DISSERTATION

AN AGE ESTIMATION TECHNIQUE AND SOME NORMAL BLOOD VALUES FOR MOUNTAIN LIONS (*FELIS CONCOLOR*)

Submitted by

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WE HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER OUR SUPERVISION BY MARY JEAN PFILE CURRIER ENTITLED AN AGE ESTIMATION TECHNIQUE AND SOME NORMAL BLOOD VALUES FOR MOUNTAIN LIONS (FELIS CONCOLOR) BE ACCEPTED AS FULFILLING IN PART REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY.

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ABSTRACT OF THESIS

AN AGE ESTIMATION TECHNIQUE AND SOME NORMAL

BLOOD VALUES FOR MOUNTAIN LIONS (FELIS CONCOLOR)

The objectives of this study were: (1) to devise a method for estimating the ages of individual, wild-caught mountain lions, (2) to identify the normal ranges of various physiological and morphological parameters in the mountain lions, and (3) to determine whether differences in the physiological and morphological parameters measured exist between wild-caught mountain lions captured in south-central Colorado and captive lions in other areas.

The age-estimation formula developed for females included the following blood parameters: globulins, blood urea nitrogen, total proteins, percentage monocytes, and zinc; and morphological measurements: gumline recession from the premolars and rear tarsal length.

The age-estimation formula developed for males included the following blood parameters: globulins, alkaline phosphatases, and percentage neutrophils; and morphological measurements: gumline recession from the upper canine and total body length.

Both formulas had an r^2 of about 0.80.

Blood, hair, and vibrissae samples; and tooth and body measurements were taken from 46 captive and 31 free-ranging mountain lions. Eight animals were sampled each year for three years, 22 for two years, and 50 only once, for a total of 52 female and 34 male captive lion samplings, and 21 female and 11 male wild lion samplings.

The blood samples were evaluated for hematocrit, amount of zinc in the plasma, 18 serum parameters, and white blood cell differentials. The hair and vibrissae samples were stretched until they broke to measure

elasticity. Two upper and two lower teeth were measured for gumline recession and measurements of six body characteristics were made. Normal (mean) values and 95 percent confidence intervals for all 38 parameters were determined.

The entire 118 samplings were divided into several sets of subgroups and selectively tested for significant differences ($P \leq 0.10$) in each of the 38 parameters.

Three male mountain lions were raised from age 3 weeks. Eighteen summer and 17 winter blood samples were taken and tested for summer-winter differences.

Ten blood and two morphological parameters were significantly different between the wild, and captive, non-kitten mountain lions. Two blood and five morphological parameters were significantly different between female, and male, non-kitten mountain lions. Eight blood and all morphological parameters were significantly different between wild kittens and wild non-kittens. Six blood parameters were significantly different between summer and winter blood collections.

Twenty-six parameters (the 12 determined to be significantly different between wild and captive lions were omitted) were initially evaluated for the development of the age-estimation formulas with multiple regression analysis. Of the eight blood parameters found to be significantly different between wild kittens and wild non-kittens, two were included in the female age-estimation formula and three in the male age-estimation formula.

Winter-summer significant differences were probably not seasonal differences, but for the most part due only to restraint or method of immobilization.

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INTRODUCTION

Felis concolor, under the name mountain lion, cougar, puma, el Leon, and many others, has roamed throughout most of the Western Hemisphere since about the time of the Pleistocene extinctions 10,000 years ago (Young and Goldman 1946, Robinson 1976). The subspecies once present throughout much of southeastern U.S. (Felis concolor coryi) and northeastern U.S. (Felis concolor couguar) are both listed as endangered by the United States Department of the Interior (Anonymous 1974). In the 11 western states, all eight subspecies present are listed by the states as game animals (Robinson 1976). The status of the mountain lion in Canada is much the same as in the U.S.: game animals in western Canada, endangered or rare in eastern Canada (the range of Felis concolor couguar probably originally extended into parts of Ontario, Quebec, and New Brunswick (Young and Goldman 1946)). Little is known about the status of this big cat in South America. It has probably been extirpated from heavily populated areas and offered little protection elsewhere.

Confusion and misinformation about this elusive creature abound. Nevertheless, management of the mountain lion is desirable if an optimum level of this species is to be maintained. The principle methods employed to directly manage mountain lions are harvest regulations in the west and full protection in the east. One type of information that is important in order to understand the dynamics of a wildlife population (and therefore to know which management actions would be beneficial) is the sex and age structure of a population in any given geographic area. This, combined with reproductive and mortality data, and population size, can give the manager a clear picture of the vigor

and stability of the population (Eberhardt 1971). This information can be used to calculate the turnover rate of that population, which should form the basis for management through harvest regulation.

Various types of data may be collected for utilization in an age-estimation technique. Body parts are often collected from dead animals, however both physiological and morphological data are available from live specimens. No method has been reported that is adequately objective for estimating the ages of individual mountain lions.

Physiological and morphological data are also important for the analysis of the state of health of an individual animal. Baseline information of normal (mean) blood and blood serum values would provide standards that would aid veterinarians and biologists in the diagnoses of ailments in individual mountain lions. Morphological data can reveal part of the nutritional history of the animal.

The objectives of the study were threefold:

1. To devise a method for estimating the ages of individual, wild-caught mountain lions;
2. To identify the normal ranges of various physiological and morphological parameters in the mountain lion;
3. To determine whether differences in the physiological and morphological parameters measured exist between wild-caught mountain lions captured in south-central Colorado and captive mountain lions in other areas.

METHODS

Subjects

Samples and measurements were taken between 1975 and 1977 from 40 different, known-age, captive mountain lions, and six unknown-age, captive mountain lions. Six females and two males were sampled and measured three consecutive years; 11 females and 10 males, two consecutive years; and 12 females and eight males one year only; for a total of 52 female, and 34 male, captive samplings. The age distribution of the captive, known-age animals was representative (Table 1).

Samples and measurements were taken between 1975 and 1977 from 20 different, free-ranging, females mountain lions, and 11 different, free-ranging, male lions. One female was caught, sampled, and measured in two consecutive years. The total number of wild samplings was, therefore, 21 female and 11 male.

The entire 118 samplings were divided into several sets of subgroups, then tested for differences between them in all of the parameters measured (Table 2). Appropriate subgroups were then subjected to age regression analysis (see Statistical Analyses for specific testing methods).

Blood samples were taken from three captive, hand-raised, male mountain lions born in the Dickerson Park Zoo, Springfield, Missouri, on 12 June 1977, at ages 6, 8, 12, 16, and 20 weeks; and 6, 7, 8, 9, 10, 12, and 14 months. They were weighed at ages 10, 19, 29, 36, 40, 45, 51, 53, 56, 60, 76, and 83-84 days; and 14, 16, 20, 26, 31, 36, 40, 44, 53, and 62 weeks. Tooth eruption pattern was checked weekly until age 1.5 months, then monthly.

TABLE 1. Age distribution of known-age, captive mountain lions sampled.

Age(yrs) No.	1-1.9	2-2.9	3-3.9	4-4.9	5-5.9	6-6.9	7-7.9	8-8.9	9-9.9	> 10
Female	4	2	1	7	7	6	5	4	0	3
Male	3	4	3	3	4	4	5	1	0	2

TABLE 2. Classification of the mountain lions studied and the testing pattern for significant differences between subgroups.

	Female				Male			
	Wild		Captive		Wild		Captive	
	Kitten	Non-kitten	Kitten	Non-kitten	Kitten	Non-kitten	Kitten	Non-kitten
No. sampled once	3	16	0	12	6	5	0	8
No. sampled twice	0	1	0	11	0	0	0	10
No. sampled thrice	0	0	0	6	0	0	0	2
Total samples	3	18	0	52	6	5	0	34
A's vs B's		A		B		A		B
C's vs D's		C		C		D		D
E's vs F's	E	F			E	F		
G vs. H		G		H				
I vs. J						I		J

Immobilization

Three immobilizing agents were used at various times: phencyclidine hydrochloride, ketamine hydrochloride, and tiletamine hydrochloride. The latter two substances are derivatives of the former. Phencyclidine and ketamine were supplied in liquid form, and tiletamine was supplied as a powder. Sterile saline or distilled water was used to hydrate the powder and fill the dart. The tiletamine had been previously mixed with zolazepam, a muscle relaxant, and occasionally a muscle relaxant, either acepromazine or diazepam, was used in conjunction with the other two immobilizing agents.

Drug delivery was by one of four methods. They were: darted with the dart shot from a Cap-Chur[1] pistol or rifle, darted with the dart propelled from a blow gun, hand-syringed with the animal in a squeeze cage, or jabbed with a syringe on the end of a pole (jab stick).

Blood and urine collection and preparation

A 10-15 cc blood sample was drawn from the dorsal branch of the medial saphenous vein in the hind leg at about the level of the knee (Fig. 1 in Appendix A). About 7 cc were put in a plastic tube and allowed to clot. The remainder was put in a plastic tube with two drops of zinc-free heparin. At least two good blood smears were made from either the blood remaining in the syringe immediately after the two tubes had been filled, or from the heparinized sample.

The bladder of seven of the captive males was catheterized with a sterile French size 10 urethral catheter and the bladder emptied. A timed, 1-2 minute sample of urine was collected.

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[1]Palmer Chemical and Equipment Co., Douglasville, GA.

Both the clotted sample and all but 3 cc of the heparinized sample were centrifuged at about 7000 rpm for five minutes. The serum was removed from the top of the clotted blood, labelled, and frozen. The plasma was removed from the top of the heparinized blood, labelled, and frozen. The hematocrit was measured by the standard method using heparinized blood.

Blood smears were stained by the standard method using Wright's stain (Seiverd 1972:233-240). Two hundred leucocyted were differentiated into neutrophilic segmented cells, eosinophilic segmented cells, basophilic segmented cells, lymphocytes, and monocytes. Percentages of each were recorded.

Plasma analysis

Plasma was prepared for zinc analysis according to the method for non-lyophilized plasma of Prasad et al. (1963) with two exceptions: (1) to fully utilize the plasma, the entire amount of plasma, not just 1 ml, was used (this also led to use of varying amounts of trichloroacetic acid to maintain the proper ratio), and (2) the samples were centrifuged 40 minutes at a time instead of 20 minutes because the rpm of the centrifuge used was lower than the suggested rpm. Concentration of zinc in the plasma was determined by use of an atomic absorption spectrophotometer. Dilute solutions of heparin and trichloracetic acid were tested for zinc content.

Serum analyses

Seventeen parameters of the blood serum (Table 3 and Appendix C) were determined by personnel at the C.S.U. Veterinary Hospital with the automated Hycel Mark-17 Discretionary Multi-Channel Analyzer. Albumins were calculated by subtracting globulin values from total protein values.

Triiodothyronine uptake analysis

In 11 instances (seven females and four males), 3 cc of heparinized whole blood were incubated at room temperature with I^{125}-labelled triiodothyronine (T3), using the methods of Soltz et al. (1963). The hematocrit was measured by the standard method, and radioactivity of the washed red blood cells was measured with a solid scintillation counter. The efficiencey of the counting system and the effect of volume on the count were measured using standard, known-activity solutions of various volumes. Radioactivity attached to the red blood cells (percentage T3* uptake) was calculated as percentage of that added from the following formulas.

Expected activity: $A = A_o e^{-\lambda t}$, where:

A = activity/ml

A_o = initial activity/ml

e = 2.718 (the base of natural (Napierian) logarithms)

λ = decay constant

$= \frac{\ln 2}{T_{1/2}}$

$T_{1/2}$ = half-life

t = time elapsed since initial activity measured

Volume red blood cells: $V = hV_{wb}$, where:

V = volume red blood cells

h = hematocrit (fraction red blood cells)

V_{wb} = volume whole blood

System efficiency: $E_V = \frac{A_V}{A}$, where:

E_V = efficiency of system at V ml

A_V = activity at V ml

A = known activity

Percentage T3*uptake: $\frac{Am}{E_V x V x A}$

A_m = measured activity

E_V = efficiency at volume V

V = volume red blood cells

A = expected activity/ml

Urine analysis

The following standard formula for renal clearance of creatinine was used (modified from Ganong 1973:514 by using serum concentrations instead of plasma concentrations).

$$C_{CREA} = \frac{U_{CREA} XV}{S_{CREA}}$$

C_{CREA} = clearance of creatinine

U_{CREA} = concentration of creatinine in urine

S_{CREA} = concentration of creatinine in serum

V = rate of urine production

TABLE 3. Source and symbol of each blood and morphological parameter measured in this study.

Source	Parameter	Symbol
-	Sex	
-	Age	
Plasma	Zinc concentration	ZN
Red blood cells	Hematocrit	HKT
	Triiodothyronine uptake	T3
White blood cells	Percentage neutrophilic segmented cells	NEU
	Percentage eosinophlic segmented cells	EO
	Percentage lymphocytes	LYM
	Percentage monocytes	MONO
Serum	Lactic dehydrogenase	LDH
	Creatine phosphokinase	CPK
	Serum glutamic-pyruvic transaminase	SGPT
	Serum glutamic-oxalacetic transaminase	SGOT
	Alkaline phosphatase	ALK
	Sodium concentration	NA
	Potassium concentration	K
	Chloride concentration	CL
	Calcium concentration	CA
	Phosphorous concentration	P
	Glucose concentration	GLU
	Cholesterol concentration	CHOL
	Bilirubin concentration	BIL
	Creatinine concentration	CREA

TABLE 3. (continued)

Source	Parameter	Symbol
Serum	Blood urea nitrogen concentration	N
	Total protein concentration	PRO
	Globulin concentration	GLOB
	Albumin concentration	ALB
Hair	Young's Modulus	HA
Vibrissae	Young's Modulus	WH
Teeth	Gumline recession from upper canine	UC
	Gumline recession from lower canine	LC
	Gumline recession from upper premolar	UP
	Gumline recession from lower premolar	LP
	Total gumline recession from both canines	C
	Total gumline recession from both premolars	PR
Body	Body weight	WT
	Total body length	LG
	Girth	GI
	Skull arch length	SK
	Right rear tarsal length	TL

Hair and vibrissae

Hair cut from the hind leg prior to blood collection was saved in an envelope. Six vibrissae were snipped off close to the skin and saved in a straw taped on both ends.

Three hairs and three vibrissae from each animal were prepared in the following way. A loose knot was tied at each end, then the hair or vibrissa was glued at the knots to a piece of paper with one or two drops of epoxy glue. After the glue had thoroughly dried, the sample diameters were measured with a micrometer and tested for tensile strength and stretch properties on an Instron Universal Testing Instrument. Load Cell A, capable of measuring up to 500 g tension, was used to test the hairs, and Load Cell B, capable of measuring up to 2000 g tension, was used to test the vibissae. The hair of vibrissa was mounted in the holder, then stretched at a rate of 0.2 in/min until a break occurred. Young's Modulus, a measure of elasticity, was calculated from the following formula (Hodgman et al., ed. 1960:3087).

Young's Modulus of Elasticity: $M = \frac{mgl}{\pi r^2 s}$, where:

M = Young's Modulus N/m^2

m = mass, kg

g = gravitational acceleration, 9.8 m/sec^2

l = length of hair or vibrissa before stretching, m

r = radius of hair or vibrissa before stretching, m

s = elongation, m

Gumline recession

Measurements were taken from four teeth on one side of each mountain lion's mouth: upper canine, lower canine, second upper premolar (the first upper premolar is vestigial), and first lower premolar (Fig. 4 in Appendix A). A stainless steel periodontal probe graduated in millimeters was used to take measurements. All measurements were from the cemento-enamel junction midline, represented by a lateral ridge on the tooth (Fig. 5 in Appendix A), to the gingiva (gumline). If the gingiva extended over the cemento-enamel junction, the measurement was recorded as positive. If the gingiva had receded beyond the cemento-enamel junction, it was recorded as negative.

Body measurements

Six body measurements were made: weight, total body length, tail length, girth, rear tarsal length, and skull arch. Details on how each was measured are included in Appendix A.

Statistical analyses

Multiple regression analysis

Two computer programs were used. One was entitled "STAT 40R: Regression Screen" from the Colorado State University Statistical Laboratory. The other one was entitled "STAT 38R: Stepwise Regression-Version of Nov., 1972, originally BMD02R" also from the Colorado State University Statistical Laboratory. Output of STAT 40R is an ordered list of the n best k-variable regressions for k = 1, 2, ..., n, where n is the number of independent variables. It helps determine the least-biased model. The output of STAT 38R is a stepwise series of regression equations. First STAT 38R was run to determine correlations,

then STAT 40R was run with the 19 variables that were most highly correlated with age (Stat 40R will not accept more that 19 independent variables). STAT 38R was run again with those variables forced into the stepwise equations that STAT 40R had indicated would lead to the least biased model. Thirty-eight variables were initially evaluated (Table 3 and Appendix C). The dependent variable was age. The maximum number of steps for STAT 38R was 76. F-value for both inclusion and deletion was 3.84. Three regressions were run: females, males, and females and males together. STAT 40R was also used to determine if any ond source of data (blood or morphological measurements) could be used to predict age.

Analysis of variance

The Statistical Package for the Social Sciences (SPSS), Version 7.0 - March 15, 1978, computer program from the Colorado State University Statistical Library was used for the analyses of variance. In addition to the 40 variables listed previously, two more variables were added: status (wild or captive), and kitten or non-kitten (non-kitten mountain lions are those without obvious spots and with an adult set of teeth; probably all animals greater than one year of age).

Six differences were tested (Table 1):

Wild, non-kitten mountain lions vs. captive, non-kitten mountain lions;

All non-kitten females (wild and captive) vs. all non-kitten males (wild and captive)

Wild kittens vs. wild non-kittens;

Wild females vs. captive females if both wild-captive and female-male significant differences existed in the morphological measurement;

Wild male vs. captive male if both wild-captive and female-male significant differences existed in the morphological measurement;

Summer (16 April through 14 October) blood collection vs. winter (15 October through 15 April) blood collection from the three captive, hand-raised mountain lions.

The hypothesis that the two sub-groups in each case were drawn from the same population (null hypothesis) was tested, and the probability that the difference was due to chance was calculated with the computer program.

RESULTS

Immobilization

The 77 animals were immobilized 118 times. Thirty-two wild and 22 captive lions were immobilized with tiletamine hydrochloride, 41 captive lions with ketamine hydrochloride, eight captive lions with phencyclidine hydrochloride, and 14 captive lions with various drug combinations (Table 4). Fifty-nine were immobilized with one dose, 44 with more than one, and 14 with an unknown dose (part of the dose was sprayed into the air, or otherwise not delivered).

Tiletamine usually acted within 3-5 minutes, ketamine within 5-10 minutes, and phencyclidine within 15-20 minutes. No drug-attributed mortalities occurred.

TABLE 4. Immobilizing agents, recommended dose rates, and delivered dose rates administered during the study.

Immobilizing Agent	Planned Dose Rate (mg/lb)	Status	Actual Effective Dose Rate (mg/lb)	Number of Animals	Initial Ineffective Dose Rate (mg/lb)	Number of Animals	Number Animals Unknown Dose
Tiletamine	3.0-3.5	wild	2.3-5.5	23	1.2-6.6	9	0
		captive	2.4-5.5	11	2.5-4.7	8	3
Ketamine	7	captive	5.0-8.7	17	4.8-10.0	15	9
Phencyclidine	0.5	captive	0.5-0.8	3	0.3-1.0	4	1
Combination:							
Phencyclidine-Ketamine		captive	0.3P+2.2K-0.8P+1.7K	3	0.3P+1.3K-0.4P+1.5K	7	0
Ketamine-Xylazine		captive	8.6K+0.1X-8.8K+0.1X	2	6.6K+0.1X	1	1
Total numbers:				59		44	14

Blood Parameters

A significant difference (probability that the difference is due to chance is less than or equal to 0.10) existed between the non-kitten, wild and non-kitten, captive mountain lions sampled for 10 blood parameters (Table 5).

TABLE 5. Blood parameters with values that were significantly different between wild and captive mountain lions.

Parameter	P	Number in sample* Wild	Captive
SGOT	0.0000	17	68
NA	0.0000	17	68
CL	0.0000	17	68
BIL	0.0001	17	68
CHOL	0.0027	17	68
HKT	0.0040	18	52
K	0.0072	17	68
CPK	0.0081	17	68
P	0.0675	17	68
LDH	0.1046	17	68

*The number of samples might vary in each case due to some incomplete sampling.

A significant difference ($P \leq 0.10$) existed between summer (16 May through 14 October) and winter (15 October through 15 April) collection of blood from the three male, captive, hand-raised mountain lions for six blood parameters (Table 6).

TABLE 6. Blood parameters and means with values that were significantly different between summer and winter collection of blood.

Parameter	P	Number in sample Winter	Summer	Mean Winter	Summer
K	0.0007	17	18	4.6 mEq/l	5.5 mEq/l
CPK	0.0016	17	18	71 U/l	144 U/l
P	0.0086	17	18	6.6 mg/dl	7.6 mg/dl
LDH	0.0129	17	18	137 U/l	255 U/l
CREA	0.0154	17	18	1.53 mg/dl	2.66 mg/dl
NA	0.0451	17	18	146.8 mEq/l	148.4 mEq/l

A significant difference ($P \leq 0.10$) existed between wild kittens and wild non-kittens for eight blood parameters (Table 7).

TABLE 7. Blood parameters and means with values that were significantly different between wild, kitten and wild, non-kitten mountain lions.

Parameter	P	Number in sample Kitten	Non-kitten	Mean Kitten	Non-kitten
ALK	0.0000	8	11	25.48 U/l	7.58 U/l
GLOB	0.0090	8	11	3.13 g/dl	3.65 g/dl
MONO	0.0205	8	11	0.7 %	1.5 %
LYM	0.0339	8	11	50.7 %	35.4 %
NEU	0.0388	8	11	46.4 %	61.3 %
LDH	0.0354	8	11	241.63 U/l	166.09 U/l
CREA	0.0782	8	11	1.59 mg/dl	2.51 mg/dl
CA	0.1019	8	11	11.81 mg/dl	10.96 mg/dl

A significant difference ($P \leq 0.10$) existed between non-kitten females and non-kitten males for two blood parameters (Table 8).

TABLE 8. Blood parameters with values that were significantly different between non-kitten, femlale and non-kitten, male mountain lions.

Parameter	P	Number in sample Female	Male
K	0.0074	50	29
GLOB	0.0679	50	29

The correlation coefficient between triiodothyronine uptake and age in the 11 mountain lions tested was 0.105.

The normal (mean) value and the 95 percent confidence interval of each blood parameter evaluated are listed in Table 9. In the cases where a significant difference existed between wild and captive, or female and male, the normal value and confidence interval for each are given.

Urine Analysis

Catheterization of the bladder was not possible in the females, and quite difficult in the males. The results in Table 10 were obtained from the seven males in which it was accomplished.

TABLE 9. Source, normal (mean) values, and 95 percent confidence intervals of blood parameters of non-kitten mountain lions.

Source	Parameter	Units	Status	Mean	95% Confidence Interval	Number in sample*
Plasma	ZN	µg/ml	all	1.66	1.18 - 2.14	85
Whole blood	HKT	%	wild	46.9	44.9 - 48.8	18
			captive	41.8	38.9 - 43.7	52
White blood cells	NEU	%	all	60.7	58.5 - 52.9	85
	EO	%	all	2.3	1.9 - 2.7	92
	LYM	%	all	35.1	32.7 - 37.5	92
	MONO	%	all	1.9	1.6 - 2.2	92
Serum	LDH	U/l	wild	165	133 - 197	17
			captive	141	128 - 153	68
	CPK	U/l	wild	108	82 - 135	17
			captive	62	46 - 78	68
	SGPT	U/l	all	52	48 - 57	85
	SGOT	U/l	wild	68	58 - 79	17
			captive	44	40 - 47	68
	ALK	U/l	all	6.1	4.9 - 7.3	85
	NA	mEq/l	wild	144.6	143.4 - 145.7	17
			captive	147.5	147.0 - 148.0	68
	K	mEq/l	wild	4.6	4.4 - 4.7	17
			captive	4.2	4.1 - 4.3	68
			female	4.4	4.3 - 4.6	50
			male	4.1	3.9 - 4.2	29
	CL	mg/dl	wild	114.2	111.9 - 116.5	17
			captive	118.3	117.6 - 119.1	68
	CA	mg/dl	all	10.8	10.6 - 11.1	85
	P	mg/dl	wild	5.6	4.8 - 6.3	17
			captive	4.8	4.4 - 5.2	68
	GLU	mg/dl	all	145	135 - 154	92
	CHOL	mg/dl	wild	167	148 - 185	17
			captive	203	192 - 214	68
	BIL	mg/dl	wild	0.3	0.2 - 0.3	17
			captive	0.6	0.5 - 0.7	68
	CREA	mg/dl	all	2.77	2.46 - 3.07	85
	N	mg/dl	all	33.0	30.0 - 36.1	85
	PRO	g/dl	all	7.2	7.1 - 7.3	85
	GLOB	g/dl	female	3.83	3.70 - 3.98	50
			male	4.06	3.85 - 4.27	29
	ALB	g/dl	all	3.16	3.03 - 3.30	70

*The number of samples may vary in each case due to some incomplete sampling.

TABLE 10. Creatinine clearance in seven male mountain lions.

Age (years)	Clearance (ml/min)
1-2	494
3-4	248
4-5	30
5-6	4.6
6-7	405
7-8	200
12-13	7

Morphology

A significant difference ($P \leq 0.10$) existed between non-kitten, wild and non-kitten, captive mountain lions for two morphological parameters measured (Table 11).

TABLE 11. Morphological parameters with values that were significantly different between wild and captive mountain lions.

Parameter	P	Number in sample	
		Wild	Captive
GI	0.0000	21	68
WT	0.0218	21	68

A significant difference ($P \leq 0.10$) existed between wild kittens and wild non-kittens for five morphological parameters measured (Table 12).

TABLE 12. Morphological parameters with values that were significantly different between wild, kitten and wild, non-kitten mountain lions.

Parameter	P	Number in sample	
		Kittens	Non-kittens
WT	0.0000	8	11
LG	0.0001	8	11
GI	0.0015	8	11
SK	0.0042	8	11
TL	0.0114	8	11

A significant difference ($P \leq 0.10$) existed between non-kitten females and non-kitten males for five morphological parameters (Table 13).

TABLE 13. Morphological parameters with values that were significantly different between non-kitten, female and non-kitten, male mountain lions.

Parameter	P	Number in sample	
		Females	Males
WT	0.0000	50	29
LG	0.0000	50	29
GI	0.0000	50	29
SK	0.0000	50	29
TL	0.0000	50	29

The normal (mean) value and the 95 percent confidence interval of each morphological parameter evaluated are listed in Table 14. In the cases where a significant difference existed between wild and captive, or female and male lions, the normal value and confidence interval for each is given.

TABLE 14. Normal (mean) values and 95 percent confidence intervals of morphological parameters measured for non-kitten mountain lions.

Parameter	Units	Status	Mean	95% Confidence Interval	Number in Sample[1]
WH	N/m^2	captive	2.0×10^6	1.5×10^6-2.5×10^6	68
HA	N/m^2	captive	2.7×10^6	2.1×10^6-3.4×10^6	68
UC	mm	all	(-0.7)	(-1.1)-(-0.3)	91
LC	mm	all	(-0.6)	(-0.9)-(-0.3)	91
UP	mm	all	(-0.4)	(-0.7)-(-0.2)	91
LP	mm	all	(-0.5)	(-0.7)-(-0.3)	91
C	mm	all	(-1.3)	(-1.9)-(-0.6)	91
PR	mm	all	(-1.0)	(-1.5)-(-0.5)	91
WT	kg	wild			
		female	40.5	36.4 - 44.5	16
		male	61.6	57.7 - 65.4	31[2]
		captive			
		female	45.5	42.1 - 48.9	39
		male	61.6	57.7 - 65.4	31[2]
LG	cm	female	199	195 - 203	50
		male	222	218 - 226	29
GI	cm	wild			
		female	69.9	66.7 - 73.1	16
		male	76.0	73.7 - 78.3	4
		captive			
		female	78.3	75.3 - 81.2	39
		male	85.7	83.7 - 87.8	27
SK	cm	female	17.4	17.1 - 17.7	50
		male	20.7	20.1 - 21.2	29
TL	cm	female	17.7	17.3 - 18.0	50
		male	19.4	19.0 - 19.8	29

[1]The number of samples may vary in each case due to some incomplete sampling.

[2]Wild male values were not significantly different ($P \leq 0.10$) from captive male values.

Age-Estimation Formula

Female

Values of 25 parameters from 39 known-age, captive, non-kitten, female mountain lions were used to construct a model from which the ages of individual, female mountain lions could be estimated. None of the parameters that were significantly different between the wild and captive mountain lions were used. The following is the least-biased model that resulted.

$$\hat{Y} = (-7.39) + \underbrace{3.63GLOB + 0.12N - 1.56PRO + 0.37MONO + 0.31ZN}_{\text{blood}} - \underbrace{0.71PR}_{\text{teeth}} + \underbrace{0.27TL}_{\text{body}}$$

$\hat{Y}$ = age estimate in years

The coefficient of multiple determination (r^2), or the percent of variability in age due to the independent variables in the model (PR, GLOB, etc.) is 81 percent. The standard error of the estimate is 1.55.

The least-biased formula using only blood parameters of the female mountain lions resulted in an r^2 of only 51 percent. Using only morphological parameters (teeth, body, vibrissae, and hair measurements), the r^2 was only 56 percent.

Male

Values of 25 parameters from 29 known-age, captive, non-kitten, male mountain lions were used to construct a model from which the ages of individual, male mountain lions could be estimated. None of the parameters that were significantly different between wild and captive lions were used. The following is the least-biased model that resulted.

$$\hat{Y} = 3.43 + \underbrace{0.12NEU - 0.28ALK + 2.07GLOB}_{\text{blood}} - \underbrace{0.85UC}_{\text{teeth}} - \underbrace{0.06LG}_{\text{body}}$$

$\hat{Y}$ = age estimate in years

The coefficient of determination (r^2) is 80 percent. The standard error of the estimate is 1.45.

The least-biased formula using only blood parameters of the male mountain lions resulted in an r^2 of only 39 percent. Using only morphological parameters, the r^2 was only 49 percent.

Kittens: Growth and Tooth Development

The following tables and figures (Tables 15, 16, 17, 18, and 19; Figures 1 and 2) can be used to illustrate the weight increases, tooth development, and eye color and pelage changes in the three hand-raised, male mountain lions from ages 10 days to 14 months. Mitzli, Schunta, and Radar are their names.

TABLE 15. Weights of the three hand-raised, male mountain lions during the first 84 days of life.

Age (days)	Weight (kg)		
	Mitzli	Schunta	Radar
10	1.48	1.31	1.36
19	1.65	1.65	1.65
29	2.00	2.10	2.05
33		2.55	
36	2.45	2.72	2.45
40	2.98	3.15	2.83
45	3.22	3.20	3.20
51	3.80	3.80	3.70
53	4.05	4.00	4.00
56	4.40	4.30	4.25
60	4.95	4.80	4.80
64	5.20	5.00	5.10
76	6.35	6.30	6.25
83			7.40
84	7.60		

Figure 1. Weights of the three hand-raised, male mountain lions during the first 84 days of life.

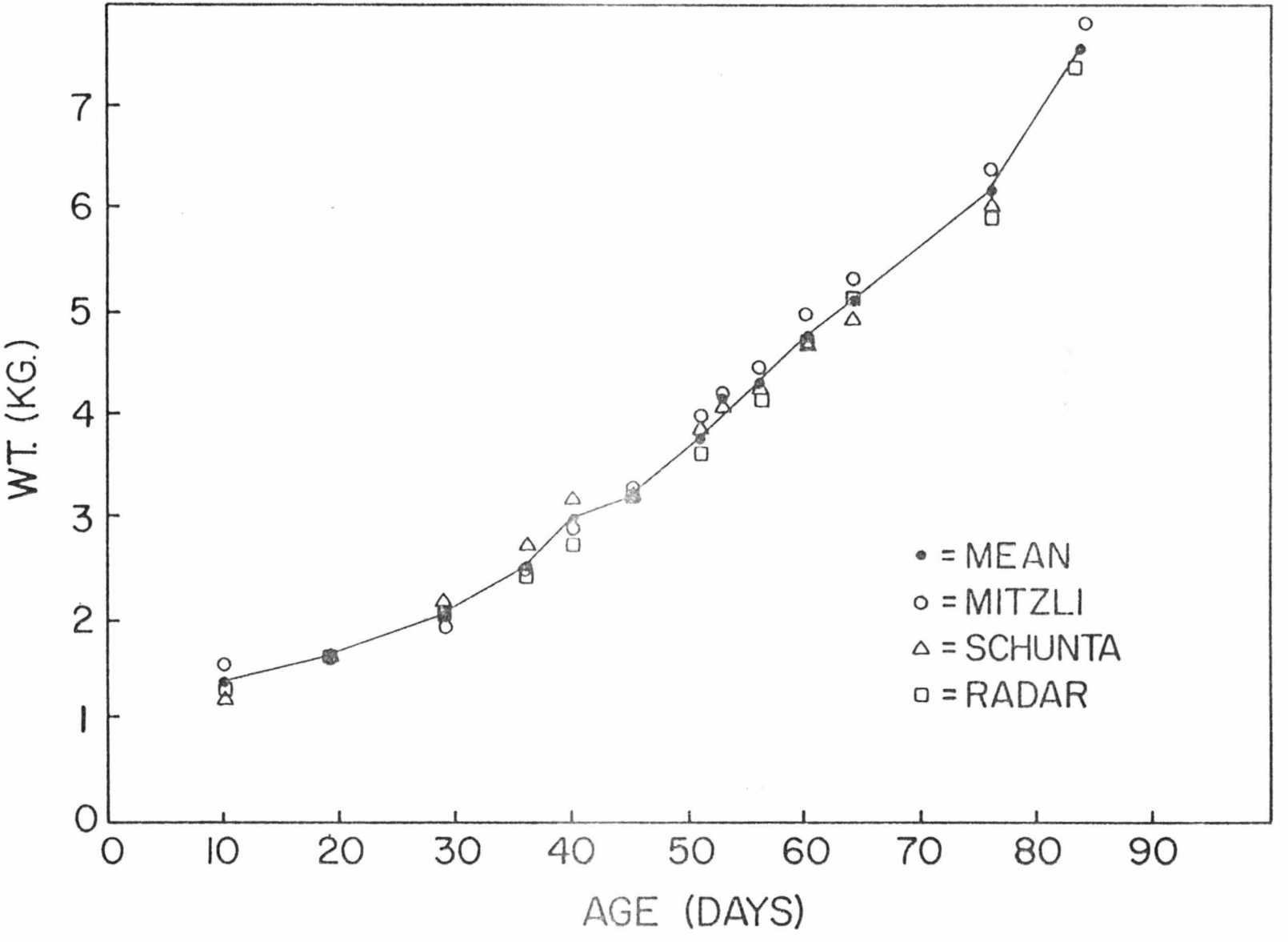

WT. (KG.)
0
1
2
3
4
5
6
7
0
10
20
30
40
50
60
70
80
90
AGE (DAYS)
• = MEAN
○ = MITZLI
△ = SCHUNTA
□ = RADAR

TABLE 16. Weights of the three hand-raised, male mountain lions from age 4 weeks through age 62 weeks.

Age (weeks)	Weight (kg)		
	Mitzli	Schunta	Radar
4	2.0	2.1	2.1
8	4.4	4.3	4.3
14	10.0	9.5	9.5
16	11.8	11.8	12.7
20	16.4	16.4	15.9
26	25.5	25.0	25.0
31	30.9	30.9	30.9
36	35.5	33.6	36.4
40	39.5	38.2	41.8
44	42.7	40.9	43.6
53	50.9	50.5	48.2
62	54.5	56.3	52.7

TABLE 17. Tooth eruption and replacement schedule of the three hand-raised, male mountain lions.

Age	Erupted teeth	Type of tooth
17 days	upper and lower incisors	primary
23 days	upper and lower canines	
30 days	lower premolars	
33 days	upper premolars	
5½ months	upper and lower (just erupting) incisors	permanent
8 months	lower canines	
8½ months	upper canines (for a short time, both primary and permanent remained)	

Figure 2. Weights of the three hand-raised, male mountain lions from age 4 weeks through age 62 weeks.

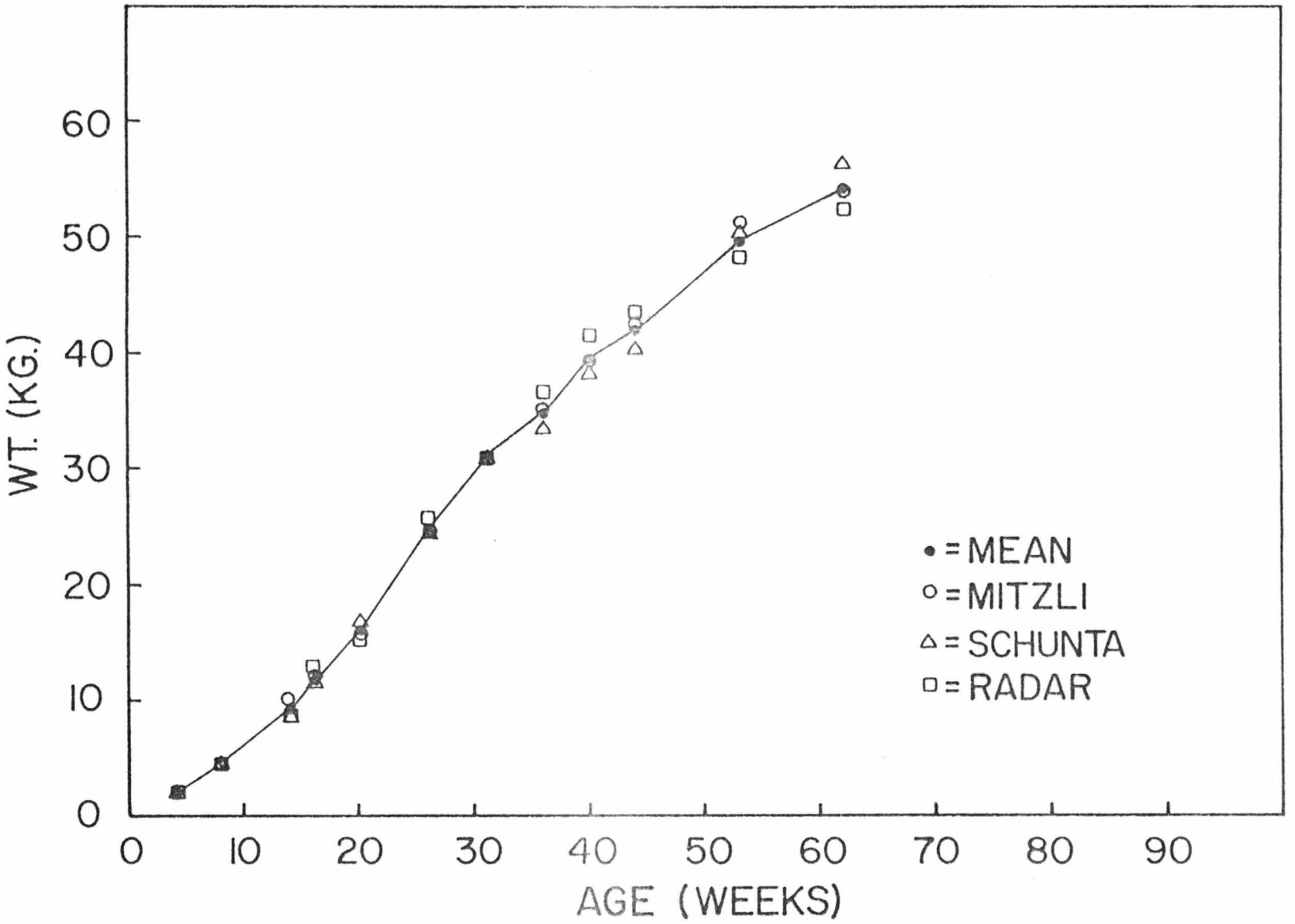

WT. (KG.)
0
10
20
30
40
50
60
0
10
20
30
40
50
60
70
80
90
AGE (WEEKS)
• = MEAN
○ = MITZLI
△ = SCHUNTA
□ = RADAR

TABLE 18. Eye color changes in the three hand-raised, male mountain lions.

Age	Color
Birth	Entire iris blue
3 months	Approximately 3mm next to contracted pupil brown
4 months	Approximately 5 mm next to contracted pupil brown
? months	Entire iris brown
9 months	Approximately 3 mm gold ring around brown

TABLE 19. Pelage changes in the three hand-raised, male mountain lions.

Age	Color
Birth	Dark gold with vivid black spots
12-14 weeks	Spots faded, still visible
1 year	Spots on body barely visible, but leg stripes still obvious

TABLE 20. Estimated ages in years of unknown-age, non-kitten mountain lions.

		1975		1976		1977	
		Age est.		Age est.		Age est.	
Number	Status	formula	Appearance	formula	Appearance	formula	Appearance
Females							
107	captive	4.7		9.6			
108	captive	9.1		8.9			
109	captive	3.9		5.3			
112	captive	6.4		7.5			
1*	wild	3.3	2-3				
2*	wild	9.5	9-10				
4*	wild			3.7			
5*	wild			8.0	5-6		
8	wild			0.5	2-3		
9	wild			6.5	6-7		
10	wild			3.0	2-3		
11	wild			8.0	9-10		
12	wild			2.1	3-4		
15	wild			10.1	3-4		
17	wild			13.4	9-10	8.3	5-6
20	wild			4.9	2-3		
21	wild			13.8	8-9		
23	wild					3.9	2-3
29	wild					5.8	4-5
32	wild					4.5	3-4
Males							
110	captive	4.2		9.7			
6	wild			6.9	6-7		
7	wild			6.6	9-10		
16	wild			8.4	8-9		
18	wild			6.8	2-3		
19	wild			2.6	2-3		

*incomplete data, some variables estimated

DISCUSSION

Immobilization

Unlike many anesthetic drugs, phencyclidine hydrochloride, ketamine hydrochloride, and tiletamine hydrochloride do not depress respiratory or cardiac functions. They may cause hallucinations.

Phencyclidine, the parent compound, is the most potent anesthetic of the group. We used a dose rate of 1.1 mg/kg of phencyclidine, 4.4 - 5.1 mg/kg of tiletamine (it was combined with a muscle relaxant in a ratio of 2:1, for a total dose rate of 6.6 - 7.1 mg/kg), and 15.4 mg/kg of ketamine. The volumes of drugs in solution necessary to immobilize a 45 kg mountain lion were 0.5cc phencyclidine, 2cc or less tiletamine (the tiletamine-muscle relaxant was supplied as a powder, so we mixed it with enough distilled water or saline to fill a 2cc dart), and 7cc ketamine.

The drugs of the phencyclidine family are central nervous system exciting agents. They cause the animal to go through an excitation phase, then into a hallucinatory and catatonic phase. Central nervous system depressants cause the animal to go through the excitatory and hallucinatory (catatonic) phases, then into one or two depressant phases (these are the phases in which respiratory and cardiac functions are affected). Increased doses of phencyclidine family drugs result in further excitation rather than depression, so convulsions can result.

Phencyclidine and its derivatives cause excess salivation, but they do not affect the pharyngeal or laryngeal reflexes (Corssen et al, 1968). The animal can still swallow.

Sometimes a tranquilizing (muscle relaxant) agent, acepromazine, was used in conjunction with phencyclidine or ketamine. The purpose was to forestall possible convulsions.

Tiletamine combines the favorable qualities of the other two drugs: a low volume is required like phencyclidine, thus allowing better accuracy and more distance with the dart, but it is fast acting like ketamine, so the animal is not able to travel very far after administration of the drug (not as important in a captive situation, but possibly the difference between success or failure in finding the animal in the wild).

Blood and Urine Parameters

Significance

Each of the 23 blood or urine parameters evaluated reflected some condition of the internal environment of the mountain lion's body. Before the differences between wild and captive, female and male, kitten and non-kitten, can be interpreted, the role of each parameter must be understood.

Zinc, a mineral essential to mammals in trace amounts, is incorporated in several metaloenzymes, such as carbonic anhydrase (important in the blood buffer system). Although trace amounts are necessary in the diet, excessive ingestion of zinc does not seem to result in excessive uptake of zinc from the gut. Low levels of zinc in the plasma indicate either a zinc-deficient diet or a problem with zinc absorption from the digestive tract. (Bos et al. 1977, Brown et al. 1978, Kang et al. 1977, Deeming and Weber 1977).

The thyroid gland secretes two main hormones: thyroxin and triiodothyronine (T3). Most of the secreted T3 is bound to plasma protein, but red blood cells also have T3-specific binding sites. Both hormones have

very widespread, general effects on the body: they stimulate oxygen consumption in almost all metabolically active tissues and affect growth and maturation. Soltz et al. (1963) have shown that T3* uptake by the red blood cells increases during the growth phase of rats, then decreases steadily thereafter; in humans, the red blood cell uptake of T3* was higher in young adults than old ones. In both cases a negative correlation existed between age and T3* uptake in adults. In the 11 mountain lions tested in this study, almost no correlation between age and T3* uptake was observed (the small positive correlation was probably a sampling artifact). U.S.Seal (Veterans Administration Hospital, Minneapolis, Personal communication) found that thyroid activity varies more with coat molt changes in the carnivores he has studied, than with age.

The circulating white blood cell population is composed of five types of cells: neutrophilic granulocytes (also called neutrophilic segmented cells), eosinophilic granulocytes (segmented cells), basophilic granulocytes (segmented cells), lymphocytes, and monocytes. Neutrophils quickly phagocytize bacteria in the blood stream. An increased percentage of neutrophils is seen during acute infections, such as pneumonia or abcesses, and granulocytic leukemia. Monocytes also phagocytize bacteria and debris, but are usually slower in acting than neutrophils. An increase in the percentage of monocytes is seen in tuberculosis, monocytic leukemia, and subacute endocarditis. Eosinophils apparently phagocytize antigen-antibody complexes, so increased levels of eosinophils are often seen when an animal has either an allergic reaction or a parasitic infection. Daily and seasonal fluctuations in eosinophils have been noted in humans (Diem, ed. 1962). Basophils contain heparin, but their function is not well understood.

An increase of circulating basophils is seen in chronic granulocytic leukemia, polycythemia vera (a disease which causes an increase in red cell mass and total blood volume), irradiation, hemolytic anemia, and after removal of the spleen. Lymphocytes contain antibodies, and are increased in lymphocytic leukemia and in acute infectious lymphocytosis. (Diem, ed. 1962).

The hematocrit is the volume percentage of red blood cells in the whole blood. It is increased in acclimitization to high altitudes. Polycythemia (an increase in the total number of circulating red blood cells) can cause an increase in the hematocrit, and anemia can cause a reduction in the hematocrit. The hematocrit is affected by the size and shape of the red blood cells, so any disease which alters the size or shape of the red blood cells, genetic or otherwise (such as sickle cell anemia), will affect the hematocrit. Dehydration (often the result of excessive vomitting or diarrhea, or diseases causing a high body temperature) causes an elevated hematocrit. In humans, women generally have a lower hematocrit than men possibly due to menstrual bleeding, which doesn't occur in other animals. At birth, the hematocrit in humans is quite high, then it drops until about age 1 year, at which time it begins a steady rise until maturity. (Diem, ed. 1962).

Lactic dehydrogenase (LDH) is an enzyme found in most animal tissues, but the highest concentration is in the heart, liver, kidney, and skeletal muscle. If any of these tissues are damaged, LDH is released into the blood stream. The serum LDH level will begin to rise two to three days after a heart attack, and will remain elevated for about two weeks. Liver and kidney diseases also cause an elevation

of serum LDH levels, as does cancer. Strenuous exercise increases LDH release from skeletal muscle. (Anonymous 1976d, Diem, ed. 1962).

Creatine phosphokinase (CPK) is involved with ATP formation. It catalyzes formation of creatine and ATP from creatine phosphate and ADP. CPK is found mainly in skeletal muscles, and is released into the bloodstream following injury or disease of the muscles and during exercise. Muscular dystrophy is such a disease that causes an increase in CPK levels in the serum. CPK also increases after a heart attack, beginning 4 to 6 hours later, and peaking 24 to 36 hours after the attack. It returns to normal in about three days. (Anonymous 1976a).

Glutamic-pyruvic transaminase (SGPT) is present in the liver and, to a lesser extent, the kidney, heart, and skeletal muscle. Elevated SGPT levels indicate liver damage. (Anonymous 1974b, Diem, ed. 1962).

Glutamic-oxalacetic transaminase (SGOT) is a widely-distributed enzyme, but the greatest concentrations of it are found in the heart and liver, with slightly lower concentrations in skeletal muscle, kidney, pancreas, spleen, and lung. Greatly elevated SGOT levels indicate liver or muscle damage (if SGPT is also elevated, it is liver damage, otherwise probably muscle). Moderately elevated SGOT levels result from muscle injuries, muscular dystrophy, pulmonary emboli (obstruction of the pulmonary artery), or pancreatitis. (Anonymous 1976e, Diem, ed. 1962).

Several enzymes are grouped under the term alkaline phosphatases (ALK), but they all catalyze the conversion of organic phosphate esters to alcohol at an alkaline pH. ALK levels are normally highest in growing animals with a lot of bone development and during pregnancy. Pathologically high levels of ALK are found in obstructive jaundice (blockage of bile flow), bone cancer, osteomalacia (softening of fully-formed bones), parenchymal liver disease, and hyperthyroidism. (Anonymous 1977a,

Diem, ed. 1962). Serum levels rise in cases of vitamin D deficiency (Searcy 1969), and fall with zinc deficiency (Bos et al. 1977) in humans.

The major cation of the extracellular fluid is sodium. Lowered serum levels of sodium result from diabetes insipidus, metabolic acidosis (in some cases, in other cases metabolic alkalosis causes it), Addison's disease (hypofunction of the adrenal cortex), diarrhea, and renal tubular disease. Elevated serum sodium levels are found in hyperadrenalism, severe dehydration, and certain types of brain injury. (Anonymous 1974a).

Potassium is the major intracellular cation. Prolonged diarrhea or vomitting, hyperadrenalism, and some types of alkalosis result in lowered serum levels of potassium. Addison's disease, oliguria (reduced urine secretion), anuria (no urine secretion), and urinary obstruction cause elevated serum potassium levels. Any change in the serum level of potassium causes a disturbance in myocardial function. (Anonymous 1974d, Diem, ed. 1962). Potassium levles are low in the serum of malnourished infants (Searcy 1969).

Chloride is the major extracellular anion. High serum levels are seen in dehydration and conditions causing decreased renal flow (such as congestive heart failure or renal tubular disease). Low chloride levels are found in cases of salt-losing nephritis, metabolic acidosis (and in some cases metabolic alkalosis), and prolonged vomitting. (Anonymous 1975c, Diem, ed. 1962).

Calcium exists in the serum in three forms: a protein-bound fraction, a fraction complexed with ions such as citrate or phosphate, and an ionized fraction (Ca^{++}). Calcium levels are high in growing mammals, then level off as growth stops. Hyperparathyroidism (overproduction of the parathyroid glands) causes an initially elevated level of calcium in the serum, but as the body's supply of calcium is exhausted, the serum

level may fall below normal. Too much vitamin D, multiple myeloma (cancer of the bone marrow), and some other cancers of the bone also can cause high levels of serum calcium. Low levels are observed in hypoparathyroidism, steatorhea (excess fat in the feces), nephrosis, nephritis (both diseases of the kidney), and pancreatitis (inflammation of the pancreas). (Anonymous 1975b, Diem, ed. 1962). Calcium levels in the serum decreased in nutritionally deficient wolves (Seal et al. 1975).

Most of the body's phosphorous is present as calcium phosphate in the bones. The remaining phosphorous is involved in carbohydrate metabolism, phospholipid and nucleic acid formation, ATP production, and other important processes. Phospholipids and inorganic phosphorous are present in the serum. A decrease in the calcium in the serum is accompanied by an increase in serum inorganic phosphorous. Therefore, too much vitamin D, hypoparathyroidism, and renal failure result in an increase in inorganic phosphorous in the serum as well as a decrease in serum calcium; and rickets and hyperparathyroidism initially result in lowered inorganic phosphorous levels. Later, as calcium supplies are exhausted and the calcium level drops, the phosphorous levels rise. (Anonymous 1976c, Diem, ed. 1962). Amount of phosphorous in the diet has a slight effect on serum levels (Searcy 1969).

The serum glucose level is regulated by insulin and various other hormones. The liver is the major site of serum glucose regulation. The serum glucose level is elevated during diabetes, and lowered during Addison's disease. It is also slightly higher after eating. (Anonymous 1976b, Diem, ed. 1962).

Cholesterol is the principle animal sterol, $C_{27}H_{46}O$. Many things affect the cholesterol level in the serum, including age, sex, diet, reproductive status, stress, time of day, and season. It is decreased

by thyroid hormones and estrogens. It is elevated by obstructed biliary flow, by untreated diabetes mellitus, and by hypothyroidism. (Anonymous 1977b, Diem, ed. 1962). Serum levels of cholesterol increased in swine fed diets high in fat and vitamin D_3 (Huang et al, 1977). Caloric restriction reduced serum cholesterol in children (Widhalm et al. 1978).

Bilirubin is a pigment produced by the degradation of hemoglobin in the reticuloendothelial cells (highly phagocytic cells found in the liver, pituitary and suprarenal glands, and elsewhere). Serum bilirubin levels are usually elevated after birth, but rapidly drop. Obstructive jaundice, hemolytic jaundice, neonatal jaundice, and hepatitis cause elevated serum levels of bilirubin. (Anonymous 1975a, Diem, ed. 1962).

Creatinine is formed from creatine phosphate during muscle contraction. It is passively removed from the blood plasma by glomerular filtration and excreted in the mountain lion (in humans, it is also actively removed from the blood). High protein intake increased creatinine in humans (Searcy 1969). Serum levels of creatinine do not increase until kidney function is greatly impaired. Creatinine level measurement is the most unreliable measurement of the Hycel machine, so not to be depended upon unless it is found to be very elevated in several trials. (Anonymous 1975d, Diem, ed. 1962).

Amino acids are deaminated by the liver to form urea, which is then carried by the blood to the kidney. There it is filtered through the glomerulus, then partially reabsorbed. Serum urea nitrogen levels are directly related to protein intake. The serum level tripled within 12 hours after ingestion of 230 g of readily digested protein in humans (Searcy 1969). Increased serum urea nitrogen levels are seen in cases of renal insufficiency. (Anonymous 1975e, Diem, ed. 1962).

Two types of serum proteins exist: albumins and globulins. They serve as part of the body's amino acid pool and can be broken down and used to build new proteins, or broken down for energy, or transformed into carbohydrates or lipids. Some serum proteins carry vital ions, hormones, or lipids from one part of the body to another. They are also important in maintaining osmotic pressure and pH of the blood. Dehydration causes an increase in the concentration of serum proteins (Arhammer et al. 1972, Seal et al. 1975). Various diseases and injuries cause a decrease in serum protein levels: kidney disease causes albumin loss through the kidneys, and severe burns or bleeding also cause a drop in serum proteins. Inadequate intake of protein is also reflected in the serum protein level. (Anonymous 1976f, Dien, ed. 1962).

Globulins are one of the two fractions of serum proteins. They are further subdivided into α_1, α_2, β, and γ globulins. Many of the globulins are sythesized in the liver. Serum globulin levels are moderately increased in many infections, mainly due to increased antibody formation (antibodies are γ-globulins). Large elevations of serum globulins are caused by multiple myeloma (a type of bone cancer). Globulins increase during pregnancy and stress. (Anonymous 1974c, Diem, ed. 1962).

Albumins, the second fraction of serum protein, are reduced in the serum in animals with cancer, but collect abundantly in the cancer cells themselves (Diem, ed. 1962).

Healthy kidneys are important in maintaining a proper balance in the internal environment of an animal. Several methods have been developed to ascertain the state of health of the kidney without actually examining it, but in order to understand those methods, one must first understand how the kidney functions. Blood comes into intimate contact

with the kidneys at the glomerulus. There, it is passively filtered, and a fluid resembling plasma is carried on down the renal tubules. Some substances, such as glucose, are then removed from the filtrate and returned to the blood in capillaries surrounding the tubules while other substances, such as hydroxyindoacetic acid (the prinicple metabolite of the vasoconstrictor, serotonin), are actively removed from the blood and secreted into the filtrate. (Ganong 1971).

To measure the efficiency of glomerular filtration, a major determinant of kidney health, one can measure the concentration in the plasma or serum of a substance that is neither actively reabsorbed nor secreted in the kidney, and compare it with the concentration in the urine. Such a substance in many animals, including the mountain lion, is creatinine. Unfortunately, there are three difficulties in using creatinine clearance. We could not catheterize the females (the opening to the ureter was too recessed in the vagina). As stated previously, the Hycel machine is fairly unreliable as far as creatinine measurements are involved. It is also very difficult to completely empty the bladder, so faulty measurements are often made. Therefore, the test was discontinued after seven trials. Macy (1978) used a more reliable method to test renal function. It involved intravenous administration of sodium sulfanilate, then collection of blood samples 30, 60, and 90 minutes after injection. The sulfanilate clearance halftime was then calculated, and used as an indication of renal function.

Differences

1. Wild-Captive

Although nine of the 22 blood parameters evaluated in the wild mountain lions studied were significantly different from those of the captive mountain lions, the only one that is of great biological significance is cholesterol. It is probably due to a difference in diet and the sedentary life of the captive lions. Further difference might be due to a difference in amount and frequency of meals.

In domestic cats, SGOT levels can go as high as 100 U/l without being abnormal, so the difference between the means of 68 U/l (wild) and 44 U/l (captive) is not really remarkable. Likewise the differences in CPK (108 U/l in the wild lions and 141 U/l in the captive lions) are not very significant from a biological disorder standpoint. Several factors are probably involved in causing the differences. The wild lions were subject to more strenuous exercise prior to darting which caused elevation in CPK and LDH. Since serum levels of all three enzymes gradually rise after muscle damage (which the dart certainly caused), the slightly elevated serum levels of SGOT, CPK, and LDH probably also reflected the greater amount of time that elapsed between darting the wild lions and drawing their blood (the darted lion would often leap from the tree and run, so often 15 to 20 minutes would be spent in relocating it). Another possible factor could be the injection procedure: all wild lions were darted with a Cap-Chur gun, while several of the captive lions were injected with a hand-held or pole syringe, which would presumably be less damaging to the muscles.

Sodium levels in the serum have a normal range of about 20 mEq/l in the domestic cat, so once again, the difference between the wild (144.6 mEq/l) and captive (147.5 mEq/l) mountain lions does not seem to be very great. The difference might be attributable to diet, and a similar difference in chloride levels in the serum would be expected. The wild lions had slightly lower chloride levels (114.2 mg/dl) than the captive lions (118.3 mg/dl). A slight, but opposite difference in potassium would also tend to balance the difference in sodium levels. Indeed it is slightly higher in the wild lions (4.6 mEq/l) than in the captive lions (4.2 mEq/l). Diet might also be involved in the difference in inorganic phosphorous levels in the serum of the wild lions (5.5 mg/dl) and captive lions (4.8 mg/dl). It is obviously not a serious difference, or a difference would also be seen in the serum levels of calcium.

In domestic cats, any serum bilirubin level less than 1.0 mg/dl is not considered very abnormal, so the difference between the wild (0.3 mg/dl) and captive (0.6 mg/dl) lions is not as consequential as it may at first seem. Perhaps cholesterol and diet lead to a minor obstruction of the bile flow, causing a slightly higher level of bilirubin in the captive mountain lions.

The difference in hematocrits probably reflected the difference between the elevations where the wild mountain lions were found (most of them above 2100 m (Currier 1976)), and the elevations where the captive lions were located (most of them in the Midwest below 500 m).

2. Winter-Summer Blood Collection

The three male mountain lions raised by hand were restrained, but not immobilized, when their blood was taken until age 20 weeks. Therefore, 12 of the 18 summer samples and three of the 17 winter samples

were obtained without any drug influence. From age 20 weeks until 10 months, they were restrained briefly and injected by hand. For the final two summer dates on which blood was taken at ages 12 and 14 months, it was necessary to use a CO_2 pistol. It is possible that restraint results in bruising the animal's muscles and is therefore more damaging to the muscles than the quick immobilization with a hand-held syringe. A dart almost certainly causes more muscle damage than a hand-held syringe. Therefore, the difference in the CPK and LDH levels is probably not a reflection of seasonal differences, but rather due to the level of muscle damage: the dart is the most damaging, restraint less damaging but nonetheless bruising, and the hand-held syringe the least damaging. Summer values were all obtained in the two most damaging ways (six with darts and 12 with restraint), while winter values were obtained in the two least damaging ways (three with restraint and 14 with hand-held syringe). Further support for this hypothesis is the fact that the values for these two enzymes from the wild lions (collected in the winter) were higher than the values of the captive lions (collected in the summer). The reverse of the situation with the captive kittens: winter values were lower than summer values.

LDH is also significantly different between wild kittens and wild non-kittens. The mean of the LDH levels for the first three months of the hand-raised kittens' lives was 306 U/l, then dropped to a mean of 142 U/l for the final 11 months. Therefore, LDH levels appear to be very high during the first three months or so of life, then drop off sharply.

The creatinine difference could be due to the Hycel machine. Such a small difference (1.13 mg/dl) is not biologically very significant. The sodium and potassium differences are also not biologically significant (the difference in sodium is 1.6 mEq/l, and the normal range in the domestic cat is 2o mEq/l). The sodium difference might be a seasonal difference because the level is higher in both summer and the captive lions. The potassium difference might be due to use of an immobilizing agent to obtain 14 of the 17 winter samples, but only six of the 18 summer samples Seal et al. (1972) noticed a decrease in serum potassium following phencyclidine administration in white-tailed deer. The final difference to be discussed is inorganic phosphorous in the serum. Since phosphorous levels are closely tied to calcium levels, which are not significantly different, one can assume the phosphorous difference is not biologically important.

3. Wild Kitten - Wild Non-kitten

Differences between wild kittens and wild non-kittens were tested for significance to help clarify whether some blood parameters do indeed change with age. Two (GLOB, MONO) of the five blood parameters that appear in the female age-estimation formula also appear on the list of significantly different wild kitten/non-kitten blood parameters. Globulins in humans at birth are about 1.78 g/dl, increase to 2.01 g/dl at 5-6 months, and are 2.34 g/dl for adults (Diem, ed. 1962), so one could anticipate the noted difference between kittens and non-kittens. In humans, the percentage of monocytes in the blood gradually decreases with age (Diem, ed. 1962). All three blood parameters (GLOB, ALK, NEU) that appear in the male age-estimation formula also appear on the wild kitten/wild non-kitten significantly different list. Serum alkaline

phosphatase levels are increased in the presence of growth hormone (Diem, ed. 1962), so one would expect a difference between growing kittens and non-kittens. The precentage of neutrophils in human blood is high at birth (53 - 82.5 percent). It drops rapidly to 18 - 46 percent at nine to 11 days, and then gradually rises to about 66 percent in adulthood (Diem, ed. 1962). Lymphocytes and neutrophils generally make up about 95 percent of the circulating white blood cell population, so a change in one is generally reflected by a change in the other. One would not expect them both to appear in an aging equation because after the first has been added, the other can give no additional information. It is to be expected that if one shows a significant difference between two groups, the other would reflect that difference.

A possible reason for the kitten/non-kitten significant difference in LDH levels has been mentioned previously: LDH levels appear to be quite elevated during the first three months of life. They then drop and level off at about half of the former level. The reason could be that LDH is made in different amounts in different tissues at different stages of development (Balinsky 1965). Some final organ development (such as the kidney) could be occurring during the first three months of the mountain lion's life, after which only growth takes place.

The creatinine difference between wild kittens and wild non-kittens is not biologically significant, although statistically significant. It might be only an artifact due to the unreliability of the Hycel machine. Calcium levels, as stated earlier, are high in growing mammals, so one would expect a significant difference between wild kittens and wild non-kittens.

Morse and Follis (1974) took blood samples every two weeks from two African lion cubs, starting at age two weeks, and ending at about age 32 weeks. Their serum analyses covered 19 of the 22 blood parameters that were measured in this study. Seven of the eight parameters that were different between wild kittens and wild non-kittens were measured (only the globulins were not), but in the African lions, only two appeared to change with age: creatinine increased, and alkaline phosphatase decreased. At 22 months of age, blood samples were again taken from the African lions. At this time, the calcium levels had dropped. The four other parameters that were different between wild mountain lion kittens and wild mountain lion non-kittens (percentage monocytes, lymphocytes, and neutrophils; and LDH) did not appear to change as the two African lions aged.

4. Female-Male

Only two of the blood parameters were significantly different between non-kitten females and non-kitten males: potassium and globulins. The difference between mean levels of potassium (0.3 mEq/l) is even less than that between captive and wild mountain lions (0.4 mEq/l) or between winter and summer (0.9 mEq/l) and is probably of little biological significance. Globulins are a fraction of the plasma protein. Testosterone, the male sex hormone, has a greater anabolic, or protein-forming action,than estrogens, the female sex hormones (Diem et al. 1962). The slightly higher mean level of globulins in the serum of the males (4.06 g/dl) than the females (3.83 g/dl) might be the result of this anabolic effect.

Morphological Parameters

The classification of Felis concolor into subspecies is based mainly on characteristic patterns of cranial structure (size, and relative proportions of other measurements), although body size is greater for the subspecies occupying the northern and southern limits of the mountain lion range than in the central portion of the range, and slightly different colors in the pelage have been noted (Young and Goldman 1946). Since the only skull measurement made in this study was skull arch, and most of the captive mountain lions were of mixed or unknown ancestry, one would not expect a significant difference between wild and captive lions in morphological measurements unless they are greatly affected by nutrition, exercise, or both. Non-kitten female/male differences are expected for all non-tooth body measurements since males are reputed to be 50 percent heavier than females. This indeed seemed to be the case. None of the tooth measurements were significantly different. Weight was significantly different both between wild and captive lions, and females and males. Although when wild males were compared with captive males, no significant difference was noted, it could have been due to the small (five) sample size of wild, non-kitten males. The captive females weighed an average of 12 percent more than the wild females. Wild males weighed an average of 52 percent more than wild females, but captive males weighed only an average of 35 percent more than captive females.

Differences in girth generally tended to follow the differences in weight with one notable exception: the average,captive female girth was three percent greater than the average,wild male girth. Differences in both weight and girth illustrate the difference in nutrition and

exercise. In the wild, a premium is put on the lion's ability to make a quick, powerful dash at its prey. That movement demands strong leg muscles. One would expect a lion in the wild to have more weight concetrated in the muscular legs, with less body fat. Therefore, even though wild males outweigh captive females, the average girth of a captive female (78.3 cm) is greater than that of a wild male (76.0 cm), and much greater than a wild female (69.9 cm), in fact a 12 percent difference. The girth of the average captive male (85.7 cm) is 13 percent greater than that of the average wild male. Non-kitten males were on the average 12 percent longer, had a 19 percent larger skull arch, and a 10 percent longer rear tarsal measurement than non-kitten females.

Since kittens do not have permanent teeth, gumline recession measurements were not made for them. All of the other morphological measurements predictably show a significant difference between wild kittens and wild non-kittens. Means of kitten size were not compared with non-kittens due to the great increase in body size during the first year of life, making such a comparison of little value.

Age Estimation Models

Aging can be defined as the effect of the passage of time on an organism: conception, growth, maturity, physical decline, and death. Different species age at different rates. To make the matter even more complicated, different organs, and even different cells within organs, age at different rates. Fortunately, much of the aging process is genetically determined, so individuals of the same species tend to age at similar rates, and generalizations can be made.

Age estimation is important to the study of population dynamics, and the study of population dynamics should be a fundamental part of any management program. Population dynamics involves conception, birth, growth and maturation, onset of sexual maturity, periodicity of reproduction, fecundity, decline of reproductive capacity, and death. The thread linking all of these is the passage of time. Without knowledge of the average age at which these events occur in a species, and without knowledge of the age structure of a population, the study of that population becomes static, not dynamic, and predictions made as to what can happen to that population become more subject to error. Reduction below desirable levels or even extirpation of that population can result.

Methods of Age Estimation

Farmers have long used tooth wear as a method of estimating the ages of individual horses. This method was (and to some extent, still is) also used to estimate the ages of individual white-tailed deer, mule deer, elk, moose, bison, pronghorn antelope, caribou, reindeer, and coyote (Taber 1971). Unfortunately, tooth-wear is highly dependent upon several factors not directly related to age: individual habit (diet selection, degree of mastication, etc.), substrate (if sand is usually on the food, teeth will wear down quicker), and so on. D. Ashman (Nevada Fish and Game Department, Personal communication) ages mountain lions based on tooth wear and tooth staining. Tooth-wear estimation has largely been replaced by counting cemental annuli. Periodical changes in the rate of calcification of the tooth root tissue cause annual layers to be laid down, much like tree rings (Klevezal' and Kleinenberg 1967). This method has been found to be effective for deer

(Erickson and Seliger 1969, Low and Cowan 1963, Thomas and Bandy 1973), elk (Keiss 1969), moose, bison, seals, bats, rabbits, beaver, rodents, cetaceans (except those without teeth), bears, wolves, and foxes (generally) (Klevezal' and Kleinenberg 1967), and bobcats (Crowe 1972). M.G. Hornocker (University of Idaho, Personal communication) found that mountain lions do not consistently form cemental annuli.

Annular rings also are often laid down of horns. Counting these rings is a method used to estimate the ages of individual big horn sheep and mountain goats (Taber 1971). Other bony structures are also used in age estimation, but often only to separate juveniles from adults. Montana Fish and Game Department personnel are developing an age-estimation method for mountain lions based on cleaned skull characteristics (K. Greer, Montana Fish and Game Department, Personal communication).

Weight of the eye lens has been used to estimate the ages of individual seals, cottontail rabbits, jackrabbits, and moose (Taber 1971). The eye lens has also been used to determine age even more accurately by the determination of the amount of insoluble protein in the lens. This method has been successfully used for field mice (Dapson and Irland 1972) and other vertebrates (Otero and Dapson 1972).

Sometimes age-estimation methods are needed for humans, too. Furukawa et al. (1975) used multiple regression analysis to develop such a method. The best formula they obtained was as follows.

Age in years = 95.232 - 0.138 HT - 0.180 WT + 0.142 SBP - 0.072 DBP - 0.003 VC - 0.252 PSP - 1.433 OAR - 0.816 OAL + 0.262 VSR + 0.315 VSL, where:

HT = height

WT = weight

SBP = systolic blood pressure

DBP = diastolic blood pressure

VC = vital capacity of the lungs

PSP = percentage phenosulfophthalein excretion in 15 minutes

OAR = ocular accomodation on right

OAL = ocular accomodation on left

VSR = threshold of vibratory sensation on right

VSL = threshold of vibratory sensation on left

The standard error of this model is 5.15.

The only completely accurate method for aging a mountain lion or any other animal is by capturing and marking it when it is still quite immature, then recapturing it in later years.

Physiological Changes with Age

Theories

Various theories have been proposed to explain why an animal ages. Five of the most popular are: tissue wear and tear, random mutations, autoimmunity, the accumulation of harmful substances, and cross linkages forming between macromolecules. Kohn (1971) considered the most likely cause to be a progressive intermolecular cross-linkage of collagen, a substance that makes up 25 - 30 percent of the total body protein in most mammals, and which is found throughout the body. Cross-linkage of the collagen would tend to make tissues inflexible, making it more difficult and less efficient for the heart and other muscles to contract, and therefore perfusion of, and diffusion into, the organs of the body would be reduced. In short, the whole physiological machine would gradually slow to a halt. This seems to be the case in humans, but very few animals in the wild have the opportunity to reach that stage of debilitation. As long as an animal is growing, new collagen

is being laid down. This synthesis of collagen effectively ceases when growth ceases, and at that stage the cross-linkage process begins. An increased metabolic rate could increase the enzyme action that actually causes the cross-linkage. An increased metabolic rate would also increase the amount of stress on the tissues, which would also lead to increased cross-linkage. Therefore, one would expect animals with high metabolic rates to age faster than those with slower ones.

Physiological Changes

No matter which theory is correct, physiological changes do occur with age. Riegle and Nellor (1966) have shown that average percentages of neutrophils increased, and percentages of lymphocytes decreased, with age in dairy bulls, dairy and beef cows, sheep and rats. Total leucocyte counts decreased with increasing age in dairy bulls, and dairy and beef cows, but increased with increasing age in rats. The average percentage of eosinophils was significantly higher with increasing age in sheep. Levels of plasma protein increased with increasing age in all of the mentioned species, and percentages of gamma globulins increased with increasing age in dairy bulls, dairy and beef cows, and goats, but decreased in rats. Percentages of albumins decreased in dairy bulls.

Soltz et al. (1963) found that radioactive triiodothyronine (T3*) uptake by the red blood cells increased during growth in rats, then leveled off at sexual maturity, and gradually decreased with advancing age. They also found that T3* uptake by the red blood cells was higher in young people than old ones. Lindemann et al. (1971) have shown that plasma levels of zinc are higher in men than women, and that there is a significant linear decrease of plasma zinc with increasing age. Red

blood cell zinc concentrations were similar for men and women, and had no correlation with age. Threatt et al. (1971) found a brain-reactive antibody in the sera of old mice. This antibody attacked neurons in sections of brain from both old and young mice. The sera from young mice contained no such antibody. This finding tends to support the autoimmune theory of aging.

Physiological changes have been noted in other parts of the body, too. Rosenquist and Rosenquist (1974) studied uptake of the dye Alcian blue by sections of the kidney in spider monkeys. They found that uptake increased until maturity was reached, then decreased thereafter. Schmucker et al. (1977) found that the surface area of the hepatic endoplasmic reticulum increased in rats from one to 20 months, then decreased fron 20 to 30 months.

Morphological Changes

Morphological changes also occur as a result of aging. Glickman (1964) reported the following periodontal changes: tissue dessication, reduced elasticity, altered cell permeability, thinning of dermis and epidermis, recession and diminished keratinization of the gingiva (gumline), and reduced oxygen consumption in the gum. Viidik (1969) found that the tensile strength of collagen in the skin increased with age, but became less elastic (the stress-strain curve became steeper).

Age-Estimation Technique

Needs

Even if an age estimation model is very accurate, it is useless if it is not applied. For the technique to gain acceptance, the data necessary for it should not be too difficult to collect and handle in the field. If data treatment and analysis are too complex, the amount of time necessary to utilize the technique will be too great, and both the time delay and the expense might stymie its usage. The technique should be fairly inexpensive to apply. The technique should be reliable, and it is helpful if the data for the technique can come from either live or dead specimens.

Techniques Developed

The age estimation techniques developed in this study fulfill most of the requirements. The field data collection is not complex. One body measurement and one or two tooth measurements are made, one blood sample is drawn and is put in either one or two test tubes, and blood smears are made. Later, the test tubes of blood must be centrifuged, the serum and, for females, plasma drawn off and frozen. Practically all hospitals and large veterinary hospitals have automatic serum analyzers (the two most common brand names are HYCEL and SMAC). Price varies from hospital to hospital; our cost was $9 per sample. An added bonus from the serum analysis is that the state of the individual's health can be determined. White blood cell differentials and plasma zinc analysis can be done by any competent laboratory technician. Although all of the data collected during the study was from live animals, it is possible that fresh-killed animals could also be used.

Both physiological and morphological data are preferred for these age-estimation formulas. The coefficient of variation (r^2) is a measure of the reliability of the formula. Using both physiological and morphological parameters, the r^2 for the female formula is 81 percent. If only physiological (blood) parameters are used, the r^2 drops to 51 percent. If only morphological parameters are used, it is only 56 percent. The same situation holds for the male formula: 80 percent with both, 39 percent with just physiological parameters, and 49 percent with just morphological parameters. A great deal of reliability is lost without use of both types of data.

Further testing of the formula with known-age, wild mountain lions would help substantiate its accuracy. Although no parameter was used that was significantly different between the wild and captive mountain lions, it is possible that some parameter used in either the female of male formula changes differently with respect to age in wild as opposed to captive mountain lions. Another unknown quality is the effect of pregnancy on the blood serum parameters in the females. No known-age, captive females that were used in the study were known to pregnant. Pregnancy probably affects the globulins, and possibly the total proteins of blood urea nitrogen, or both. It might ve possible to calculate the effect, and alter the formula accordingly.

Two additional sources of error are possible in this technique: humean and machine. The human error can be minimized if personnel are properly trained, if there is a low turnover of personnel used, and if care is taken in data collection and processing. Machine error can usually not be minimized, but can be calculated using duplicate samples.

Future research needs are as follows.

1. The formulas should be tested with use of known-age, wild mountain lions, both alive and dead.
2. Other age indicators should be tested, such as testing renal function using Macy's (1978) method.
3. Blood values of pregnant, female mountain lions should be compared with those of non-pregnant females.

Hand-Raised Kittens

Weight

Weight gains during the first 11 weeks of life of the three kittens hand-raised for this study were about 10 percent greater than for the kitten with the highest weight gain of the six kittens reported by Eaton and Verlander (1977) (weight gain was only reported to the second week for two kittens, third week for one, eighth week for two, and eleventh week for the kitten with the highest weight gain). Later weight gain of our three was also greater than that predicted by the graph of Robinette et al. (1961). They predicted attainment of 10 kg at about 20 weeks (ours reached 10 kg at about 14 weeks), 20 kg at about 32 weeks (ours: 23 weeks), 30 kg at about 44 weeks (ours:30 weeks), and 40 kg at about 58 weeks (ours: 36 weeks). The variation is probably due to both genetic and nutritional differences.

The graph developed by Robinette et al. (1961) was based on a few records of known-age mountain lions in zoos, plus the probable weights at sexual maturity. All three sources (Eaton and Verlander (1977), Robinette et al. (1961), and this study) are based either entirely or mainly on known-age, captive mountain lions. The situation is probably

similar in the wild. Possibly similar genetic and nutritional variation occur, but with an additional difference from captive lions: amount of exercise. The kittens start following their mother to kills as soon as they start eating meat (probably at about two months of age).

Tooth Eruption and Replacement

Volf (1972) reported initial eruption of the incisors of two mountain lion kittens from 18 to 34 days. The incisors of the one kitten reported by Eaton and Verlander (1977) erupted between 13 and 15 days. The three kittens hand-raised for this study had all of their incisors by 17 days. Upper and lower canines for Volf's kittens erupted between 30 and 34 days. Our three had both sets of canines by 23 days. Lower premolars in Volf's study erupted on day 45, and upper premolars between days 48 and 53. Lower premolars erupted by the 30th day, and upper premolars by the 33rd day in our three. These results indicate that precocious weight gain was accompanied by precocious tooth development in the three kittens hand-raised for this study, and was probably due to similar genetic and nutritional differences.

Eye Color and Pelage Changes

The kittens were born weighing about 0.4 kg, covered with black spots, and with their eyes closed. A female mountain lion probably gains more weight from gorging at a kill than at full-term pregnancy. A large weight gain would be quite detrimental to her hunting, since speed and balance may often be the deciding factors between making a kill or not. Spotted coats would help camouflage the kittens while the mother is away hunting. Blue eyes in neonates usually indicate vision is not fully developed. Good eyesight is not necessary for the

kittens until they start to follow their mother. The spots rapidly fade between the 12th and 14th weeks, probably about the time the kittens start to accompany their mother on her hunts (not just follow her to a kill), and a tawny moving coat would be less conspicuous than a spotted one.

Estimated Ages of Unknown-Age Mountain Lions

Two of the five unknown-age, captive lions had estimated ages of almost exactly one year difference when they were sampled one year apart. A third female lion (No. 107) developed renal problems the second year and had a high blood urea nitrogen, which made the aging formula unreliable. If her N would have been average, her estimated age would have been 6.7 years, or an estimated age difference of two years instead of the one that it was. Another female (no. 109) was estimated to be about the same age both years (she was known to be old, which could have led to less accurate aging). The male (No. 110) was aggressive and resistant to the drug the first year, and never was sompletely immobilized, so I was unable to obtain accurate measurements. This led to what was probably an inaccurate age estimate. The age estimate for the second year was probably closer to reality.

The one wild lion that was captured in two separate years (No. 17) was aged as 13 the first year, and 8 the second. She was accompanied by seven- to eight-month-old kittens (according to the graph of Robinette et al. (1961)) the second year, so was probably pregnant when captured 10 months earlier the first year. This could have caused the elevated blood urea nitrogen (11 mg/dl higher the first year) and globulins (0.7 U/l higher the first year, which would have added almost four (apparent) years to her age.

Of the remaining non-kitten, wild lions, the estimated ages of six are within the range of the age estimated by appearance, seven are within a year of that range, three are within two and one-half years of that range, three are more than three years different from that range. The age of one was not estimated by appearance. Of the three with large differences between estimated ages, the ages of two females (Nos. 15 and 21) estimated by the formula developed in this study are greater than those based on appearance. Palpation of the abdomen of No. 21 revealed at least three fetuses. Female No. 15 also could have been pregnant. This might have led to elevated blood urea nitrogen and globulins. This would result in an over-estimateion of age. One male was estimated by the study formula to be 6.8 years old, but only two to three years old by appearance. The latter estimate is more likely to be correct since he still had stripes on his legs (they probably remain until the animal is two or three years old). His blood serum analyses revealed possible liver problems, such as hepatitis (LDH and ALK were greatly elevated, cholesterol depressed, and globulins elevated).

It appears that the age estimation formulas developed during this study are probably reliable unless a female is pregnant (it might be possible to use an early pregnancy test kit to determine if a female is pregnant), or the other blood serum values indicate illness.

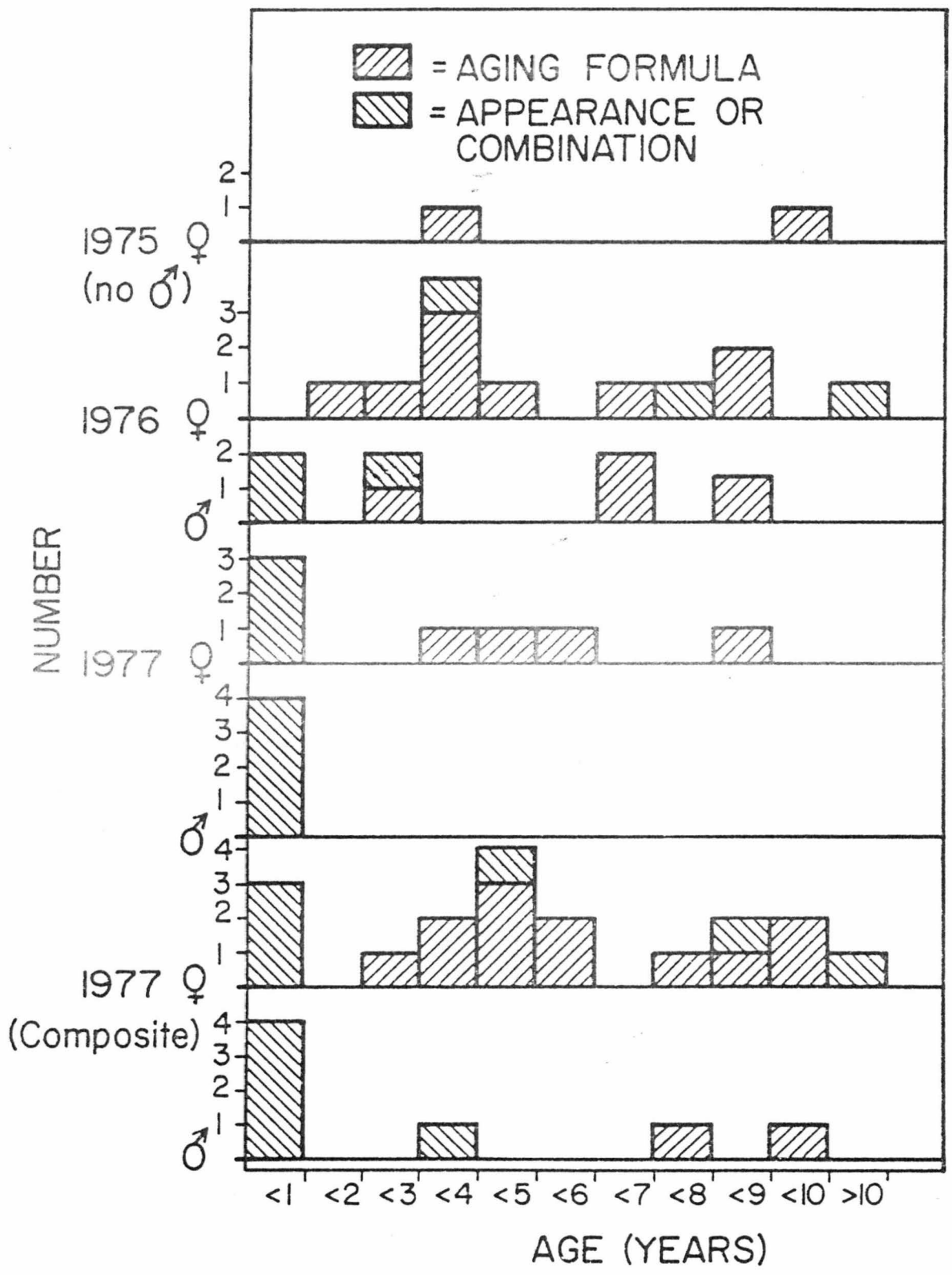
The age structure of the mountain lions caught all three years is shown in Figure 3. These age estimates are based on the assumption that all of the age estimates from the formulas developed in this study are correct except the four with greater than 2.5 years difference between the study formula and appearance method. The four with large

differences in estimates have been put either in the age class of the appearance estimate, or between the appearance and study estimates, depending upon the possible reasons for the differences. All four are marked differently on the graph so can be distinguished. Assuming the mortality of the two kittens caught the second year that were probably orphaned by a hunter, and the known mortality before May 1977 of the two males Nos. 7 and 19, a composite graph of 1977 has been drawn (also assuming that each of the remaining lions aged one year from 1976, and two years from 1975) (Figure 3).

Several possible conclusions can be drawn from the graph. Non-kitten females outnumber non-kitten males by five to one in the captured animals. Either this reflects the actual ratio in the population, or females are more likely to be caught than males. Sitton et al. (1976, P-R Job Prog. Rep., Proj. W-51-R, Calif. Dept. Fish and Game) hypothesized that males travel more, and are more likely to be caught. This might be true on their study area, but there is probably a difference in lion movement and tracking conditions between the damp, coastal study area in California, and the arid study area in Colorado. It is probable that females do indeed outnumber the males on the Colorado study area due to hunter pressure on the males (only male mountain lions are usually considered "trophy" animals). In the three years corresponding to the field study (1975, 1976, and 1977) 42 males and 21 females were reported killed on the Big Game Units containing the study area where the wild lions were caught (Colo. Div. Wildlf. 1975-77, Fed. Aid Proj. W-121-R).

Figure 3

Estimated age structure of the wild mountain lions captured near Canon City, Colorado.


= AGING FORMULA
= APPEARANCE OR COMBINATION
1975 ♀
(no ♂)
1976 ♀
♂
1977 ♀
♂
1977 ♀
(Composite)
♂
NUMBER
<1 <2 <3 <4 <5 <6 <7 <8 <9 <10 >10
AGE (YEARS)

Although no non-kitten, male mountain lions were caught in 1977, it is reasonable to assume some of the ones caught in 1976 survived the year (although some might have been killed illegally), but the paucity in numbers is cause for concern. The females appeared to be doing well. The composite graph indicates that there is a peak in the four to seven year old age range, which is presumably when a female is most productive.

CONCLUSIONS

The three objectives of the study were fulfilled. Normal ranges of various blood and morphological parameters were identified for the mountain lion. These can be used to gauge the state of health of individual mountain lions both in zoos and in the wild.

Some minor differences in blood and morphological values between wild-caught and captive mountain lions were found. These were probably due to diet and amount of exercise, but not due to a differing state of health of either group as a whole.

Two formulas, one for females and one for males, were developed for estimating the ages of individual, wild-caught mountain lions. With both formulas I have utilized combined information from blood samples and morphological data. If these formulas are field-tested with known-age, wild lions and found to be accurate, their application can lead to more comprehensive analyses of mountain lion population dynamics in the wild state.

LITERATURE CITED

Anonymous. 1974a. Endangered fauna. U.S. Dept. of the Interior. 22pp.

Anonymous. 1974b. For the quantitative determination of serum glutamic-pyruvic transaminase. Hycel, Inc., Houston. 4pp.

Anonymous. 1974c. For use in the quantitative determination of globulin in serum. Hycel, Inc., Houston. 4pp.

Anonymous. 1974d. For use in the quantitative determination of sodium and potassium on the Hycel 17™. Hycel, Inc., Houston. 2pp.

Anonymous. 1975a. For the quantitative determination of bilirubin in serum. Hycel, Inc., Houston. 4pp.

Anonymous. 1975b. For the quantitative determination of calcium in serum and urine. Hycel, Inc., Houston. 4pp.

Anonymous. 1975c. For the quantitative determination of chloride in serum. Hycel, Inc., Houston. 4pp.

Anonymous. 1975d. For the quantitative determination of creatinine in serum and urine. Hycel, Inc., Houston. 6pp.

Anonymous. 1975e. For the quantitative determination of urea nitrogen in serum and urine. Hycel, Inc., Houston. 4pp.

Anonymous. 1976a. For the quantitative determination of creatine phosphokinase in serum. Hycel, Inc., Houston. 4pp.

Anonymous. 1976b. For the quantitative determination of glucose in serum, spinal fluid and urine. Hycel, Inc., Houston. 4pp.

Anonymous. 1976c. For the quantitative determination of inorganic phosphorous in serum and urine. Hycel, Inc., Houston. 4pp.

Anonymous. 1976d. For the quantitative determination of lactic dehydrogenase in serum. Hycel, Inc., Houston. 4pp.

Anonymous. 1976e. For the quantitative determination of serum glutamic-oxalacetic transaminase. Hycel, Inc., Houston. 5pp.

Anonymous. 1976f. For the quantitative determination of total serum protein. Hycel, Inc., Houston. 4pp.

Anonymous. 1977a. Alkaline phosphatase test. Hycel, Inc., Houston. 4pp.

Anonymous. 1977b. For the quantitative determination of cholesterol in serum. Hycel, Inc., Houston. 5pp.

Arhammer, G., K. B. Bjornesjo, R. Jagenburg, and O. Mellander. 1972. Plasma and erythrocyte amino acids during treatment of protein caloric malnutrition: C.N.U. report 56. ACTA Paediatr. Scand. 61(2): 145-148.

Balinsky, B. I. 1965. An introduction to embryology. W. B. Saunders Co., Philadelphia and London. 673pp.

Bos, L. P., W. A. Van Vloten, A. F. D. Smith, and M. Nube. 1977. Zinc deficiency with skin lesions as seen in acrodermatitis enteropathica, and intoxication with zinc during parental nutrition. Neth. J. Med. 20(6): 263-266.

Brown, E. D., W. Chan, and J. C. Smith. 1978. Bone mineralization during a developing zinc deficiency. Proc. Soc. Exp. Biol. Med. 157(2): 211-214.

Corssen, G., M. Miyasaka, and E. F. Domino. 1968. Changing concepts in pain control during surgery: dissociative anesthesia with CI-581, a progress report. Anesth. and Analg. 47: 746-759.

Crowe, D. M. 1972. The presence of annuli in bobcat tooth cementum layers. J. Wildlf. Manage. 36(4): 1330-1332.

Currier, M. J. P. 1976. Characteristics of the mountain lion population near Canon City, Colorado. M. S. Thesis. Colo. State Univ., Fort Collins. 81pp.

Dapson, R. W. and J. M. Irland. 1972. An accurate method of determining age in small mammals. J. Mammal. 53(1): 100-106.

Deeming, S. B. and C, W. Webber. 1977. Evaluation of hair analysis for determination of zinc status using rats. Am. J. Clin. Nutr. 30(12): 2047-2052.

Diem, K., ed. 1962. Documenta Geigy: scientific tables, sixth edition. Geigy Pharmaceuticals, Ardsley, N.Y. 778pp.

Eaton, R. L. and K. A. Verlander. 1977. Reproduction in the puma: biology, behavior and ontogeny. Pages 45-70 in R. L. Eaton, ed. The world's cats, Vol. III, No. 3: contributions to breeding and husbandry. Feline Res. Group, Woodland Park Zoo, Seattle.

Eberhardt, L. L. 1971. Population analysis. Pages 457-495 in R. H. Giles, Jr., ed. Wildlife management techniques. The Wildlife Society, Washington, D. C.

Furukawa, T., M. Inoue, F. Kajiya, H. Inada, S. Takasugi, S. Fukui, H. Takeda, and H. Abe. 1975. Assessment of biological age by multiple regression analysis. J. Geron. 30(4): 422-434.

Ganong, W. F. 1971. Review of medical physiology, sixth edition. Lange Medical Publ., Los Altos, CA. 577pp.

Glickman, I. 1964. Aging and the periodontium. Pages 54-57 in Clinical periodontology. W. B. Saunders Co., Philadelphia and London.

Haung, W. Y., A. Kamio, S. J. C. Yeh, and F. A. Kummerar. 1977. The influence of vitamin D on plasma and tissue lipids and arthero-sclerosis in swine. Artery 3(5): 439-455.

Hodgman, C. D., R. C. Weast, and S. M. Selby, eds. 1960. Handbook of chemistry and physics: 42nd edition. Chem. Rubber Publ. Co., Cleveland. 3481pp.

Kang, H. K., P. W. Harvey, J. L. Valentine, and M. E. Swenseid. 1977. Zinc, iron, copper, and magnesium concentrations in tissues of rats fed various amounts of zinc. Clin. Chem. 23(10): 1834-1837.

Keiss, R. E. 1969. Comparison of eruption-wear patterns and cementum annuli as age criteria in elk. J. Wildlf. Manage.33(1): 175-180.

Klevezal', G. A. and S. E. Kleinenberg. 1969. Age determination of mammals from annual layers in teeth and bones. U.S. Dept. of the Interior and Natl. Sci. Found., Washington, D. C. 128pp.

Kohn, R. R. 1971. Principles of mammalian aging. Prentice-Hall, Inc., Englewood Cliffs, NJ. 171pp.

Lindeman, R. D., M. L. Clark, and J. P. Colmore. 1971. Influence of age and sex on plasma and red-cell zinc concentrations. J. Geron. 26(3); 358-363.

Low, W. A. and I. McT. Cowan. 1963. Age determination of deer by annular structure of dental cementum. J. Wildlf. Manage.27(3): 466-471.

Macy, D. W. 1971. The effects of bicarbonate and manitol on amphoteri-cin B nephrotoxicity in the dog. M.S. Thesis Univ. of Illinois, Urbana. 128pp.

Morse, R. W. and T. B. Follis. 1974. Physiological normal research in lions. Pages 188-203 in R. L. Eaton, ed. The world's cats, Vol. II: biology, behavior and management of reproduction. Feline Res. Group, Woodland Park Zoo, Seattle.

Otero, J. G. and R. W. Dapson. 1972. Procedures in the biochemical estimation of age of vertebrates. Res. Pop. Ecol. 13: 152-160.

Prasad, A. S., D. Oberleas, and J. A. Halstead. 1965. Determination of zinc in biological fluids by atomic-absorption spectrophotometry in normal and cirrhotic subjects. J. Lab. and Clin. Med. 66(3): 508-516.

Riegle, G. D. and E. Nellor. 1966. Changes in blood cellular and protein components during aging. J. Geron. 21(3): 435-438.

Robinette, L. G., J. S. Gashweiler, and O. W. Morris. 1961. Notes on cougar productivity and life history. J. Mammal. 42(2): 204-216.

Robinson, D. 1976. Puma! Puma! Defenders. 51(2): 97-104.

Rosenquist, T. H. and J. W. Rosenquist. 1974. Histochemical changes in the affinity of Alcian Blue 8GX for aging monkey kidneys, quantified by atomic absorption spectrophotometry. J. Geron. 29(2): 149-152.

Schmucker, D. L., J. S. Mooney, and A. L. Jones. 1977. Age-related changes in the hepatic endoplasmic reticulum: a quantitative analysis. Sci. 197: 1005-1008.

Searcy, R. L. 1969. Diagnostic biochemistry. McGraw-Hill Book Co., New York. 660pp.

Seal, U. S., L. D. Mech, and V. Van Ballenberghe. 1975. Blood analysis of wolf pups and their ecological and metabolic interpteta-tion. J. Mammal. 56(1): 69-75.

Seal, U. S., J. J. Ozoga, A. W. Erickson, and L. J. Verme. 1972. Effects of immobilization on blood analysis of white-tailed deed. J. Wildlf. Manage.36(4): 1034-1040.

Soltz, W. B., A. Horonick and B. F. Chow. 1963. Agewise difference in the uptake of radioactive triiodothyronine (T3) by red blood cells. J. Geron. 18: 151-154.

Taber, R. D. 1971. Criteria of sex and age. Pages 325-402 in R. H. Giles, Jr., ed. Wildlife management techniques. The Wildlife society, Washington, D. C.

Thomas, D. C. and R. J. Bandy. 1973. Age determination of wild black-tailed deed from dental annulations. J. Wildlf. Manage. 37(2): 232-235.

Threatt, J., K. Nandy, and R. Fritz. 1971. Brain-reactive antibodies in serum of old mice demonstrated by immunofluorescence. J. Geron. 26(3): 316-323.

Viidik, A. 1969. The aging of collagen as reflected in its physical properties. Pages 125-148 in A. Engel and T. Larsson, eds. Thule international symposia: aging of connective and skeletal tissues. Nordkska Bokhandelns Forlag, Stockholm.

Volf, J. 1972. Food requirements and dentition of three species of felids. (Transl. from French.) Mammalia 36: 683-686.

Widhalm, K., E. Maxa, and H. Zyman. 1978. Effect of diet and exercise upon the cholesterol and triglyceride content of plasma lipoprotein in overweight children. Eur. J. Pediatr. 127(2): 121-126.

Young, S. P. and E. A. Goldman. 1946. The puma: mysterious American cat. Amer. Wildlf. Inst. 358pp.

APPENDIX A

Instructions for collection of the data.

A. Field methods after immobilization

1. Record on the data sheet the date and lion's identification number, date initially captured, and estimated or known age at that time.

2. Blood and hind leg hair collection.

   a. Apply pressure to the inner hind leg just above the knee (see Fig. 1) to block blood flow and raise the vein.

   b. When the vein is located, release the pressure momentarily and clip off a patch of hair about 1.5 inches in diameter along the vein with scissors. Save the hair in an envelope and mark the animal's number, sex, age, and the data of capture on the envelope.

   c. Either have an assistant apply pressure above the lion's knee, or apply a tourniquet. After the vein has risen, stabilize it in the clipped patch by placing the thumb and finger of one hand on either side of the vein and spread them slightly apart (don't spread hard enough to cause the vein to disappear!). With the other hand hold the needle with the syringe attached parallel to the vein at about a 15 degree angle from the lion's skin surface (see Fig. 2 and Fig. 3). With a swift, shallow jab, puncture the vein with the needle, beveled surface up, still keeping it fairly parallel to the vein. Do not insert the needle so deep as to go through the vein. Hold the needle and base of syringe steady with the hand used to stabilize the vein and apply

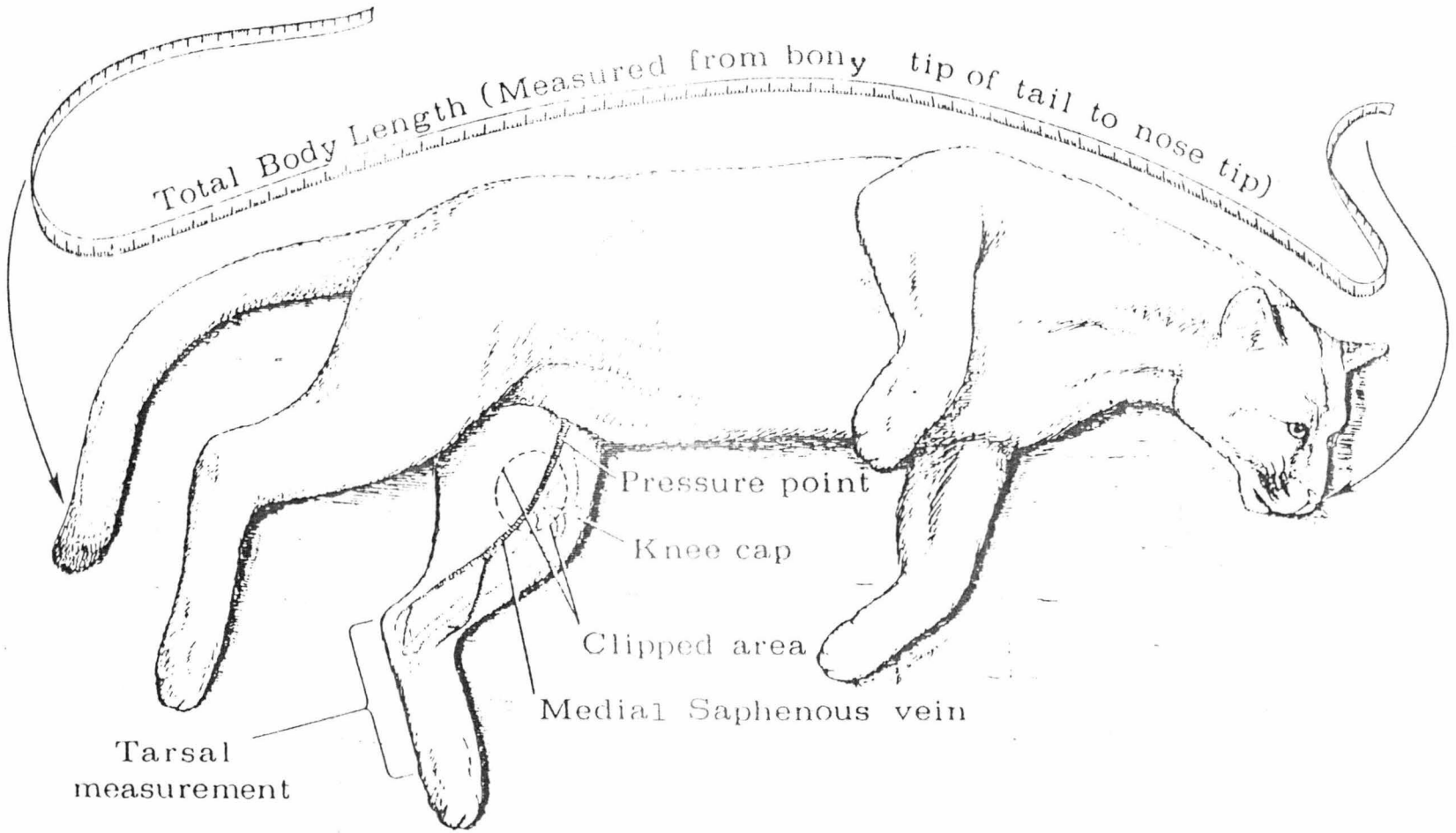


Fig. 1. Orientation for taking blood from, and measuring a comatose mountain lion.

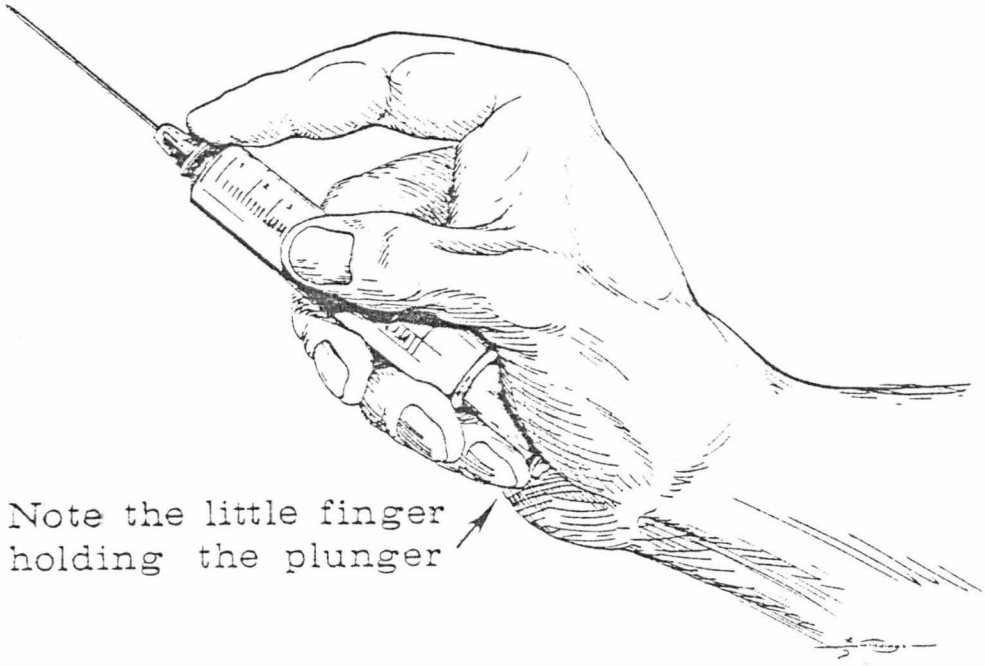


Fig. 2. Proper grasp of the syringe prior to drawing blood sample.

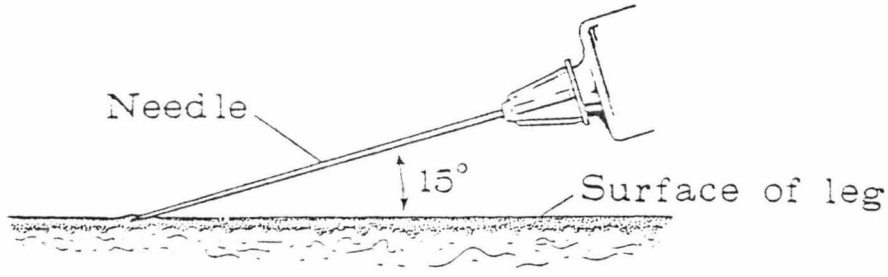


Fig. 3. Position of the needle with respect to the leg surface when drawing blood sample.

slight suction by pulling the plunger out with the other hand about 1/2 inch. If the blood does not enter the syringe, feel for the tip of the needle with your finger to determine if it has gone through the vein, not far enough, or slipped to the side of the vein. If it has gone through the vein, withdraw the needle slowly, still applying slight suction, until the blood rushes in. If the needle has not gone far enough, or is off to one side, try to get it into the vein (by swift, short jabs) but without withdrawing it from the leg. When blood enters the syringe, continue to pull the plunger out slowly (if it is pulled too quickly, the blood cells will burst and the vein may collapse). Collect about 10 cc of blood. Have the assistant release the pressure, or release the tourniquet before you remove the needle from the vein, or a hematome (blood clot under the skin) will form. Remove the needle from the syringe. Slowly squirt about 6 cc of the blood down the side of a plastic test tube. Label with the animal's number, age, date and the word "serum". Slowly squirt all but a few drops of the remaining blood down the side of a plastic test tube containing two drops of heparin; cap the tube and slowly mix the blood by tilting the tube from side to side about 20 times. Label with the animal's number, sex, and age, the date, and the word "plasma". Immediately, put a drop of blood from the syringe on the slide (same side as the roughened surface). Place the drop close to but not on the roughened part. Hold another slide at

about a 30 degree angle, touch the edge of the drop of blood, and take a full second to draw it down the slide. The slide is not usable if the blood is drawn off the end of the slide. Three good slides are necessary. Mark the animal's number, age, sex, and the date in pencil on the roughened surface and place the slide in the carrying container. Be careful not to let anything touch the blood smear itself.

3. Gum line recession measurements

   a. A mountain lion has one molar, one large premolar and one very small premolar on each side of its upper jaw, and one molar and two large premolars on each side of its lower jaw. You will be measuring the large premolar on both the upper and lower jaws (that means the large premolar in the upper jaw, and the first premolar in the lower jaw, see Fig. 4), and the corresponding canine, or four teeth in all. The side of the mouth measured is not important unless some abnormality is apparent.

   b. Close to the gumline on all of the teeth is a ridge. This ridge can be felt by running the dental probe up and down the buccal (cheek) side of the tooth. Sometimes in young mountain lions the gumline covers the ridge, but the dental probe can be slipped between the gum and the tooth for measurement.

   c. The dental probe is lined up parallel to the tooth axis, prependicular to the gumline. The probe is marked in mm. The distance from the gumline to the crest of the ridge

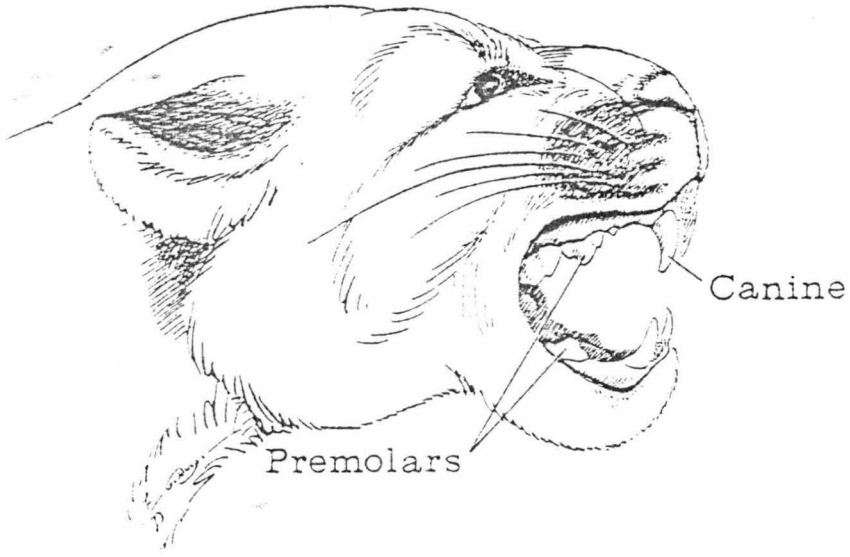
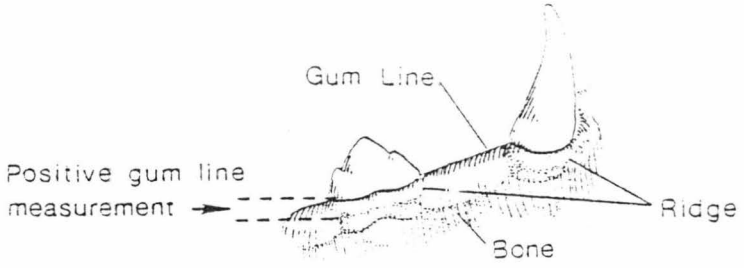
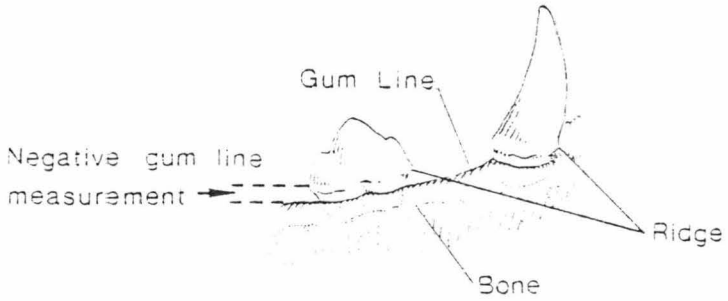


Fig. 4. Teeth from which gumline recession measurements are obtained in the mountain lion.



A. Young specimen



B. Old specimen

Fig. 5. Gumline recession measurements in the mountain lion.

is measured (see Fig. 5). If the gum covers the ridge, the distance is recorded as (+). If the gum line has receded to the point where the ridge is uncovered, the distance is recorded as (-). (Hint: the ridge is generally more pronounced on the premolars.) Record all measurements in the space provided on the data sheet.

4. Snip off six whiskers close to the skin and save them in a straw with the ends covered with tape and the lion's number, sex, and age and the date written on it.
5. Weigh the lion and record its weight in the space provided on the data sheet.
6. Measure the following with a tape measure graduated in cm:
   a. Total body length: measure from the end of the nose, down the center of the back (between shoulder blades), to the bony tip of the tail;
   b. Girth: measure around the animal's chest, directly behind the forelegs;
   c. Skull arch: measure from the top of zygomatic arch on one side of the skull, over the skull to the top of the zygomatic arch on the other side (from the bony ridge caudal to one eye over the bony ridge caudal to the other eye);
   d. Right rear tarsal length: measure the distance from the base of the hind pad (opposite the toe end of the pad and not including the pad) to the "heel" of the foot (see Fig. 1);
   e. Tail length: measurement from the tip of the bony tail to the point where the tail vertebrae join the pelvic girdle.

B. Processing methods at camp

1. No more than 6 hours should elapse before refrigeration, but do not let the blood freeze (the red cells will burst and ruin the serum or plasma). No more than 24 hours (hopefully only 6 or less) should elapse before centrifuging the blood. Gently mix the blood labelled "plasma". Fill a hematocrit tube with that blood and centrifuge in a hematocrit centrifuge. Run a pippette around the edge of the test tube without heparin to loosen the clot. Centrifuge both "serum" and "plasma" test tubes at about 7000 rpm for about 5 minutes. After the elapsed time, stop the machine and draw off the serum (the non-red part) from the test tube labelled "serum", and plasma from the test tube labelled "plasma" with a pipette and put it into other plastic tubes labelled with the animal's number, age, sex, and the date the sample was taken and either "serum" or "plasma" as appropriate. There should be approximately the same amount of serum or plasma and red blood cells. If there is very little serum, mash the clear clot and recentrifuge.
2. Dip the slides in methanol and let dry. Return to a slide holder.

APPENDIX B

Data Sheet

Zoo: _____

Mountain lion number: _____

Age: _____

Sex: _____

Colorado Cooperative Wildlife Research Unit
Mountain Lion Study

_____ Date (Day, Mo, Yr)

_____ Time (24 hr)

Yes No Female pregnant

Yes No Female lactating

_____ Estimated weight (lbs)

_____ Actual weight (lbs)

_____ Total body length (cm)

_____ Girth (cm)

_____ Skull arch (cm)

_____ Blood Uptake Time

_____ Urine Collected (Min.)

_____ RR tarsal ln (cm)

_____ Tail ln (cm)

Yes No 4 vibrissae collected

Yes No Hind leg hair collected

Yes No Photos taken

Yes No Right hind foot printed

_____ Drug

_____ Volume

_____ Rate (mg/lb)

_____ Injection site

_____ Injection time

_____ Ataxia time

_____ Immobilization time

_____ Down time

_____ Acepromazine time

_____ Acepromazine volume (ml)

_____ Atropine time

_____ Atropine volume

_____ Antibiotics volume

_____ Pulse rate _____

_____ Pulse rate _____

_____ Pulse rate _____

_____ Resp. rate _____

_____ Resp. rate _____

_____ Resp. rate _____

_____ Rectal temp. _____

_____ Rectal temp. _____

_____ Rectal temp. _____

_____ Ambient temp. _____

+	-	Tooth No. Up	Tooth No. Lo	mm from gum edge to cemento-enamel junction

Injection site

Number	
1	Hip or hind leg
2	Abdominal region
3	Thoracic region
4	Back
5	Neck
6	Shoulder or fore leg
7	Head
8	Tail
0	Unknown

APPENDIX C

Alphabetical listing of the symbols used

Symbol	Variable
ALB	Albumin concentration
ALK	Alkaline phosphatase
BIL	Bilirubin concentration
C	Total gumline recession from upper and lower canines
CA	Calcium concentration
CHOL	Cholesterol concentration
CL	Chloride concentration
CPK	Creatine phosphokinase
CREA	Creatinine concentration
EO	Percentage eosinophilic segmented cells
GI	Girth
GLOB	Globulin concentration
GLU	Glucose concentration
HA	Young's Modulus for hind leg hair
HKT	Hematocrit
K	Potassium concentration
LC	Gumline recession from lower canine
LDH	Lactic dehydrogenase
LG	Total body length
LP	Gumline recession from lower premolar
LYM	Percentage lymphocytes
MONO	Percentage monocytes
N	Blood urea nitrogen concentration

Symbol	Variable
NA	Sodium concentration
NEU	Percentage neutrophilic segmented cells
P	Phosphorous concentration
PR	Total gumline recession from upper and lower premolars
PRO	Total protein concentration
SGOT	Serum glutamic-oxalacetic transaminase concentration
SGPT	Serum glutamic-pyruvic transaminase concentration
SK	Skull arch length
T3	Triiodothyronine uptake
TL	Right rear tarsal length
UC	Gumline recession from upper canine
UP	Gumline recession from upper premolar
WH	Young's Modulus for vibrissa
WT	Body weight
ZN	Zinc concentration