{percentage}}}$ degrees.

FRACTION		9	8	7	6	5	4	3	2	+1	-1	2
captive, male, adult, *O. h. columbianus*, Wolf Lake, B.C.												
without aid of succinylcholine chloride N = 2	$\bar{X}$	39.9	9.5	12.0	17.3	12.9	6.5	8.3	15.8	21.6	8.0	20.0
	± S.E.	2.0	0.0	0.2	0.5	0.4	0.4	0.3	0.6	0.6	0.4	0.6
with aid of succinylcholine chloride N = 2	$\bar{X}$	37.2	8.8	12.1	19.2	14.9	7.0	8.5	15.3	24.3	9.0	18.0
	± S.E.	0.1	0.2	0.2	0.0	0.3	1.1	0.2	0.1	0.1	0.4	0.2

N = number of aliquots

TABLE 16

EFFECT OF SUCCINYLCHOLINE CHLORIDE - Student's t-test comparisons of the percent composition (in arcs~in $\sqrt{\text{percentage}}$ degrees) of the serum protein fractions.

FRACTION	9	8	7	6	5	4	3	2	+1	-1	2
captive, male, adult, *O. h. columbianus*, Wolf Lake, B.C.											
with vs. without aid of succinylcholine chloride	=	=	=	=	=	=	=	=	=	=	=

= no significant difference between the two samples.

CONDITION OF ANIMAL

Individual variation

Serum samples from eight wild, female, adult, O. v. texanus were obtained from Sinton, Texas from April 19 - May 3, 1963. These yellow samples were stored for slightly more than one year. The numerical data for the mobility and for the percent composition of the protein fractions are given in Tables 17 & 19 respectively.

Individual variation is found in all parameters used in taxonomy. That there is also a very great individual variation in the serum proteins is readily seen in Tables 18 & 20 where the t-test comparisons for mobility and percent composition respectively are given. Mobility appears to be a more variable parameter than percent composition.

Each individual animal has its own particular immune history which will have an effect on the gamma-globulins and possibly also on the alpha- and beta-globulins (see section on Age). This immune response must account for some of the variability between individuals which is shown here in the majority of the protein fractions. That fraction +10 may be albumin (as it is the fraction of greatest mobility) is supported by its stability in mobility.

There is no obvious explanation as to why the mobilities of fraction +1 and +2 and the percent compositions of

fractions +1 and +3 do not vary significantly between individuals. The effect of individual variation has been accounted for in further comparisons by using groups of at least two individuals.

TABLE 17

INDIVIDUAL VARIATION - mobilities in 10^{-5} cm^2 / volt seconds.

FRACTION		10	9	8	7	6	5	4	3	2	+1	-1	2
wild, female, adult, *O. v. texanus*, Texas													
D 210	$\bar{X}$	.850	.510	.439	.401	.338	.256	.211	.175	.077	.011	.011	.143
N = 4	± S.E.	.036	.011	.007	.000	.012	.017	.004	.009	.009	.001	.001	.010
D 237	$\bar{X}$	.863	.481	.435	.359	.330	.254	.197	.155	.092	.026	.010	.190
N = 2	± S.E.	.057	.004	.001	.010	.000	.011	.006	.000	.023	.016	.000	.000
D 242	$\bar{X}$	.804	.412	.349	.334	.291	.222	.164	.154	.064	.016	.010	.154
N = 2	± S.E.	.000	.021	.010	.006	.006	.000	.006	.006	.011	.006	.000	.006
D 243	$\bar{X}$	.809	.391	.339	.317	.285	.217	.159	.148	.084	.010	.016	.164
N = 2	± S.E.	.005	.000	.000	.000	.000	.006	.000	.000	.000	.000	.006	.006
D 247	$\bar{X}$	.821	.439	.381	.352	.321	.259	.177	.154	.060	.014	.010	.111
N = 4	± S.E.	.012	.013	.009	.009	.005	.016	.011	.000	.006	.005	.000	.020
D 249	$\bar{X}$	.803	.509	.469	.436	.392	.339	.275	.169	.097	.011	.009	.064
N = 5	± S.E.	.007	.004	.002	.005	.003	.004	.009	.009	.022	.002	.000	.003
D 250	$\bar{X}$	.841	.469	.407	.379	.332	.254	.161	.149	.072	.010	.010	.128
N = 4	± S.E.	.032	.021	.012	.012	.002	.012	.001	.002	.007	.000	.000	.030

N = number of aliquots

TABLE 18

INDIVIDUAL VARIATION - Student's t-test comparisons of the mobilities of the serum protein fractions.

FRACTION	10	9	8	7	6	5	4	3	2	$\overset{+}{1}$	$\overset{-}{1}$	2
wild, female, adult, *O. v. texanus*, Texas												
D 210 vs. D 237	=	=	=	--	=	=	=	=	=	=	=	++
vs. D 242	=	--	--	--	=	=	--	=	=	=	=	=
vs. D 243	=	--	--	--	--	=	--	=	=	=	=	=
vs. D 247	=	--	--	--	=	=	=	=	=	=	=	=
vs. D 249	=	=	++	++	++	++	++	=	=	=	=	--
vs. D 250	=	=	=	=	=	=	--	=	=	=	=	=
D 237 vs. D 242	=	=	--	=	--	=	=	=	=	=	=	--
vs. D 243	=	--	--	=	--	=	--	--	=	=	=	=
vs. D 247	=	=	--	=	=	=	=	--	=	=	=	=
vs. D 249	=	++	++	++	++	++	++	=	=	=	--	--
vs. D 250	=	=	=	=	=	=	--	=	=	=	=	=
D 242 vs. D 243	=	=	=	=	=	=	=	=	=	=	=	=
vs. D 247	=	=	=	=	=	=	=	=	=	=	=	=
vs. D 249	=	++	++	++	++	++	++	=	=	=	--	--
vs. D 250	=	=	=	=	++	=	=	=	=	=	=	=
D 243 vs. D 247	=	=	++	=	++	=	=	++	=	=	=	=
vs. D 249	=	++	++	++	++	++	++	=	=	=	=	--
vs. D 250	=	=	++	++	++	=	=	=	=	=	=	=
D 247 vs. D 249	=	++	++	++	++	++	++	=	=	=	--	=
vs. D 250	=	=	=	=	=	=	=	=	=	=	=	=
D 249 vs. D 250	=	=	--	--	--	--	--	=	=	=	++	=

= no significant difference between the two samples.
++ second item significantly faster than the first item to the .01 level.
-- second item significantly slower than the first item to the .01 level.

TABLE 19

INDIVIDUAL VARIATION - percent composition in arcsin $\sqrt{\text{percentage}}$ degrees.

FRACTION		10	9	8	7	6	5	4	3	2	+1	-1	2
wild, female, adult, *O. v. texanus*, Texas													
D 210 N = 4	$\bar{X}$	36.3	17.8	14.9	10.6	8.0	5.3	9.2	9.9	13.3	13.5	18.7	23.0
	± S.E.	0.2	0.9	0.7	0.7	1.3	1.1	0.8	1.1	1.4	1.0	1.6	1.4
D 237 N = 3	$\bar{X}$	42.7	17.6	15.4	15.1	6.9	8.1	11.6	11.8	16.7	12.3	14.7	20.0
	± S.E.	1.1	1.7	0.6	1.1	0.6	1.6	1.0	0.7	0.4	1.5	0.8	0.6
D 242 N = 2	$\bar{X}$	37.6	12.5	11.3	11.0	4.1	7.1	10.8	10.6	16.9	13.3	21.1	22.6
	± S.E.	0.1	2.1	0.3	0.0	2.0	1.7	0.1	0.6	0.9	0.1	0.3	0.8
D 243 N = 2	$\bar{X}$	39.6	12.2	13.5	11.6	10.7	10.3	10.3	11.5	10.8	12.7	15.8	23.0
	± S.E.	1.9	1.4	0.4	1.1	0.2	0.0	0.2	0.0	0.9	1.8	3.2	4.6
D 247 N = 3	$\bar{X}$	37.3	17.9	13.1	15.5	10.3	5.6	11.4	10.7	11.8	10.4	12.5	24.0
	± S.E.	0.6	2.1	1.0	0.3	0.4	0.7	0.8	0.2	0.5	2.1	1.8	2.0
D 249 N = 5	$\bar{X}$	36.6	19.9	14.0	12.0	18.1	8.7	5.7	13.1	11.5	15.3	14.0	14.6
	± S.E.	0.5	0.3	1.0	0.6	1.4	0.5	0.9	2.6	1.2	1.7	0.5	0.5
D 250 N = 4	$\bar{X}$	42.0	17.5	10.6	10.5	10.9	5.0	7.4	10.5	10.4	12.9	12.9	23.5
	± S.E.	0.3	1.2	1.4	0.6	1.4	0.3	0.9	1.7	1.6	0.8	0.9	0.8

N = number of aliquots

TABLE 20

INDIVIDUAL VARIATION - Student's t-test comparisons of the percent composition (in arcs ⌒in $\sqrt{\text{percentage}}$ degrees) of the serum protein fractions.

FRACTION	10	9	8	7	6	5	4	3	2	+1	-1	2
wild, female, adult, *O. v. texanus*, Texas												
D 210 vs. D 237	++	=	=	=	=	=	=	=	=	=	=	=
vs. D 242	++	=	--	=	=	=	=	=	=	=	=	=
vs. D 243	=	=	=	=	=	++	=	=	=	=	=	=
vs. D 247	=	=	=	++	=	=	=	=	=	=	=	=
vs. D 249	=	=	=	=	++	=	=	=	=	=	=	--
vs. D 250	++	=	=	=	=	=	=	=	=	=	=	=
D 237 vs. D 242	--	=	--	=	=	=	=	=	=	=	++	=
vs. D 243	=	=	=	=	++	=	=	=	--	=	=	=
vs. D 247	--	=	=	=	++	=	=	=	--	=	=	=
vs. D 249	--	=	=	=	++	=	--	=	--	=	=	--
vs. D 250	=	=	=	=	=	=	=	=	--	=	=	=
D 242 vs. D 243	=	=	=	=	=	=	=	=	=	=	=	=
vs. D 247	=	=	=	++	=	=	=	=	--	=	--	=
vs. D 249	=	=	=	=	++	=	--	=	=	=	--	--
vs. D 250	++	=	=	=	=	=	=	=	=	=	--	=
D 243 vs. D 247	=	=	=	=	=	--	=	=	=	=	=	=
vs. D 249	=	++	=	=	++	=	--	=	=	=	=	=
vs. D 250	=	=	=	=	=	--	=	=	=	=	=	=
D 247 vs. D 249	=	=	=	--	++	=	--	=	=	=	=	--
vs. D 250	++	=	=	--	=	=	=	=	=	=	=	=
D 249 vs. D 250	++	=	=	=	--	--	=	=	=	=	=	++

= no significant difference between the two samples.
++ second item significantly greater than the first item to the .01 level.
-- second item significantly smaller than the first item to the .01 level.

Sex

Electrophoretic studies considering the effect of the sex of the animal on a serum sample are few. Using micro-electrophoresis in agar-gel, van Sande and Karcher (1960) found no significant sexual differences in the protein patterns of many species of insects. Free electrophoresis was utilized by Brandt et al (1950), Deutsch & Goodloe (1945), and Moore (1945) to detect sexual differences in the protein patterns of chickens. Brandt et al (op. cit.) found that laying hens exhibited increased total protein, an extra pre-albumin fraction, increased alpha-globulin, and decreased albumin when compared to non-laying hens and cockerels. Sibley and Johnsgard (1959a) found that in one year old pheasants the laying females had very low albumin levels compared to those of male pheasants and in adult jungle fowl the laying and non-laying females exhibited lower albumin levels than the males. Juvenile pheasants, however, evinced no sex difference in serum protein patterns.

Moore (1945) examined the free electrophoretic profiles of a wide variety of mammals and could detect no sexual differences. Utilizing filter paper electrophoresis, Cowan & Johnston (1962) found sexual differences in the

protein patterns of the captive deer O. h. sitkensis and O. v. ochrourus. The adult female deer had a significantly lower percent composition of fraction I (albumin?) and a significantly higher percent composition of fractions II and V (globulins?) than the males.

Thus it appears that where sexual differences in protein concentration are detected they will usually be exhibited by lowered albumin and increased globulin levels in the female.

Yellow samples of wild adult, O. h. columbianus - Chemainus, Vancouver Island, B.C. (collected in December, 1961 and stored less than two years) and of wild fawns of O. h. columbianus - Mendocino County, California (collected in October, 1962 and stored less than three weeks) were examined electrophoretically for the effect of sex on the serum proteins. The wild, female, fawns of O. h. columbianus - Mendocino County, California (Tables 23 & 24) have a significantly lower percent composition of fraction +3 (globulin?) than the corresponding male fawns. As the precise age of the fawns is not known this one significant difference may be linked not only with sex but also with maturation (see section on Age).

The wild, female, adults of O. h. columbianus - Chemainus, Vancouver Island, B.C. show a significantly lower percent composition of fraction +10 than the corresponding male adults (Tables 23 & 24). As this fraction is possibly albumin (see section on Individual variation) then this result is in agreement with previous work.

Cowan & Johnston (1962) found no significant differences in the mobilities of the protein fractions of adult male and female captive O. h. sitkensis and O. v. ochrourus using filter paper electrophoresis. The use of starch-gel electrophoresis in the present study indicates that the wild, adult O. h. columbianus - Chemainus, Vancouver Island, B.C. differ significantly in the mobilities of three fractions, the female having significantly slower fractions +3, +6, and +8 (Tables 21 & 22). Table 22 also shows no significant differences between the mobility of any of the protein fractions of male and female fawns of O. h. columbianus - Mendocino County, California.

In summary, sexual differences in the mobility of the protein fractions were not observed between male and female fawns but were observed between male and female adults. Sexual differences in the percent composition of the protein

fractions were observed in both male and female fawns and adults. Therefore, the sex of the deer must be taken into consideration in further comparisons.

TABLE 21

EFFECT OF SEX - mobilities in 10^{-5} cm^2 / volt seconds.

FRACTION		10	9	8	7	6	5	4	3	2	+1	-1
wild, *O. h. columbianus*, California												
male, fawn	$\bar{X}$	.977	.542	.460	.431	.394	.330	.224	.182	.101	.015	.011
N = 3	± S.E.	.037	.021	.002	.005	.014	.031	.031	.005	.015	.005	.001
female, fawn	$\bar{X}$	.893	.527	.443	.407	.371	.272	.183	.179	.095	.034	.011
N = 3	± S.E.	.024	.043	.020	.023	.017	.007	.006	.006	.014	.013	.001
wild, *O. h. columbianus*, Chemainus, B.C.												
male, adult	$\bar{X}$		.829	.494	.423	.383	.313	.260	.165	.133	.022	.012
N = 2	± S.E.		.013	.008	.008	.005	.011	.012	.005	.011	.012	.003
female, adult	$\bar{X}$		.775	.421	.383	.340	.286	.221	.115	.080	.017	.013
N = 2	± S.E.		.006	.006	.006	.001	.013	.007	.006	.006	.001	.001

N = number of animals

TABLE 22

EFFECT OF SEX - Student's t-test comparisons of the mobilities of the serum protein fractions.

FRACTION	10	9	8	7	6	5	4	3	2	+1	-1
wild, *O.h. columbianus*, California											
male fawn vs. female fawn	=	=	=	=	=	=	=	=	=	=	=
wild, *O.h. columbianus*, Chemainus, B.C.											
male adult vs. female adult	=	--	=	--	=	=	--	=	=	=	

= no significant difference between the two samples.
-- second item significantly slower than the first item to the .01 level.

TABLE 23

EFFECT OF SEX - percent composition in arcs in $\sqrt{\text{percentage}}$ degrees.

FRACTION		10	9	8	7	6	5	4	3	2	+ 1	- 1
wild, *O. h. columbianus*, California												
male, fawn	$\bar{X}$	35.0	17.3	13.6	11.2	9.6	11.2	13.1	12.2	20.8	9.8	23.7
N = 3	± S.E.	2.2	0.8	0.3	2.1	0.9	3.4	1.5	0.6	0.5	1.6	0.9
female, fawn	$\bar{X}$	41.0	19.1	10.9	13.7	8.7	6.2	8.8	9.1	22.8	11.7	17.1
N = 3	± S.E.	0.8	0.3	0.6	1.5	0.8	1.0	1.4	0.1	1.2	0.8	2.0
wild, *O. h. columbianus*, Chemainus, B.C.												
male, adult	$\bar{X}$		44.6	19.2	14.2	7.4	6.9	7.2	10.4	11.3	18.0	19.9
N = 2	± S.E.		0.1	2.4	0.5	1.4	0.8	1.8	0.1	1.8	3.2	0.6
female, adult	$\bar{X}$		39.2	16.3	13.7	11.1	8.1	12.8	14.7	12.7	15.5	22.9
N = 2	± S.E.		0.5	0.5	0.9	1.4	0.3	3.7	3.2	2.6	3.1	2.4

N = number of animals

TABLE 24

EFFECT OF SEX - Student's t-test comparisons of the percent composition (in arcs$\hat{}$in $\sqrt{\text{percentage}}$ degrees) of the serum protein fractions.

FRACTION	10	9	8	7	6	5	4	3	2	+1	-1
wild, *O.h. columbianus*, California											
male fawn vs. female fawn	=	=	=	=	=	=	=	--	=	=	=
wild, *O.h. columbianus*, Chemainus, B.C.											
male adult vs. female adult	--	=	=	=	=	=	=	=	=	=	=

= no significant difference between the two samples.
-- second item significantly smaller than the first item to the .01 level.

Age

Examinations of the blood serum of chickens by means of free electrophoresis (Brandt et al, 1950, Marshall & Deutsch, 1950) and of pheasants by means of filter paper electrophoresis (Sibley & Johnsgard, 1959a) all show an increase in gamma-globulin with age. The results involving the effect of the animal's age on albumin concentration are varied: Brandt et al found no change in albumin concentration while Marshall & Deutsch and Moore found albumin increased with age in chickens and Sibley & Johnsgard found it decreased with age in pheasants. Brandt et al also found an increase in total protein and alpha-globulin with no change in the beta-globulin concentration of chickens.

König et al (1949) used free electrophoresis to observe an increase in gamma-globulin as lambs mature. Munkácsi (1961) used paper electrophoresis to study horses from the newborn stage to twenty year olds. He found that the total protein rose steadily with increasing age, that this increase was due to the addition of beta-globulin up to 1½ years, and that the increase thereafter was due to the addition of gamma-globulin. He found no change in albumin or alpha-globulin. Cowan & Johnston (1962) used paper electrophoresis to study the effect of age on the serum

proteins of captive O. h. columbianus - Vancouver Island, B.C. The percent composition of the protein fractions changed up to the age of fifteen months. Fraction I (albumin?) decreased, Fractions II & III remained constant, and Fraction IV and Fraction V (gamma-globulin?) increased.

The only consistent finding in the above work is the increase in gamma-globulin as the animal ages. The investigators agree that this increase is probably a response to the increasing number of antigenic substances the animal comes into contact with as it grows older.

Most samples received for the present study were labelled either "fawn" or "adult". However, yellow samples taken from wild, female, O. h. columbianus - Mendocino County, California in October, 1962, were classified as fawns, 1 year olds, 2 year olds, 4 year olds, and 6 year olds. Due to the small number of samples in each age group, the latter three ages were grouped and are considered as "adults" in the following tables. These "adults" represent the possible range of ages that would be included in the "adult" labels on samples received from other sources. The fawn sera were stored less than three weeks while the remaining sera were stored less than two years.

Numerical data for the mobility and the percent composition of the protein fractions are given in Tables 25 & 27 respectively. All ages exhibit the same total number of protein fractions.

Table 26 shows that there is no significant change in the mobility of the protein fractions from fawns to adults. This is in agreement with Cowan & Johnston's (1962) findings on mobility.

That the percent composition of the protein fractions does change with age is seen in Table 28. Fraction +10, which is assumed to be albumin, has decreased with age sufficiently to show a significant difference between fawns and adults. This decrease in albumin agrees with that found in pheasants (Sibley & Johnsgard, 1959a) and in captive O. h. columbianus - Vancouver Island, B.C. (Cowan & Johnston, 1962).

The fawns and the one year olds are similar in the percent composition of all their protein fractions. The fawns and the adults differ not only in the albumin fraction but the adults also show an increase in fraction +3 (globulin?) over the fawns. The adults also show an increase in fractions +3 and +4 (globulins?) over the one year olds.

As the identity of the globulins with a particular fraction number has not been determined fractions +3 and +4 must remain only "globulins".

In summary, no changes in the mobility of the protein fractions are shown to occur with age. Changes in percent composition are found between fawns, one year olds, and adults. As the majority of the serum samples received in this study were from adults, the fawns and one year olds were eliminated from further comparisons.

TABLE 25

EFFECT OF AGE - mobilities in 10^{-5} cm^2 / volt seconds.

FRACTION		10	9	8	7	6	5	4	3	2	+1	-1
wild, female, *O. h. columbianus*, California												
fawn	$\bar{X}$	.901	.517	.442	.411	.375	.290	.212	.181	.099	.029	.011
N = 4	± S.E.	.019	.032	.014	.017	.013	.019	.023	.005	.011	.011	.001
1 year old	$\bar{X}$	.952	.515	.460	.431	.403	.295	.228	.174	.077	.012	.011
N = 4	± S.E.	.046	.027	.025	.026	.025	.019	.017	.024	.017	.002	.001
adult	$\bar{X}$	.979	.540	.475	.434	.406	.288	.225	.149	.087	.012	.012
N = 3	± S.E.	.044	.016	.012	.012	.013	.028	.040	.042	.024	.002	.001

N = number of animals

TABLE 26

EFFECT OF AGE - Student's t-test comparisons of the mobilities of the serum protein fractions.

FRACTION	10	9	8	7	6	5	4	3	2	+1	-1
wild, female *O. h. columbianus*, California											
fawn vs. 1 year old	=	=	=	=	=	=	=	=	=	=	=
fawn vs. adult	=	=	=	=	=	=	=	=	=	=	=
1 year old vs. adult	=	=	=	=	=	=	=	=	=	=	=

= no significant difference between the two samples.

TABLE 27

EFFECT OF AGE - percent composition in arcs⌒in $\sqrt{\text{percentage}}$ degrees.

FRACTION		10	9	8	7	6	5	4	3	2	+1	-1
wild, female, *O. h. columbianus*, California												
fawn	$\bar{X}$	41.2	18.9	11.7	12.8	8.3	7.0	9.0	9.2	22.2	11.6	17.6
N = 4	± S.E.	0.6	0.3	0.9	1.5	0.7	1.0	1.1	0.2	1.1	0.6	1.8
1 year old	$\bar{X}$	40.4	20.0	12.2	8.8	7.0	6.4	9.5	9.8	18.5	13.3	22.2
N = 4	± S.E.	0.3	1.3	1.9	0.8	0.8	1.7	0.2	0.3	3.5	0.3	1.6
adult	$\bar{X}$	37.2	16.7	13.1	10.6	7.9	12.2	12.9	13.3	19.6	10.9	21.2
N = 3	± S.E.	0.8	0.9	1.9	1.1	2.5	2.7	0.2	0.6	0.6	1.1	4.1

N = number of animals

TABLE 28

EFFECT OF AGE - Student's t-test comparisons of the percent composition (in arcs⁀in $\sqrt{\text{percentage}}$ degrees) of the serum protein fractions.

FRACTION	10	9	8	7	6	5	4	3	2	+1	-1
wild, female, *O. h. columbianus*, California											
fawn vs. 1 year old	=	=	=	=	=	=	=	=	=	=	=
fawn vs. adult	--	=	=	=	=	=	=	++	=	=	=
1 year old vs. adult	=	=	=	=	=	=	++	++	=	=	=

= no significant difference between the two samples.
++ second item significantly greater than the first item to the .01 level.
-- second item significantly smaller than the first item to the .01 level.

Season

Deer, like many other animals, are affected by seasonal changes in the quantity and the quality of their food and maintain an annual cycle of reproduction. As electrophoretic patterns of mammalian serum proteins are affected both by diet (see section on Captivity) and pregnancy (Moore, 1959) there is the possibility of the deer's protein pattern showing seasonal effects.

Reports of seasonal effects on blood proteins are scarce. Sibley & Johnsgard (1959a) give the following illustration of the effect of season on the electrophoretic pattern of the serum proteins of mallards. They found that female mallards had lower albumin levels in November than in July. However, the first samples were from wild birds in July which were subsequently kept in captivity until the November sampling (see section on Captivity). Cowan & Johnston (1962) in a paper electrophoretic study of captive, adult, male, O. h. columbianus report no significant differences in either the mobility or the percent composition of the serum proteins associated with the onset of rut.

In the present study, serum samples were compared from wild, female, adult, O. h. columbianus - Mendocino County, California; three in October, 1962 and two in

January, 1963.

In January the nutritional status of the deer in Mendocino County, California is markedly poorer than in October (Bissel & Strong, 1955, Taber & Dasmann, 1958). That there were no significant differences observed in the mobilities of any of the protein fractions (Tables 29 & 30) is in agreement with the findings of Cowan & Johnston (1962). Significant differences were found, however, in the percent composition of the protein fractions as separated by starch-gel electrophoresis (Tables 31 & 32). The January group shows an increased albumin (fraction +10) and lowered globulin (fraction +4) over the October group. These results agree with those of Goldstein & Scott (1956) who studied the effects of a Vitamin E deficiency in chickens.

Taber (1953) found the peak of conception in 1949 of O. h. columbianus - Mendocino County, California occurred during the last week in October and the first two weeks in November. It is not known if the groups of females used in the present study were pregnant at the time of sampling. It is doubtful, however, that early pregnancy would have a greater effect on the electrophoretic pattern than the drastic change in nutrition.

In summary, a change of season does not affect the

mobility but does affect the percent composition of the protein fractions. These changes can be linked to the nutritional status of the season. It is apparent, therefore, that studies intending to explore serum protein variation in deer can only get comparable samples by using phenologically equivalent individuals.

TABLE 29

EFFECT OF SEASON - mobilities in 10^{-5} cm^2 / volt seconds.

FRACTION		10	9	8	7	6	5	4	3	2	+1	-1
wild, female, adult, *O. h. columbianus*, California												
October, 1962	$\bar{X}$	.979	.540	.475	.434	.406	.288	.225	.149	.087	.012	.012
N = 3	± S.E.	.044	.016	.012	.012	.013	.028	.040	.042	.024	.002	.001
January, 1963	$\bar{X}$	.961	.533	.463	.435	.402	.297	.209	.156	.099	.010	.011
N = 2	+ S.E.	.083	.049	.032	.037	.040	.001	.001	.025	.006	.001	.001

N = number of animals

TABLE 30

EFFECT OF SEASON - Student's t-test comparisons of the mobilities of the serum protein fractions.

FRACTION	10	9	8	7	6	5	4	3	2	+1	-1
wild, female, adult, *O. h. columbianus*, California											
October, 1962 vs. January, 1963	=	=	=	=	=	=	=	=	=	=	=

= no significant difference between the two samples.

TABLE 31

EFFECT OF SEASON - percent composition in arcs $\widehat{\ }$ in $\sqrt{\text{percentage}}$ degrees.

FRACTION		10	9	8	7	6	5	4	3	2	+ 1	- 1
wild, female, adult, *O. h. columbianus*, California												
October, 1962	$\bar{X}$	37.2	16.7	13.1	10.6	7.9	12.2	12.9	13.3	19.6	10.9	21.2
N = 3	± S.E.	0.8	0.9	1.9	1.1	2.5	2.7	0.2	0.6	0.6	1.1	4.1
January, 1963	$\bar{X}$	46.4	20.5	11.5	11.2	6.3	9.2	8.5	9.5	13.7	10.9	16.9
N = 2	± S.E.	0.5	0.4	1.4	1.3	1.1	0.9	0.4	0.5	3.5	0.6	1.9

N = number of animals

TABLE 32

EFFECT OF SEASON - Student's t-test comparisons of the percent composition (in arcs$\frown$in$\sqrt{\text{percentage}}$ degrees) of the serum protein fractions.

FRACTION	10	9	8	7	6	5	4	3	2	+1	-1
wild, female, adult, *O. h. columbianus*, California											
October, 1962 vs. January, 1963	++	=	=	=	=	=	--	=	=	=	=

= no significant difference between the two samples.
++ second item significantly greater than the first item to the .01 level.
-- second item significantly smaller than the first item to the .01 level.

Captivity

The blood protein fractions of a wide variety of animals have been found to be affected both by diet (Bandy et al, 1957, Goldstein & Scott, 1956, Helgebostad & Martinsons, 1958, Kitts et al, 1956, Weech et al, 1935) and by disease (Foreman, 1960, Gleason & Friedberg, 1953, Ingram, 1956, Jencks et al, 1955, Johnson et al, 1958, Pert et al, 1959, Sanders et al, 1944, Schinazi, 1957). Captivity has been shown to have an effect on both structure and growth (Darwin, 1859, Spurway, 1952).

At the University of British Columbia (U.B.C.) deer were brought from the wild and put under captive conditions (Wood et al, 1961) at the age of two to three weeks. These captive conditions are such that the captive deer used in the present study are all in good health and are all on an identical high plane diet (Wood et al, 1961). The food and the state of health are very different from those found in the wild state (Linsdale & Tomich, 1953). Bandy et al (1956) found increased body size in U.B.C. captive O. h. columbianus on a high plane diet as compared to those on a low plane diet. Dasmann & Dasmann (1963) found a similar relationship between food quality and deer size in wild populations of mule deer (O. hemionus) in California.

Table 33 shows the number of electrophoretically separated protein fractions for captive and wild O. h. columbianus - Wolf Lake, Vancouver Island, B.C. (samples collected December, 1961) and from captive (samples collected March, 1962) and wild (samples collected April, 1963) O. h. sitkensis - Alaska. The captive deer of both races differ obviously from their wild counterparts in having an additional negatively migrating serum protein fraction. As it is also obvious that the limited data will not allow further numerical comparisons they are listed only in Appendices A & B.

Disease affects the electrophoretic pattern significantly only in the acute stages and then only in the percent composition of the protein fractions. It is suspected that the great difference seen between captive and wild animals (Table 33) is not due to disease but is due mainly to the extreme differences in diet.

In summary, the U.B.C. captive conditions are such that serum samples from captive deer cannot be utilized in further comparisons which also involve serum samples from deer in the wild state. Nutritional differences are suspected of causing the great differences between captive and wild deer.

TABLE 33

EFFECT OF CAPTIVITY - total number of serum protein fractions.*

		number of positively migrating fractions	number of negatively migrating fractions
wild			
female, adult *O.h. columbianus*, Wolf Lake, B.C.	N = 1	9	1
male, adult, *O.h. columbianus*, Wolf Lake, B.C.	N = 1	9	1
male, adult, *O.h. sitkensis*, Kupreanof Is. Alaska	N = 1	9	1
captive			
female, adult *O.h. columbianus*, Wolf Lake, B.C.	N = 1	9	2
male, adult, *O.h. columbianus*, Wolf Lake, B.C.	N = 1	9	2
female, adult, *O.h. sitkensis*, Petersburg, Alaska	N = 1	9	2
male, adult, *O.h. sitkensis*, Petersburg, Alaska	N = 1	9	2

N = number of animals
* see Appendix A for the mobilities and Appendix B for the percent composition of the serum protein fractions.

Geographic separation, subspecies, and species

After removal of all the hemolysed and cloudy samples, samples from captive deer, and samples from wild fawns and one year old deer, very little comparable material remained. Furthermore, as the majority of this remaining material consisted of one sample from one individual of each sex the numerical comparisons involving the mobility and the percent composition could not be made. These data are given only in Appendices C & D.

One possible electrophoretic criterion for comparing these subspecies and species is the number of protein fractions (Table 34). There appear to be 9 to 10 fractions that will migrate in a positive direction in the genus Odocoileus. All the O. hemionus group have but 1 negatively migrating fraction while the O. virginianus group have up to 4 negatively migrating fractions. O. h. columbianus from Wolf Lake and from Chemainus, Vancouver Island, B.C. have a total number of 10 fractions. This number is the same as that found for two other subspecies, O. h. crooki and O. h. sitkensis. However, O. h. columbianus from California differs from the other subspecies and from Vancouver Island specimens presently regarded as of the same subspecies in having a total number of 11 fractions. Thus, the possible criterion of the

number of fractions for comparing subspecies or species of Odocoileus is not valid. It has already been shown that captivity can alter the number of fractions.

Three groups remain for quantitative comparison. These are O. h. columbianus - Chemainus, Vancouver Island, B.C. (2 females - samples collected December, 1961), O. h. columbianus - California (5 females - samples collected October, 1962 - January, 1963), and O. v. texanus - Texas (8 females - samples collected April - May, 1963). Because seasonal differences were exhibited in the percent composition but not in the mobility of the protein fractions, these three groups were compared only on the basis of their mobilities. These numerical data are given in Table 35. These groups are clearly separated into their respective taxonomic categories on the basis of morphology (Cowan, 1956, Kellogg, 1956).

As each group of animals has its particular number of protein fractions, the fractions of each group were separated into 14 categories on the basis of their mobilities (Table 36). Those fractions of each group that belonged to the same category of mobility were then compared by means of Student's t-test (Table 37). The results were further condensed into 4 categories: 1a. the number of significant differences, 1b. the number of times no comparison could be made

due to a fraction occurring in one group of animals but not in the other, 2a. the number of similarities, and 2b. the number of common absences. These categories gave rise to the total number of differences and similarities between geographically separated groups of the subspecies, O. h. columbianus, and between these and another species represented by O. v. texanus (Table 38).

It was found that differences in mobility were greater between females from California and Vancouver Island genotypes of Odocoileus hemionus than between either of these and the females of the species O. virginianus represented by O. v. texanus.

In summary, there are 9 - 10 positively migrating and 1 - 4 negatively migrating fractions in the genus Odocoileus. Comparisons of mobilities in three groups of adult females of the genus Odocoileus indicate greater intra-specific differences than inter-specific differences.

TABLE 34

GEOGRAPHIC SEPARATION, SUBSPECIES, AND SPECIES - total number of serum protein fractions.*

	number of positively migrating fractions	number of negatively migrating fractions
wild, female, adult, O.hemionus columbianus, N = 1 Wolf Lake, B.C.	9	1
wild, male, adult, O.hemionus columbianus, N = 1 Wolf Lake, B.C.	9	1
wild, female, adult, O.hemionus columbianus, N = 2 Chemainus, B.C.	9	1
wild, male, adult, O.hemionus columbianus, N = 2 Chemainus, B.C.	9	1
wild, female, adult, O.hemionus columbianus, N = 5 California	10	1
wild, female, adult, O.hemionus crooki, N = 1 Arizona	9	1
wild, male, adult, O.hemionus crooki, N = 1 Arizona	9	1
wild, male, adult, O.hemionus sitkensis, N = 1 Kupreanof Is., Alaska	9	1
wild, male, adult, O.virginianus leucurus, N = 1 Oregon	10	4
wild, female, adult, O.virginianus texanus, N = 8 Texas	10	2

N = number of animals

* For all samples, female, N = 1, male, N = 1, N = 2, see Appendix C for the mobilities and Appendix D for the percent composition of the serum protein fractions.
For all samples, female, N = 2, N = 5, N = 8, see Table 35 for the mobilities of the serum protein fractions and Tables 36 - 38 for the analysis of these mobilities.

TABLE 35

EFFECT OF GEOGRAPHIC SEPARATION AND SPECIES - mobilities in 10^{-5} cm^2 / volt seconds.

FRACTION		10	9	8	7	6	5	4	3	2	+1	-1	2
wild, female, adult, *O. virginianus texanus*, Texas													
N = 8	$\bar{X}$	.829	.464	.406	.371	.327	.254	.190	.158	.079	.015	.011	.143
	± S.E.	.008	.016	.016	.013	.011	.013	.013	.003	.005	.002	.001	.015
wild, female, adult, *O. hemionus columbianus*, California													
N = 5	$\bar{X}$	.972	.537	.470	.434	.405	.292	.218	.151	.092	.011	.011	
	± S.E.	.036	.017	.013	.013	.015	.016	.022	.024	.014	.001	.001	
wild, female, adult, *O. hemionus columbianus*, Chemainus, B.C.													
N = 2	$\bar{X}$		.775	.421	.383	.340	.286	.221	.115	.080	.017	.013	
	+ S.E.		.006	.006	.006	.001	.013	.007	.006	.006	.001	.001	

N = number of animals

TABLE 36

EFFECT OF GEOGRAPHIC SEPARATION AND SPECIES - distribution of serum protein fractions into categories according to their mobilities (in 10^{-5} cm^2 / volt seconds).

MOBILITY	1.000	.600	.500	.450	.425	.400	.350	.300	.250	.175	.100	.050	+.000-	.100	.200
wild, female, adult, *O. virginianus texanus*, Texas		1	0	1	0	1	1	1	1	1	1	1	1	1	1
wild, female, adult, *O. hemionus columbianus*, California		1	1	1	1	1	0	0	1	1	1	1	1	1	0
wild, female, adult, *O. hemionus columbianus*, Chemainus, B.C.		1	0	0	0	1	1	1	1	1	1	1	1	1	0

1 serum protein fraction present.
0 serum protein fraction absent.

TABLE 37

EFFECT OF GEOGRAPHIC SEPARATION AND SPECIES - Student's t-test comparisons of the mobilities of the serum protein fractions within mobility categories (in 10^{-5} cm^2/vol seconds).

MOBILITY	1.000	.600	.500	.450	.425	.400	.350	.300	.250	.175	.100	.050	+.000	-.100	-.200
wild,female,adult *O.virginianus texanus*, Texas															
vs. wild,female,adult, *O.hemionus columbianus*, California		=	0	=	0	=	0	0	=	=	=	=	=	=	0
vs. wild,female,adult, *O.hemionus columbianus*, Chemainus, B.C.		--	/	0	/	=	=	=	=	=	--	=	=	=	0
wild,female,adult, *O.hemionus columbianus*, California															
vs. wild,female,adult, *O.hemionus columbianus*, Chemainus, B.C.		--	0	0	0	=	0	0	=	=	=	=	++	=	/

= no significant difference between the two samples.
++ second item significantly faster than the first item to the .01 level.
-- second item significantly slower than the first item to the .01 level.
0 protein fraction present in one sample but not in the other.
/ indicates the common absence of a protein fraction.

TABLE 38

EFFECT OF GEOGRAPHIC SEPARATION AND SPECIES - summary of differences and similarities in the mobilities of the serum protein fractions. *

	significant differences (++, --)*		no comparison (0)*		subtotal of differences		similarities (=)*		common absence (/)*		subtotal of similarities	total
wild, female, adult, *O.virginianus texanus*, Texas												
vs. wild, female, adult, *O.hemionus columbianus*, California	0	+	5	=	5		9	+	0	=	9	14
vs. wild, female, adult, *O.hemionus columbianus*, Chemainus, B. C.	2	+	2	=	4		8	+	2	=	10	14
wild, female, adult, *O.hemionus columbianus*, California												
vs. wild, female, adult, *O.hemionus columbianus*, Chemainus, B.C.	2	+	5	=	7		6	+	1	=	7	14

* see Table 37.

CONCLUSIONS

The original aim of this study was to compare, by means of starch-gel electrophoresis, the serum proteins of as many subspecies of the genus Odocoileus as possible in an attempt to clarify their phylogeny.

The study showed that many independent variables affect the electrophoretic pattern of the serum. It also showed that for taxonomic purposes clear yellow samples from at least two wild individuals of the same sex and age of each subspecies should be obtained at identical seasons. The effects of captivity and season indicate that the nutritional history of the deer also has a major influence on the electrophoretic pattern.

These strictures render it most difficult to obtain truly comparable samples that will permit an examination of differences that may be considered of genetic origin and related to the evolution of different species or subspecies. There is no published evidence to suggest that the same causes of variation of non genetic nature do not occur in other mammals. Under these circumstances one must approach with some skepticism the several published reports of apparent species related or subspecies related differences

(Cowan & Johnston, 1962) between mammals.

In this study it was possible to make valid comparisons between 2 populations of blacktail deer, O. hemionus, both currently bearing the same subspecific designation, and a whitetail O. virginianus.

The two species differed from each other in the number of identifiable fractions. O. virginianus had 2 instead of 1 negatively migrating fractions. Comparing the two species on point of mobility the differences are of the same order of magnitude as they are between the 2 populations of O. hemionus.

It is of great interest that two populations of O. hemionus presently known by the same subspecific name are shown to be markedly different from each other in terms of serum protein mobility characteristics. This supports other observations made at this laboratory revealing that these two genotypes differ in many features of body form, behaviour and physiology, and are probably as different from each other as any other two populations within this species currently given taxonomic recognition.

This study strongly suggests that electrophoretic studies of serum proteins indicate important differences between even the minor taxa. They are, therefore, of potential value in taxonomy. However, the large assortment

of factors that may be responsible for alterations in serum protein values of the same order as those that can be of taxonomic value make it most difficult to obtain truly comparable samples. Other body proteins may be less variable and of more value to the systematist.

SUMMARY

1. After standardization of the starch-gel electrophoretic technique, variation in the serum proteins of the genus Odocoileus due to the condition of the sample and the condition of the animal could be studied.

2. Significant changes in the serum sample were brought about by hemolysis, cloudiness, and decomposition. Cold storage of adult deer serum for two years, the addition of a bacteriostat to the sample, and the use of a muscle relaxant to procure samples from captive deer produced no significant changes in either the mobility or the percent composition of the protein fractions.

3. A large individual variation was found in both the mobility and the percent composition of the protein fractions.

4. The percent composition of the protein fractions was affected by sex, age, and season. The mobility of the protein fractions was affected by sex, but not by age or season.

5. Deer under the captive regimen at the University of British Columbia exhibited an additional negatively migrating protein fraction when compared to their wild counterparts.

6. Comparisons of the mobilities in three groups of adult females of the genus Odocoileus indicate intra-specific

differences of the same order as those between species. The species however, differ additionally in the number of protein fractions.

7. The technique of starch-gel electrophoresis, while potentially useful to the systematist studying mammals, suffers from the large number of influences that can induce changes in the serum proteins that are of the same order of magnitude as those between taxa.

LITERATURE CITED

Allison, A. C., Blumberg, B. S., and W. ap Rees. 1958. Haptoglobin types in British, Spanish Basque and Nigerian African populations. Nature 181: 824-825.

Ashton, G. C. 1957a. Zone electrophoresis of mammalian sera in starch gels. Nature 179: 824-825.

Ashton, G. C. 1957b. Serum protein differences in cattle by starch gel electrophoresis. Nature 180: 917-919.

Ashton, G. C. 1958. Polymorphism in the beta-globulins of sheep. Nature 181: 849-850.

Ashton, G. C. 1958a. A genetic mechanism for "Thread Protein" polymorphism in cattle. Nature 182: 65-66.

Ashton, G. C. 1958b. Lack of "Slow-Alpha" proteins in some Guernsey cattle. Nature 182: 193-194.

Ashton, G. C. 1958c. Genetics of beta-globulin polymorphisms in British cattle. Nature 182: 370-372.

Ashton, G. C. 1958d. Serum protein variation in horses. Nature 182: 1029-1030.

Ashton, G. C. 1958e. Further beta-globulin phenotypes in sheep. Nature 182: 1101-1102.

Ashton, G. C. 1959. Beta-globulin alleles in some Zebu cattle. Nature 184: 1135-1136.

Ashton, G. C. 1960. Thread protein and beta-globulin polymorphism in the serum proteins of pigs. Nature 186: 991-992.

Ashton, G. C. and G. R. Fallon. 1962. Beta-globulin type, fertility and embryonic mortality in cattle. J. Reprod. Fertil. 3: 93-104.

Ashton, G. C. and E. I. McDougall. 1958. Beta-globulin polymorphism in cattle, sheep, and goats. Nature 182: 945-946.

Auernheimer, A. H., Cutter, W., and F. O. Atchley. 1960. Electrophoretic studies on blood serum proteins of rodents. J. Mamm. 41: 405-407.

Baier, J. G. and H. R. Wolfe. 1942. Quantitative serologic relationships within the Artiodactyla. Zoologica 27: 17-23.

Bain, J. F. and H. F. Deutsch. 1947. An electrophoretic study of the egg-white proteins of various birds. J. Biol. Chem. 171: 531-541.

Bandy, P. J., Cowan, I. McT., Kitts, W. D., and A. J. Wood. 1956. A method for the assessment of the nutritional status of wild ungulates. Canad. J. Zool. 34: 48-52.

Bandy, P. J., Kitts, W. D., Wood, A. J., and I. McT. Cowan. 1957. Effect of age and plane of nutrition on the blood chemistry of the Columbian Black-Tailed Deer (Odocoileus hemionus columbianus). B. Blood glucose, non-protein nitrogen, total plasma proteins, plasma albumin, globulin, and fibrinogen. Canad. J. Zool. 35: 283-289.

Bangham, A. D. 1957. Distribution of electrophoretically different haemoglobins among cattle breeds of Great Britain. Nature 179: 467-468.

Baril, E. F., Palmer, J. L., and A. H. Bartel. 1961. Electrophoretic analysis of young alligator serum. Science 133: 278-279.

Bissell, H. D. and H. Strong. 1955. The crude protein variations in the browse diet of California Deer. Calif. Fish and Game 41: 145-155.

Boyden, A. 1943. Serology and animal systematics. Am. Naturalist 77: 234-255.

Boyden, A. and D. Gemeroy. 1950. The relative position of the Cetacea among the Orders of Mammalia as indicated by precipitin tests. Zoologica 35: 145-151.

Brandt, L. W., Clegg, R. E., and A. C. Andrews. 1950. The effect of age and degree of maturity on the serum proteins of the chicken. J. Biol. Chem. 191: 105-111.

Buzzati-Traverso, A. A. and A. B. Rechnitzer. 1953. Paper partition chromatography in taxonomic studies. Science 117: 58-59.

Cantarow, A. and B. Schepartz. 1957. Biochemistry. 2nd edition. W. B. Saunders Co., Philadelphia, London. 867 p.

Cowan, I. McT. 1956. What and where are the Mule and Black-Tailed Deer? p. 335-359. In W. P. Taylor (ed.), The deer of North America. The Stackpole Co., Harrisburg, Pennsylvania and the Wildlife Management Institute, Washington, D. C.

Cowan, I. McT. and P. A. Johnston. 1962. Blood serum protein variations at the species and subspecies level in deer of the genus Odocoileus. Systematic Zool. 11: 131-138.

Cowan, I. McT., Wood, A. J., and H. C. Nordan. 1962. Studies in the tranquilization and immobilization of deer (Odocoileus). Canad. J. of Comparative Medicine, Veterinary Science 26: 57-61.

Craighead, J. J., Hornocker, M., Woodgerd, W., and F. C. Craighead, Jr. 1960. Trapping, immobilization and color-marking Grizzly Bears. Trans. 25th North American Wildlife and Natural Resources Conference: 346-363.

Darwin, C. 1859. The origin of species. The Modern Library, New York. 386 p.

Dasmann, R. F. and W. P. Dasmann. 1963. Mule deer in relation to a climatic gradient. J. Wildlife Management 27: 196-202.

Dessauer, H. C. and W. Fox. 1956. Characteristic electrophoretic patterns of plasma proteins of Orders of Amphibia and Reptilia. Science 124: 225-226.

Dessauer, H. C., Fox, W., and Q. L. Hartwig. 1962. Comparative study of transferrins of Amphibia and Reptilia using starch-gel electrophoresis and autoradiography. Comp. Biochem. and Physiol. 5: 17-29.

Deutsch, H. F. and M. B. Goodloe. 1945. An electrophoretic survey of various animal plasmas. J. Biol. Chem. 161: 1-20.

Deutsch, H. F. and W. H. McShan. 1949. Biophysical studies of blood plasma proteins. XII Electrophoretic studies of the blood serum proteins of some lower animals. J. Biol. Chem. 180: 219-234.

Foreman, C. W. 1960. Electromigration properties of mammalian hemoglobins as taxonomic criteria. Am. Midl. Nat. 64: 177-186.

Forsythe, R. H. and J. F. Foster. 1950. Egg white proteins. I Electrophoretic studies on whole white. J. Biol. Chem. 184: 377-392.

Fox, W., Dessauer, H. C., and L. T. Maumus. 1961. Electrophoretic studies of blood proteins of two species of toads and their natural hybrid. Comp. Biochem. and Physiol. 3: 52-63.

Gleason, T. L. and F. Friedberg. 1953. Filter paper electrophoresis of serum proteins from small animals. Physiol. Zool. 26: 95-100.

Goldstein, J. and M. L. Scott. 1956. An electrophoretic study of exudative diathesis in chicks. J. Nutrition 60: 349-360.

Goodman, L. S. and A. Gelman. 1956. The Pharmacological Basis of Therapeutics. 2nd edition. Macmillan Co., New York.

Graber, P. and C. A. Williams, Jr. 1955. Méthode Immuno-électrophorétique d'analyse de mélanges de substances antigéniques. Biochem. et Biophys. Acta 17: 67-74.

Harris, H., Robson, E. B., and M. Siniscalco. 1958a. Beta-globulin variants in man. Nature 182: 452.

Harris, H., Robson, E. B., and M. Siniscalco. 1958b. Atypical segration of haptoglobin types in man. Nature 182: 1324-1325.

Haugen, A. O. 1960. Vitamins A and E in deer blood. J. Mamm. 41: 410-411.

Helgebostad, A. and E. Martinsons. 1958. Nutritional anaemia in mink. Nature 181: 1660-1661.

Hickman, C. G. and O. Smithies. 1957. Evidence for inherited differences in the serum proteins in cattle. Proc. Gen. Soc. of Canada 2: 39.

Ingram, V. M. 1956. A specific chemical difference between the globins of normal human and sickle-cell anaemia haemoglobin. Nature 178: 792-794.

Jencks, W. P., Smith, E. R. B., and E. L. Durrum. 1955. The clinical significance of the analysis of serum protein distribution by filter paper electrophoresis. Am. J. Med. 21: 387-405.

Johnson, M. L. and M. J. Wicks. 1959. Serum protein electrophoresis in mammals - taxonomic implications. Systematic Zool. 8: 88-95.

Johnson, M. L., Wicks, M. J., and J. Brenneman. 1958. Serum protein electrophoresis of some boreal mammals. Murrelet 39: 32-36.

Johnston, P. A. 1961. The reliability of the method of paper electrophoresis as applied to deer of the genus Odocoileus. B.Sc. Thesis. Univ. of British Columbia, Dept. of Zoology.

Kellogg, R. 1956. What and where are the Whitetails? p. 31-55. In W. P. Taylor (ed.), The deer of North America. The Stackpole Co., Harrisburg, Pennsylvania and the Wildlife Management Institute, Washington, D. C.

Kitts, W. D., Bandy, P. J., Wood, A. J., and I. McT. Cowan. 1956. Effect of age and plane of nutrition on the blood chemistry of the Columbian Black-Tailed Deer (Odocoileus hemionus columbianus). A. Packed-cell volume, sedimentation rate, and haemoglobin. Canad. J. Zool. 34: 477-484.

Kohn, J. 1957. A cellulose acetate supporting medium for zone electrophoresis. Clin. Chem. Acta 2: 297-306.

König, V. L., Perrings, J. D., and F. Mundy. 1949. Electrophoretic analysis of lamb and sheep plasma and serum. Arch. Biochem. 22: 377-385.

Kunkel, H. G. and R. Trautman. 1959. Zone electrophoresis in various types of supporting media. p. 225-262. In M. Bier (ed.), Electrophoresis - Theory, Methods, and Applications. Academic Press, New York and London.

Landsteiner, K., Longsworth, L. G., and J. van der Scheer. 1938. Electrophoresis experiments with egg albumins and haemoglobins. Science 88: 83-85.

Latner, A. L. and A. H. Zaki. 1957. Starch-gel electrophoresis of animal sera. Nature 180: 1366-1367.

Leone, C. A. 1954. Further serological data on the relationships of some decapod Crustacea. Evolution 7: 192-205.

Leone, C. A. and A. L. Wiens. 1956. Comparative serology of carnivores. J. Mamm. 37: 11-23.

Leopold, A. S., Riney, T., McCain, R., and L. Tevis, Jr. 1951. The Jawbone Deer Herd. Calif. Game Bull. 4: 1-139.

Linsdale, J. M. and P. Q. Tomich. 1953. A Herd of Mule Deer. Univ. of California Press, Berkeley and Los Angeles. 567 p.

Longsworth, L. G. 1959. Moving boundary electrophoresis - practice. p. 137-177. In M. Bier (ed.), Electrophoresis - Theory, Methods, and Applications. Academic Press, New York and London.

Lowe, V. P. W. and E. I. McDougall. 1961. Serum beta-globulin types in Red Deer and other species and their stability in the presence of bacteria. Nature 192: 983-984.

McCabe, R. A. and H. F. Deutsch. 1952. The relationships of certain birds as indicated by their egg white proteins. Auk 69: 1-18.

Mainardi, D. 1958. Different heterogenetics of mouse haemoglobin according to strains. Nature 182: 947-948.

Mainardi, D. 1958. Immunology and chromatography in taxonomic studies of Gallinaceous birds. Nature 182: 1388-1389.

Marshall, M. E. and H. F. Deutsch. 1950. Some protein changes in fluids of the developing chick embryo. J. Biol. Chem. 185: 155-161.

The Merck Index of Chemicals and Drugs. 7th edition. 1960. Rahway, New Jersey, 1639 p.

Micks, D. W. 1956. Paper chromatography in insect taxonomy. Ann. Am. Ent. Soc. 49: 576-581.

Micks, D. J. and F. J. Gibson. 1957. The characterization of insects and ticks by their free amino-acid patterns. Ann. Am. Ent. Soc. 50: 500-505.

Moody, P. A., Cochran, V. A., and H. Drugg. 1949. Serological evidence on Lagomorph relationships. Evolution 3: 25-33.

Moody, P. A. and D. E. Doniger. 1956. Serological light on porcupine relationships. Evolution 10: 47-55.

Moore, D. H. 1945. Species differences in serum protein patterns. J. Biol. Chem. 161: 21-32.

Moore, D. H. 1959. Clinical and physiological applications of electrophoresis. p. 369-425. In M. Bier (ed.), Electrophoresis - Theory, Methods, and Applications. Academic Press, New York and London.

Munkácsi, F. 1961. Die Veränderungen der Bluteiweissfraktionen mit dem Fortschreitenden Alter der Pferde. Acta. Biol. Acad. Sci. hung. 11: 231-239.

Nuttall, G. H. F. 1901. The new biological test for blood in relation to zoological classification. Proc. Roy. Soc. London 69: 150-153.

Parker, W. C. and A. G. Bearn. 1961. Haptoglobin and transferrin gene frequencies in a Navajo population: a new transferrin variant. Science 134: 106-108.

Pauly, L. K. and H. R. Wolfe. 1957. Serological relationships among members of the Order Carnivora. Zoologica 42: 159-166.

Pearson, H. A., Smith, A. D., and P. J. Urness. 1963. Effects of succinylcholine chloride on Mule Deer. J. Wildlife Management 27: 297-299.

Pert, J. H., Engle, R. E., Jr., Woods, K. R., and M. H. Sleisenger. 1959. Preliminary studies on quantitative zone electrophoresis in starch gel. J. Lab. and Clin. Med. 54: 572-584.

Poulik, M. D. 1957. Starch gel electrophoresis in a discontinuous system of buffers. Nature 180: 1477-1479.

Raymond, S. 1955. Paper Electrophoresis. 3rd edition. E-C Apparatus Co., New York.

Raymond, S. and L. Weintraub. 1959. Acrylamide gel as a supporting medium for zone electrophoresis. Science 130: 711.

Ressler, N. and S. D. Jackson. 1955. Electrophoresis of serum proteins in a viscous film. Science 122: 1088-1089.

Rosa, J., Schapira, G., Dreyfus, J. C., De Grouchy, J., Mathe, G., and J. Bernard. 1958. Different heterogeneities of mouse haemoglobin according to strains. Nature 182: 947-948.

Sanders, E., Huddleson, I. F., and P. J. Schaible. 1944. An electrophoretic study of the serum and plasma from normal and leucosis-affected chickens. J. Biol. Chem. 155: 469-481.

Schinazi, L. A. 1957. Observations on a fast moving protein in avian malarial serum. Science 125: 695-697.

Sibley, C. G. 1960. The electrophoretic patterns of avian egg-white proteins as taxonomic characters. Ibis 102: 215-284.

Sibley, C. G. and P. A. Johnsgard. 1959a. Variability in the electrophoretic patterns of avian serum proteins. Condor 61: 85-95.

Sibley, C. G. and P. A. Johnsgard. 1959b. An electrophoretic study of egg-white proteins in twenty-three breeds of the domestic fowl. Am. Naturalist 93: 107-115.

Smith, A. C. 1962. The electrophoretic characteristics of Albacore, Bluefin Tuna, and Kelp Bass eye lens proteins. Calif. Fish and Game 48: 199-201.

Smithies, O. 1955a. Grouped variations in the occurrence of new protein components in normal human serum. Nature 175: 307-308.

Smithies, O. 1955b. Zone electrophoresis in starch gels: group variations in the serum proteins of normal human adults. Biochem. J. 61: 629-641.

Smithies, O. 1957. Variations in human serum beta-globulins. Nature 180: 1482-1483.

Smithies, O. 1958. Third allele at the serum beta-globulin locus in humans. Nature 181: 1203-1204.

Smithies, O. 1959a. An improved procedure for starch-gel electrophoresis: further variations in the serum proteins of normal individuals. Biochem. J. 71: 585-587.

Smithies, O. 1959b. Zone electrophoresis in starch gels and its application to studies of serum proteins. Advances in Protein Chem. 14: 65-113.

Smithies, O. and O. Hiller. 1959. The genetic control of transferrins in humans. Biochem. J. 72: 121-126.

Smithies, O. and N. F. Walker. 1955b. Genetic control of some serum proteins in normal humans. Nature 176: 1265-1266.

Smithies, O. and N. F. Walker. 1956. Notation for serum-protein groups and the genes controlling their inheritance. Nature 178: 694-695.

Snedecor, G. W. 1956. Statistical Methods applied to Experiments in Agriculture and Biology. 5th edition. Iowa State College Press. Ames, Iowa. 534 p.

Spurway, H. 1952. Can wild animals be kept in captivity? New Biology 13: 11-30.

Stallcup, W. B. 1954. Myology and serology of the avian Family Fringillidae, a taxonomic study. Univ. of Kansas Publications - Museum of Natural History 8: 157-211.

Stephen, W. P. 1961. Phylogenetic significance of blood proteins among some Orthopteroid insects. Systematic Zool. 10: 1-9.

Sutton, H. E., Neel, J. V., Binson, G., and W. W. Zuelzer. 1956. Serum protein differences between Africans and Caucasians. Nature 178: 1287.

Sutton, H. E., Neel, J. V., Livingstone, F. B., Binson, G., Kunstadter, P., and L. E. Trombley. 1959. The frequencies of haptoglobin types in five populations. Ann. of Human Genetics 23: 175-183.

Sutton, H. E., Matson, G. A., Robinson, A. R., and R. W. Koucky. 1960. Distribution of haptoglobin, transferrin, and hemoglobin types among Indians of Southern Mexico and Guatemala. Am. J. of Human Genetics 12: 338-347.

Taber, R. D. 1953. Studies of black-tailed deer reproduction on three chaparral cover types. Calif. Fish and Game 39: 177-186.

Taber, R. D. and R. F. Dasmann. 1958. The black-tailed deer of the chaparral. Calif. Fish and Game Bull. 8: 1-163.

Thompson, S., Foster, J. F., Gowen, J. W., and O. E. Tauber. 1954. Hereditary differences in serum proteins of normal mice. Soc. for Exp. Biol. and Med. 87: 315-317.

Tiselius, A. 1937. A new apparatus for electrophoretic analysis of colloidal mixtures. Trans. Faraday Soc. 33: 524-531.

van Sande, M. and D. Karcher. 1960. Species differentiation of insects by hemolymph electrophoresis. Science 131: 1103-1104.

Weech, A. A., Goettsch, E., and E. B. Reeves. 1935. Nutritional edema in the dog. I development of hypoproteinemia on a diet deficient in protein. J. Exp. Med. 62: 299-317.

Welling, W. and D. W. van Bekkum. 1958. Different types of haemoglobin in two strains of mice. Nature 182: 946-947.

Wieland, T. 1959. Applications of zone electrophoresis. p. 493-530. In M. Bier (ed.), Electrophoresis - Theory, Methods, and Applications. Academic Press, New York and London.

Williams, C. A., Jr. and P. Graber. 1956. Immunoelectrophoretic studies on serum proteins. 1. the antigens of human serum. J. Immun. 74: 158-168.

Wolfe, H. R. 1939. Serologic relationships among Bovidae and Cervidae. Zoologica 24: 309-321.

Wood, A. J., Nordan, H. C., and I. McT. Cowan. 1961. The care and management of wild ungulates for experimental purposes. J. Wildlife Management 25: 295-302.

Woods, K. R., Paulsen, E. C., Engle, R. L., Jr., and J. H. Pert. 1958. Starch gel electrophoresis of some invertebrate sera. Science 127: 519-520.

Wunderly, C. 1959. Paper electrophoresis. p. 179-223. In M. Bier (ed.), Electrophoresis - Theory, Methods, and Applications. Academic Press, New York and London.

Zweig, G. and J. W. Crenshaw. 1957. Differentiation of species by paper electrophoresis of the serum proteins of Pseudemys turtles. Science 126: 1065-1067.

APPENDIX A

EFFECT OF CAPTIVITY - mobilities in 10^{-5} cm^2 / volt seconds.

FRACTION		9	8	7	6	5	4	3	2	+1	-1	2
wild, female, adult, *O.h. columbianus*, Wolf Lake, B.C.	N = 2 $\bar{X}$	.778	.501	.448	.382	.301	.258	.181	.144	.015	.010	
wild, male, adult, *O.h. columbianus*, Wolf Lake, B.C.	N = 2 $\bar{X}$	.839	.541	.447	.400	.345	.282	.216	.114	.019	.009	
captive, female, adult, *O.h. columbianus*, Wolf Lake, B.C.	N = 11 $\bar{X}$	.924	.517	.465	.439	.395	.297	.241	.164	.018	.015	.087
captive, male, adult, *O.h. columbianus*, Wolf Lake, B.C.	N = 4 $\bar{X}$	.855	.656	.575	.465	.432	.375	.313	.128	.048	.017	.085
wild, male, adult, *O.h. sitkensis*, Kupreanof Is., Alaska	N = 3 $\bar{X}$	.884	.540	.487	.421	.381	.335	.279	.176	.032	.010	
captive, female, adult, *O.h. sitkensis*, Petersburg, Alaska	N = 2 $\bar{X}$	.892	.582	.466	.436	.359	.262	.165	.068	.039	.010	.142
captive, male, adult, *O.h. sitkensis*,	N = 2 $\bar{X}$	.961	.538	.452	.423	.336	.288	.163	.059	.012	.009	.163

N = number of aliquots from one sample from one animal

APPENDIX B

EFFECT OF CAPTIVITY - percent composition in arcs $\sin\sqrt{\text{percentage}}$ degrees.

FRACTION			9	8	7	6	5	4	3	2	+1	-1	2
wild, female, adult, *O.h. columbianus*, Wolf Lake, B.C.	N = 2	$\bar{X}$	39.1	26.0	10.3	6.2	8.4	3.7	17.0	22.4	11.6	15.5	0.0
wild, male, adult *O.h. columbianus*, Wolf Lake, B.C.	N = 2	$\bar{X}$	32.6	20.4	12.3	10.4	12.6	10.9	18.4	20.6	15.8	20.3	0.0
captive, female, adult, *O.h. columbianus*, Wolf Lake, B.C.	N = 11	$\bar{X}$	32.0	12.6	14.3	9.4	7.4	10.6	11.1	19.8	20.8	18.8	22.0
captive, male, adult, *O.h. columbianus*, Wolf Lake, B.C.	N = 4	$\bar{X}$	38.6	9.1	12.0	18.2	13.7	6.7	8.4	15.5	22.9	8.5	19.0
wild, male, adult, *O.h. sitkensis*, Kupreanof Is., Alaska	N = 3	$\bar{X}$	38.6	10.4	19.8	12.9	8.1	5.1	12.4	13.3	20.9	22.8	0.0
captive, female, adult, *O.h. sitkensis*, Petersburg, Alaska	N = 2	$\bar{X}$	30.0	15.4	14.5	16.7	10.3	5.7	15.9	14.4	22.4	20.2	13.4
captive, male, adult, *O.h. sitkensis*, Petersburg, Alaska	N = 2	$\bar{X}$	36.7	10.1	10.1	10.7	7.7	11.2	12.4	13.5	26.2	21.4	14.6

N = number of aliquots from one sample from one animal

APPENDIX C

GEOGRAPHIC SEPARATION, SUBSPECIES, AND SPECIES - mobilities in 10^{-5} cm^2 / volt seconds.

FRACTION	10	9	8	7	6	5	4	3	2	+1	−1	2	3	4
wild,female,adult, *O.h.columbianus*, Wolf Lake, B.C. N = 2	$\bar{X}$	.778	.501	.448	.382	.301	.258	.181	.144	.015	.010			
wild, male, adult, *O.h.columbianus*, Wolf Lake, B.C. N = 2	$\bar{X}$	.839	.541	.447	.400	.345	.282	.216	.114	.019	.009			
wild,male 1,adult, *O.h.columbianus*, Chemainus, B.C. N = 3	$\bar{X}$	.843	.503	.414	.378	.324	.272	.170	.144	.009	.009			
wild,male 2,adult, *O.h.columbianus*, Chemainus, B.C. N = 2	$\bar{X}$	.815	.485	.432	.388	.301	.247	.160	.121	.034	.015			
wild,female,adult, *O.h. crooki*, Arizona N = 3	$\bar{X}$	.870	.504	.436	.404	.381	.252	.171	.145	.035	.010			
wild,male,adult, *O.h. crooki*, Arizona N = 2	$\bar{X}$	.878	.499	.427	.383	.359	.282	.165	.073	.019	.010			
wild,male,adult, *O.h. sitkensis*, Kupreanof Is.,Alaska N = 3	$\bar{X}$	.884	.540	.487	.421	.381	.335	.279	.176	.032	.010			
wild,male,adult, *O.v. leucurus*, Oregon N = 4	$\bar{X}$	.829	.606	.466	.403	.379	.339	.262	.204	.146	.010	.010	.063	.131	.21

N = number of aliquots from one sample from one animal

APPENDIX D

GEOGRAPHIC SEPARATION, SUBSPECIES, AND SPECIES - percent composition in arcs^in $\sqrt{\text{percentage}}$ degrees.

FRACTION	10	9	8	7	6	5	4	3	2	+1	-1	2	3	4
wild,female,adult, O.h.columbianus, Wolf Lake, B.C. N = 2	$\bar{X}$	39.1	26.0	10.3	6.2	8.4	3.7	17.0	22.4	11.6	15.5			
wild,male,adult, O.h.columbianus, Wolf Lake, B.C. N = 2	$\bar{X}$	32.6	20.4	12.3	10.4	12.6	10.9	18.4	20.6	15.8	20.3			
wild,male 1,adult, O.h.columbianus, Chemainus, B.C. N = 3	$\bar{X}$	44.7	21.5	14.7	8.8	7.7	5.4	10.5	9.5	14.8	20.6			
wild,male 2, adult, O.h. columbianus, Chemainus, B.C. N = 2	$\bar{X}$	44.4	16.8	13.6	6.0	6.1	9.0	10.3	13.1	21.2	19.2			
wild,female,adult, O.h. crooki, Arizona N = 3	$\bar{X}$	37.1	17.2	9.2	9.1	9.6	8.2	8.7	15.0	23.6	26.2			
wild,male,adult, O.h. crooki, Arizona N = 2	$\bar{X}$	34.3	19.3	11.8	10.7	10.0	6.7	12.9	12.6	21.2	28.3			
wild,male,adult, O.h.sitkensis, Kupreanof Is.,Alaska N = 3	$\bar{X}$	38.6	10.4	19.8	12.9	8.1	5.1	12.4	13.3	20.9	22.8			
wild,male,adult, O.v. leucurus, Oregon N = 4	$\bar{X}$	31.8	17.3	18.4	18.4	13.2	8.4	13.2	22.4	13.3	4.9	6.6	5.4	12.6	9.4

N = number of aliquots from one sample from one animal