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Hematologic and Blood Biochemical Variables of Captive Chimpanzees: Cross-sectional and Longitudinal Analyses

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Hematologic and blood biochemical variables are of great importance in medical and veterinary practice. In addition, these analytes may have significance as potential biomarkers of aging. Previous reports on normative values of these variables in the chimpanzees are based on cross-sectional studies that did not include individuals of advanced age. To address this omission, we performed cross-sectional and longitudinal analyses of hematologic and blood biochemical data collected from chimpanzees over a 9-year period. One-hundred forty-six females and 106 males of ages representing the entire life span of the species were studied. We derived normative cross-sectional values of 14 commonly measured hematologic and 20 blood biochemical variables, which should provide a useful reference for clinical blood studies in chimpanzees. In addition, we found in a cross-sectional regression analysis of our data that most analytes varied significantly between males and females, and that they varied markedly with age. Most variables had year-to-year consistency within the same individuals, as indicated by statistically significant intra-year correlation coefficients. Finally, we performed a longitudinal analysis of the analytes in chimpanzees by calculating the slopes and intercepts of the best-fitting trend line for each individual. The resulting slopes were analyzed by sex and by decade of age of subjects to determine whether trends were consistent. Consistent trends detected in the longitudinal analysis were usually restricted to the first decade of life, and thus represented maturational processes. The overall lack of within-animal trends covering all or most of the period from early adulthood through old age in this 9-year study suggests that a longer period of follow-up than used here may be required to document senescence-related changes.

The constituents of blood as well as many of the chemicals it carries provide information fundamental to medical diagnosis of disease. Hematologic and blood biochemical variables also change during development, reflecting normal maturational processes. Because changes may continue into senescence, it has been proposed that some of these analytes may also be useful as biomarkers of adult aging, thus providing a tool for assessing the relative rate of "biological aging" of individuals from early adulthood to senescence. For humans and some animal species, standard values of these variables are well defined (1-4). For rare and exotic species, such as the chimpanzee, however, the published norms were often based on small numbers of individuals, or were derived from a single sample per animal. Furthermore, measurements have not been made throughout the life span of the chimpanzee (5-9).

Routine physical examination of chimpanzees housed at the Yerkes Regional Primate Research Center includes blood withdrawal for hematologic and blood biochemical analyses. We report descriptive analyses of these data. For each of 14 hematologic and 20 blood biochemical variables (Table 1), we provide normative values for female and male chimpanzees throughout the life span. In addition, we identify variables that are associated with aging, during maturation and in adult aging. The latter we define as the period from early adulthood through senescence.

The steps followed were: cross-sectional analysis to identify variables related to sex or age, study of consistency of these analytes within individuals from year to year, and analysis of longitudinal patterns in these blood variables.

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Methods

Data collection: The chimpanzee (*Pan troglodytes*) colony of the Yerkes Regional Primate Research Center was studied. Chimpanzees were maintained in an outdoor compound (approx. 1,000 m^2) in a social group, or in smaller housing areas (no smaller than 18 m^2). In both housing arrangements, chimpanzees had access to outdoor and heated indoor areas. In the interest of animal welfare, an attempt was made to socially house all chimpanzees, but some individuals were housed singly because of social incompatibility. They were fed a diet of commercial primate chow, supplemented daily with fruits and vegetables. As an integral part of clinical surveillance, chimpanzees were given annual physical examinations during which blood was drawn. Prior to these examinations, food was withheld from chimpanzees overnight, then they were anesthetized with ketamine hydrochloride or Telazol (Fort Dodge Animal Health, Fort Dodge, Iowa). Blood was taken by venipuncture of the femoral vein for the hematologic and blood biochemical determinations.

Hematologic measurements were done in house by personnel of the clinical pathology unit. For these determinations, a Sysmex model S800 (before 1992) or K1000 (1992-1998) cell counter (Sysmex Corporation of America, Long Grove, Ill.) was used. Blood biochemical analyses were done by use of a commercial laboratory (SmithKline Beecham, Decatur, Ga.). Hematologic and blood biochemical data were entered into our computerized animal records database. These data, covering the period

Table 1. Analytes and their conventional units of measurement

Variable	Abbreviation	Unit
Hematology		
Red blood cells	RBC	$10^6/\mu l$
Hematocrit	HCT	%
Hemoglobin	HGB	g/dl
Mean cell volume	MCV	fl
Mean cell hemoglobin	MCH	pg
Mean cell hemoglobin conc.	MCHC	g/dl
Platelets	PLAT	$10^3/\mu l$
White blood cells	WBC	$10^3/\mu l$
Segmented neutrophils	SEG	$10^3/\mu l$
Banded neutrophils	BAND	$10^3/\mu l$
Lymphocytes	LYMPH	$10^3/\mu l$
Monocytes	MONO	$10^3/\mu l$
Eosinophils	EOS	$10^3/\mu l$
Basophils	BASO	$10^3/\mu l$
Blood biochemistry		
Glucose	GLUC	mg/dl
Blood urea nitrogen	BUN	mg/dl
Creatinine	CREAT	mg/dl
Calcium	CALC	mg/dl
Phosphate	PHOS	mg/dl
Sodium	SOD	mEq/l
Potassium	POTAS	mEq/l
Chloride	CHLOR	mEq/l
Creatine kinase	CK	IU/l
Aspartate transaminase[1]	AST	IU/l
Alkaline phosphatase	ALKPHOS	IU/l
Alanine transaminase[2]	ALT	IU/l
Bilirubin	BILI	mg/dl
Triglycerides	TRIGLYC	mg/dl
Cholesterol	CHOLEST	mg/dl
Total protein	PROT	g/dl
Albumin	ALBU	g/dl
Globulin	GLOB	g/dl
Albumin/globulin ratio	A/G RATIO	
BUN/creatinine ratio	B/C RATIO	

from January 1, 1990 through December 31, 1998, were used in this analysis, with the requirement that only blood samples collected in periodic routine surveys, as opposed to physical examinations pursuant to an observed or known clinical problem, were included. Data meeting these criteria were available from 1,767 blood samples taken from 252 chimpanzees (146 females, 106 males), ranging in age from 55 days to 58.5 years. Because of births, deaths, and transfers during the study period, the number of individuals included in any single year ranged from 162 to 197. Table 1 lists 14 hematologic and 20 blood biochemical variables that were analyzed.

Accuracy of data entry: We compared the computerized records with the original laboratory reports for all reported hematologic and biochemical values in a random sample of 5% of the 1,767 blood samples. As a second evaluation of accuracy, we identified blood samples that had the highest or lowest value for each of the individual hematologic and biochemical variables. For each such sample, we verified the accuracy of the value of all hematologic and biochemical variables. These spot checks indicated nearly perfect accuracy of data entry.

Cross-sectional analysis: For each chimpanzee, we calculated the mean age during the study and the mean value of each variable. These means were used in multiple linear regression models to examine the relationship of each variable to sex and age. To evaluate the importance of curvilinearity of each value, we compared regression models containing linear terms for sex and age with a model containing a linear and a quadratic term. To eliminate the high correlation between age and age^2, we converted each age to the deviation from the mean age of chimpanzees in our sample. In addition to computing these regression options, we also tabulated values of each variable for each sex grouped by decade of age.

Year-to-year consistency: To evaluate the year-to-year consistency of each variable, we determined the average correlation (r) for each variable for each pair of adjacent years from 1990 through 1998. For determination of the correlation between two adjacent years, we used only chimpanzees for which data were available for both years under consideration. When an individual had two blood analyses meeting our criteria for analysis in a given year, we used only the data reported from the first blood sample. After we had obtained correlations for all eight pairs of years, we transformed these r values to Fisher Z statistics, then calculated mean Z values and retransformed the result. The resulting average correlation coefficient provides an index of the consistency of each variable.

Longitudinal analysis: The longitudinal analysis of changes in the hematologic and blood biochemical variables included only those chimpanzees for which at least four data points were available for a given variable. Data from these individuals were analyzed by use of a two-stage random effects model. In the first stage of the model, the slope and intercept of change in each variable were computed by use of conventional least-squares regression. In the second stage of the model, we placed the chimpanzees in 10-year age groups according to the mean age of each individual during the follow-up period. We then obtained the mean and standard error of the slope for each, and applied one-sample t-tests to determine whether average slopes differed significantly from zero. Because this step involved a large number of significance determinations (about 700), we adopted a stringent criterion for statistical significance (i.e., $P < 0.0001$).

Results

Hematology

Cross-sectional analysis: Tables 2 and 3 provide the mean and standard deviation (SD) values of each hematologic variable by decade of life for females and males. They also indicate number of chimpanzees in each age bracket, mean age during the study, mean duration of follow-up, and mean number of samples taken during follow-up for each age group. For example, from Table 2, it can be noted that 62 females between the ages of 0 and 10 years, with mean age of 4.5 years, were studied. These chimpanzees were studied for a mean 5.4 years, during which a mean 6.2 blood samples were taken for hematologic analysis. The value for red blood cell count (RBC) of $5.21 \times 10^6/\mu l$ (with SD of 0.40) was the unweighted mean of the 62 individual mean RBC values obtained from the female chimpanzees in this age group.

Table 4 lists the intercepts, coefficients, and significance values for the regression model based on the cross-sectional data. These parameters were based on multiple regression models in which age was recoded as the deviation from the mean age of the sample, and females and males were coded as 0 and 1, respectively. Thus, according to the regression model, mean RBC for females at the age of 15 years (i.e., mean age of our chimpanzees during the study) was $5.08 \times 10^6/\mu l$, and, according to the model in Table 4, this level remained unchanged with age, as indicated by the nonsignificant parameter for age. Because the parameter for sex (0.32) in Table 4 was significant, the model estimated that the mean value of RBC for males was $5.08 \times 10^6/\mu l \pm 0.32$, or $5.40 \times 10^6/\mu l$. When the linear and curvilinear effects of age are significant (which they were not for RBC), they can be interpreted, respec-

Table 2. Mean and standard deviation SD values of 14 hematologic variables for 146 female chimpanzees, by decade of chronologic age

Age (y)	0 to 10	10 to 20	20 to 30	30 to 40	40 to 50	50 to 60
n	62	26	29	21	5	3
Mean age (y)	4.5	14.2	24.5	34.8	42.9	54.9
Mean follow-up (y)	5.4	6.3	6.3	6.8	5.6	4.4
Mean No. of samples	6.2	7.6	7.4	7.7	6.6	5.7
	Mean	Mean	Mean	Mean	Mean	Mean
	SD	SD	SD	SD	SD	SD
RBC	5.21	5.00	5.06	5.07	5.05	4.38
	0.40	0.33	0.34	0.46	0.34	0.61
HCT	40.18	41.15	42.05	43.01	41.94	37.00
	2.26	3.06	1.80	3.24	2.72	4.99
HGB	12.99	13.44	13.47	13.69	13.32	11.78
	0.79	0.86	0.60	1.00	0.90	1.80
MCV	77.47	82.23	83.25	85.06	83.85	84.44
	4.84	3.04	3.28	3.78	0.98	2.00
MCH	25.10	26.88	26.70	27.08	26.53	26.87
	1.80	1.16	1.25	1.33	0.59	0.49
MCHC	32.38	32.71	32.07	31.85	31.72	31.81
	0.78	1.04	0.73	0.86	0.92	1.29
PLAT	312.23	280.87	255.13	240.84	262.50	299.28
	61.04	76.16	65.27	59.87	48.94	95.19
WBC	12.57	13.26	12.88	12.89	10.83	14.42
	3.33	4.74	3.62	3.49	2.51	8.91
SEG	6.08	8.00	7.49	5.90	5.19	9.51
	2.24	4.00	2.94	1.50	2.46	9.57
BAND	0.10	0.13	0.07	0.05	0.07	0.19
	0.12	0.15	0.07	0.08	0.14	0.27
LYMPH	5.81	4.53	4.72	6.32	4.94	4.23
	2.19	1.37	2.01	2.59	1.22	0.85
MONO	0.24	0.28	0.32	0.33	0.29	0.15
	0.15	0.12	0.19	0.15	0.13	0.16
EOS	0.31	0.28	0.24	0.26	0.29	0.24
	0.22	0.23	0.16	0.14	0.07	0.27
BASO	0.02	0.03	0.02	0.02	0.03	0.01
	0.02	0.03	0.03	0.02	0.03	0.02

Table 3. Mean and SD values of 14 hematologic variables of 106 male chimpanzees, by decade of chronologic age

Age (y)	0 to 10	10 to 20	20 to 30	30 to 40	40 to 50
n	55	33	10	7	1
Mean age (y)	4.7	14.9	25.8	32.6	43.4
Mean follow-up (y)	5.3	6.7	6.6	6.0	0.0
Mean No. of samples	6.4	7.7	7.5	6.9	1.0
	Mean	Mean	Mean	Mean	Mean
	SD	SD	SD	SD	SD
RBC	5.26	5.63	5.40	5.56	6.14
	0.35	0.39	0.42	0.21	NA
HCT	41.24	47.04	45.04	46.48	52.80
	3.14	3.03	4.17	2.13	NA
HGB	13.53	15.23	14.42	15.09	16.50
	1.12	1.08	1.50	0.75	NA
MCV	78.54	83.68	83.56	83.62	86.00
	4.53	4.31	4.25	2.72	NA
MCH	25.75	27.17	26.72	27.14	26.90
	1.70	1.64	1.58	0.93	NA
MCHC	32.79	32.45	31.98	32.48	31.30
	1.03	0.73	0.72	0.30	NA
PLAT	329.66	248.01	258.94	226.90	270.00
	96.37	47.12	76.97	42.81	NA
WBC	11.85	11.52	11.37	10.07	10.30
	3.45	1.97	2.53	1.57	NA
SEG	6.24	7.37	6.15	5.69	3.61
	2.42	1.84	2.52	1.08	NA
BAND	0.19	0.05	0.07	0.03	0.00
	0.53	0.05	0.10	0.06	NA
LYMPH	4.91	3.65	4.58	3.90	6.39
	1.70	1.01	1.45	0.74	NA
MONO	0.24	0.23	0.33	0.26	0.31
	0.14	0.09	0.15	0.13	NA
EOS	0.23	0.20	0.22	0.17	0.00
	0.15	0.12	0.10	0.14	NA
BASO	0.02	0.01	0.01	0.01	0.00
	0.02	0.02	0.02	0.01	NA

NA = not applicable.

tively, as the annual change in each variable, and the change in the same variable as a function of age^2 in the model. These mean values of 5.08 for females and 5.40 for males, derived from the regression model, corresponded approximately to the actual age-specific means given in Tables 2 and 3. Results from this analysis are also shown graphically for 3 representative hematologic variables (hemoglobin concentration [HGB], platelet count [PLAT], white blood cell count [WBC]) in the left-hand column of Fig. 1.

Table 4. Parameters (intercepts and coefficients) and significance levels for regression models of cross-sectional measures of 14 hematologic variables

Variable	Intercept	Sex	Age	Age^2
RBC	5.08	0.32	0.00	—
	0.0000	0.0000	0.2108	—
HCT	42.44	2.74	0.16	-0.01
	0.0000	0.0000	0.0000	0.0000
HGB	13.65	1.00	0.05	-0.002
	0.0000	0.0000	0.0000	0.0000
MCV	82.82	0.25	0.30	-0.01
	0.0000	0.6285	0.0000	0.0000
MCH	26.67	0.28	0.08	-0.003
	0.0000	0.1347	0.0000	0.0000
MCHC	32.23	0.24	-0.02	—
	0.0000	0.0133	0.0001	—
PLAT	290.10	-10.00	-2.30	—
	0.0000	0.2530	0.0000	—
WBC	12.78	-1.15	-0.01	—
	0.0000	0.0076	0.6369	—
SEG	6.80	-0.04	-0.01	—
	0.0000	0.9196	0.3762	—
BAND	0.11	-0.01	-0.002	—
	0.0000	0.5980	0.0082	—
LYMPH	5.30	-1.02	0.004	—
	0.0000	0.0000	0.6724	—
MONO	0.26	-0.02	0.003	—
	0.0000	0.1654	0.0000	—
EOS	0.29	-0.06	-0.001	—
	0.0000	0.0103	0.4966	—
BASO	0.02	-0.01	0.000	—
	0.0000	0.0609	0.2100	—

— = not computed.

The values of seven hematologic variables differed significantly between the two sexes, even when effects of age were statistically accounted for in a single regression model. On average, males had significantly higher values than did females for RBC, hematocrit [HCT], HGB, and mean cell hemoglobin concentration [MCHC], as indicated by significant positive coefficients for sex in the models for these variables. Likewise, females had significantly higher values for WBC, lymphocyte count [LYMPH], and eosinophil count [EOS], as indicated by significant negative coefficients for these analytes.

Age-related changes can be described as increases in average value across the life span, as indicated by significant positive regression coefficients for age, or as decreases in average value, as indicated by negative coefficients for these terms. Changes that are not uniform across the life span (e.g., maturational changes that may slow or reverse in direction after puberty) are represented by a significant age^2 parameter in the regression model. Only 3 of the variables had significant, monotonic changes with age that were not associated with significant curvilinearity. These were MCHC and band neutrophil count [BAND], both of which decreased significantly across the life span, and monocyte count [MONO], which increased significantly. Among the variables that had significant curvilinearity, HCT, HGB, mean cell volume [MCV], and mean cell hemoglobin [MCH] increased markedly in early maturation, whereas PLAT decreased rapidly in the first years of life. Segmented neutrophil count [SEG] also had significant curvilinearity, decreasing after 20 years of age (although high values were seen in the 3 females that were between 50 and 60 years of age). Finally, RBC, WBC, and basophil count [BASO] did not change significantly across

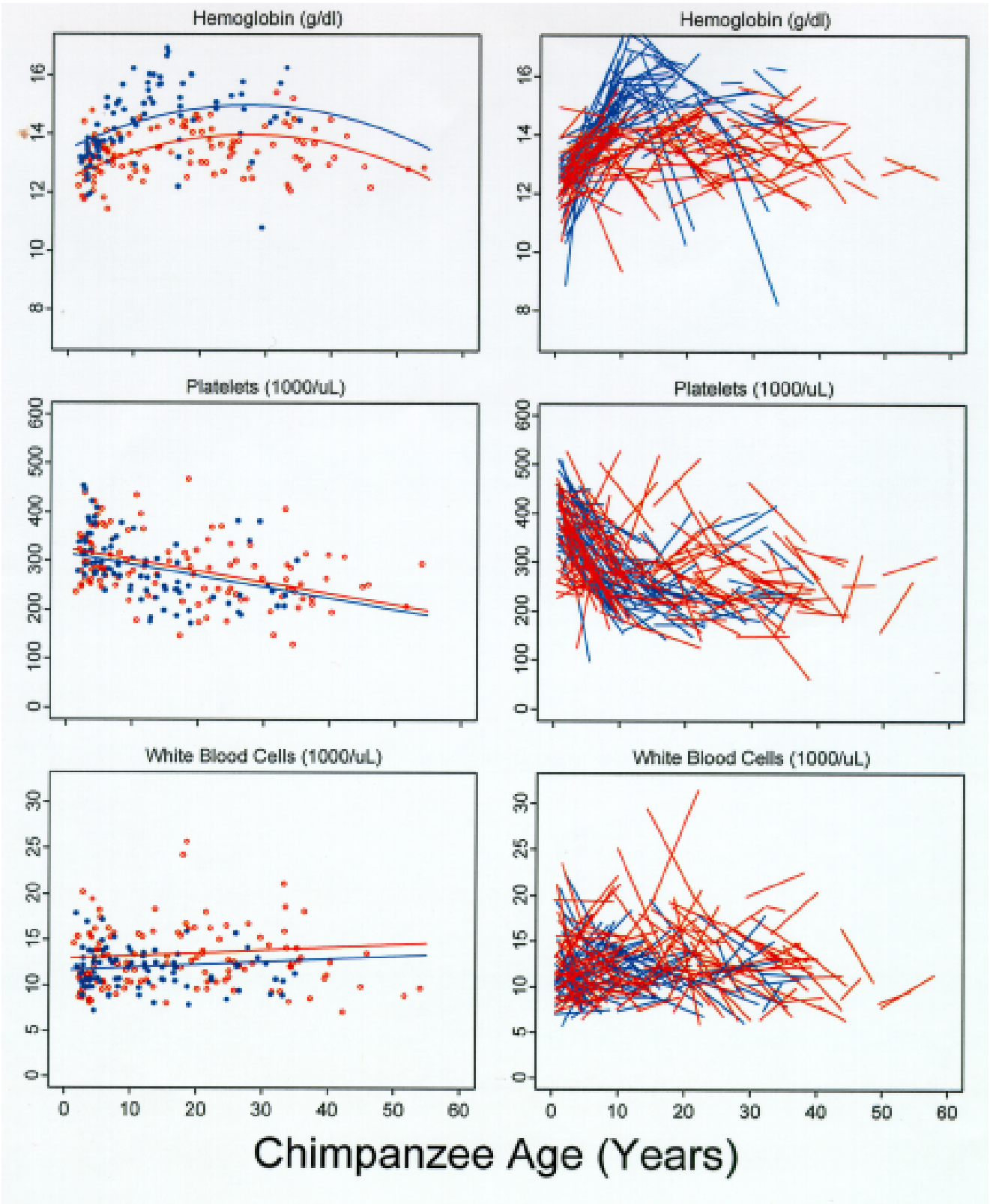

Figure 1. Results of three hematologic analytes with different, but representative patterns of results. Female and male chimpanzees are indicated by the colors red and blue, respectively. The cross-sectional analyses (left-hand column) contain a single data point for each individual, representing mean value of the analyte and mean age for that individual during the study. Lines in the left-hand column were drawn from parameters given in Table 4. The right-hand column has the longitudinal analyses, in which the best linear unbiased estimate is shown for each chimpanzee that had at least 4 blood samples taken during the follow-up period. The age range during which each individual was studied can be determined graphically by projecting of each line on the abscissa.

the life span, as indicated by non-significant coefficients associated with age (Table 4).

Year-to-year consistency: Table 5 summarizes the mean year-to-year correlation coefficients for the hematologic variables. The RBC, HCT, HGB, MCV, MCH, MCHC, PLAT, and LYMPH were stable in both sexes, with mean interyear correlations between 0.36 and 0.86. The WBC, SEG, and EOS were stable in females, as indicated by significant mean year-to-year correlation coefficients ($P < 0.05$), but not in males. The remaining analytes (BAND, MONO, BASO) were unstable from year to year for both sexes, as indicated by non-significant ($P > 0.05$) correlation coefficients.

Longitudinal analysis: We used a two-stage random effects model to examine the average slopes for males and females by 10-year age groups. Instead of tabulating all the results of this longitudinal analysis, we have graphically summarized the results of 3 representative hematologic variables (HGB, PLAT, WBC) in Fig. 1; each of the three panels in the right-hand column contains the longitudinal data for a single hematologic analyte. Each line in these panels represents the best linear unbiased estimate, determined by use of a single linear regression of that variable on age for a single chimpanzee. The projections of the lines onto the x-axis indicate the age range of that individual during the study. The reader can compare the cross-sectional with the longitudinal results of the three hematologic analytes. Thus, for the HGB cross-sectional data (Fig. 1, left-hand top panel), the red and blue lines representing the best linear unbiased estimates for females and males, respectively, were different, reflecting the significant sex difference reported for this variable in Table 4. The right-hand panel for HGB indicates graphically that the values for most males and females increased during the first decade of life (Table 10). For PLAT, Table 4 indicates a significant linear term for age, but not sex, as indicated by downward sloping, but nearly congruent lines in the left-hand panel of the center row of Fig. 1. The right-hand panel indicates apparently parallel decreases for most individuals during the first decade of life, and Table 10 confirms the statistical significance of the average slope in the first decade for males and females. For WBC, there was no significant effect of sex nor of age or age^2, as indicated by the similar, nearly horizontal regression lines in the left-hand bottom panel. The lack of any consistent pattern of longitudinal change in WBC is reflected in the apparently random directions of the individual regression lines in the right-hand bottom panel of Fig. 1. Because these slopes did not differ significantly from zero, WBC was not entered in Table 10.

The mean slopes of 7 hematologic variables (RBC, HCT, HGB, MCV, MCH, MCHC, PLAT) were significantly different from zero for at least one of the sexes. All significant changes occurred during the first decade of life. The change in HGB is detectable in Fig. 1, top row. Table 10 provides numerical estimates of the average slopes (in units of change per year) for these 7 variables. Longitudinal data for females and males of our sample in the remaining decades of life were not significantly different for any hematologic variable. Given the stringent criterion for statistical significance used ($P < 0.0001$), one concern was that some true longitudinal effects might have been missed. However, relaxation of the criterion for significance to $P < 0.05$ identified only 3 additional longitudinal changes of borderline significance. These were decreases in RBC, HCT, and PLAT for males in the second decade of life.

Table 5. Consistency of 14 hematologic variables: mean year-to-year correlations, retransformed from Fisher Z statistics

Variable	Females (n = 87.25)		Males (n = 66.25)	
	r	*P*-value	*r*	*P*-value
RBC	0.64	0.000	0.74	0.000
HCT	0.54	0.000	0.82	0.000
HGB	0.54	0.000	0.77	0.000
MCV	0.79	0.000	0.86	0.000
MCH	0.78	0.000	0.83	0.000
MCHC	0.42	0.000	0.45	0.000
PLAT	0.62	0.000	0.64	0.000
WBC	0.41	0.000	0.22	0.076
SEG	0.28	0.008	0.24	0.052
BAND	0.10	0.354	0.18	0.148
LYMPH	0.53	0.000	0.36	0.003
MONO	0.11	0.308	0.04	0.750
EOS	0.29	0.006	0.15	0.229
BASO -0.06	0.578	0.02	0.873	

n indicates the mean number of chimpanzees in the 8 separate year-to-year comparisons.

Table 6. Mean and SD values of 20 biochemical variables for 146 female chimpanzees, by decade of chronologic age

Age (y)	0 to 10	10 to 20	20 to 30	30 to 40	40 to 50	50 to 60
n	61	27	29	21	5	3
Mean age (y)	4.5	14.1	24.5	34.8	42.8	54.6
Mean follow-up (y)	5.5	6.1	6.1	6.8	5.8	4.8
Mean No. of samples	6.4	7.7	7.3	7.8	6.8	6.0
	Mean SD	Mean SD	Mean SD	Mean SD	Mean SD	Mean SD
GLUC	91.63 11.01	89.34 9.97	92.11 10.30	104.24 13.07	110.40 26.95	121.72 15.14
BUN	12.10 2.73	10.77 2.08	11.15 2.41	14.24 4.96	12.96 2.28	13.90 1.97
CREAT	0.65 0.13	0.83 0.11	0.86 0.14	1.06 0.24	0.99 0.15	1.05 0.23
CALC	9.65 0.29	9.25 0.30	9.14 0.33	9.18 0.38	9.12 0.24	9.36 0.08
PHOS	4.89 0.62	3.18 0.61	2.99 0.50	2.77 0.57	2.41 0.88	2.84 0.18
SOD	138.04 1.75	138.89 1.55	139.97 1.68	140.37 1.97	141.08 1.32	140.49 1.11
POTAS	4.23 1.05	3.69 0.21	3.39 0.43	3.15 0.42	2.84 0.35	3.05 0.57
CHLOR	100.88 1.74	100.69 2.15	98.27 2.43	96.87 2.33	96.36 1.58	94.43 3.43
CK	237.39 98.95	286.95 182.35	300.54 191.48	268.32 107.58	242.75 87.68	452.18 224.92
AST	19.03 3.76	18.90 5.14	25.01 10.82	26.13 6.89	26.86 6.78	31.44 9.68
ALKPHOS	610.73 204.94	115.10 41.57	95.67 42.71	104.42 58.92	104.00 41.32	107.79 41.61
ALT	35.08 10.26	33.84 7.89	32.57 10.76	36.03 11.36	29.92 15.14	47.49 33.60
BILI	0.25 0.05	0.26 0.05	0.28 0.08	0.25 0.07	0.22 0.05	0.27 0.08
TRIGLYC	64.48 16.64	65.63 15.54	84.00 27.63	109.12 47.80	75.20 19.11	80.84 15.59
CHOLEST	229.98 41.97	211.13 38.85	224.72 36.83	239.11 66.41	225.75 21.26	263.39 52.70
PROT	6.89 0.43	7.11 0.44	7.33 0.49	7.28 0.39	7.40 0.28	7.37 0.24
ALBU	3.57 0.21	3.58 0.21	3.34 0.21	3.26 0.28	3.49 0.09	3.37 0.17
GLOB	3.34 0.42	3.53 0.51	3.95 0.54	4.02 0.40	3.92 0.25	4.00 0.39
A/G RATIO	1.11 0.17	1.06 0.20	0.86 0.15	0.83 0.13	0.90 0.06	0.87 0.14
B/C RATIO	19.76 5.00	13.21 2.57	13.72 3.94	13.33 2.79	13.72 4.31	13.48 1.26

Blood Biochemistry

Cross-sectional analysis: Tables 6 and 7 contain the mean and SD values for each of the blood biochemical variables for female and male chimpanzees, respectively. For example, from Table 6 it can be noted that 61 females between the ages of 0 and 10 years, with a mean age of 4.5 years, were studied. These chimpanzees were studied for a mean of 5.5 years, during which a mean 6.4 blood samples were taken for blood biochemical analysis (the

Table 7. Mean SD of 20 blood chemistry variables of 106 male chimpanzees, by decade of chronologic age

Age (Years)	0 to 10	10 to 20	20 to 30	30 to 40	40 to 50
n	54	34	10	7	1
Mean age (Years)	4.8	14.7	25.9	32.7	43.9
Mean follow-up (Years)	5.5	6.3	6.7	5.8	0.9
Mean no. of samples	6.6	7.5	7.7	6.9	2.0
	Mean	Mean	Mean	Mean	Mean
	SD	SD	SD	SD	SD
GLUC	98.41	99.13	100.33	101.98	98.50
	55.89	15.63	15.18	4.14	NA
BUN	13.41	11.66	13.14	12.28	10.50
	4.31	2.82	5.43	0.96	NA
CREAT	0.75	1.16	1.12	1.10	1.20
	0.32	0.16	0.21	0.14	NA
CALC	9.63	9.57	9.18	9.03	9.30
	0.79	0.31	0.51	0.22	NA
PHOS	4.77	3.08	2.97	2.72	2.75
	1.02	0.62	0.52	0.45	NA
SOD	138.17	140.86	140.93	140.83	142.00
	2.86	1.49	2.33	1.90	NA
POTAS	4.35	3.66	3.43	3.27	3.55
	2.18	0.29	0.34	0.28	NA
CHLOR	100.19	98.13	97.04	96.44	97.00
	1.97	2.44	1.67	2.47	NA
CK	363.10	370.11	379.63	351.88	361.50
	635.68	131.43	155.43	242.58	NA
AST	23.38	36.22	35.19	33.36	27.50
	9.61	16.32	9.84	6.40	NA
ALKPHOS	651.68	169.24	93.02	93.43	91.00
	312.56	108.71	30.44	36.65	NA
ALT	37.70	52.61	42.28	43.85	41.50
	9.69	37.24	26.49	15.10	NA
BILI	0.28	0.33	0.36	0.27	0.20
	0.05	0.08	0.14	0.05	NA
TRIGLYC	67.59	83.67	82.62	69.84	119.50
	17.91	33.30	24.29	17.36	NA
CHOLEST	218.20	197.54	222.58	211.23	235.50
	36.15	28.69	31.80	25.52	NA
PROT	6.91	7.39	7.33	7.39	7.20
	0.37	0.40	0.53	0.35	NA
ALBU	3.63	3.77	3.36	3.58	3.45
	0.24	0.25	0.37	0.30	NA
GLOB	3.28	3.62	3.97	3.81	3.75
	0.41	0.48	0.77	0.40	NA
A/G RATIO	1.15	1.08	0.90	0.96	0.90
	0.20	0.17	0.24	0.17	NA
B/C RATIO	20.05	10.07	11.30	11.49	8.75
	6.00	1.93	1.97	1.56	NA

NA = not applicable.

numbers differ slightly from those of the hematologic study). The value for glucose concentration (GLUC) of 91.63 mg/dl (with SD of 11.01) is the unweighted mean of the 61 individual mean GLUC values obtained from the female chimpanzees in this age group. Results from this analysis are also shown graphically for 3 representative blood chemistry variables (alkaline phosphatase activity [ALKPHOS], potassium concentration [POTAS] and globulin concentration [GLOB]) in the left-hand column of Fig. 2.

The intercepts, coefficients, and significance values for the regression model based on the cross-sectional blood biochemical data are given in Table 8. As was the case for the hematologic data, ages entered into models were deviations from the mean age (15 years) for the sample. Thus, according to the regression model, the mean for GLUC for females at the age of 15 years was 93.62 mg/dl. The mean for males was 4.48 mg/dl higher than that of females, and there also was a significant increase of about 0.46 mg/dl each year. This general pattern of higher values for males than for females, and a general increasing trend with age was consistent with the data shown in Tables 6 and 7, at least for the first three decades of life. The higher values in females of the 30- to 40-year age group onward may have resulted from insufficient numbers of males in these higher age groups.

The regression models in Table 8 revealed values for additional analytes (creatinine [CREAT], calcium [CALC], sodium [SOD], chloride [CHLOR], creatine kinase [CK], aspartate transaminase [AST], bilirubin [BILI], cholesterol [CHOLEST], protein [PROT], albumin [ALBU], ALBU/globulin ratio [A/G RATIO], and BUN/CREAT ratio [B/C RATIO]) that were, like GLUC, affected significantly by sex and age (as indicated by a significant age or age^2 parameter). Other patterns also were evident: alanine transaminase [ALT] was related to sex, with males having significantly higher values on average than did females, but was uninfluenced by age; phosphate [PHOS], POTAS, ALKPHOS, triglycerides [TRIGLYC], and GLOB were significantly influenced by age, but not by sex.

Year-to-year consistency: Values for the blood biochemical variables were closely related from year to year within individuals (Table 9), a pattern similar to that detected for the hematologic variables. Most variables substantially exceeded chance levels of consistency, with mean r values between 0.3 and 0.9 ($P < 0.05$); however, the mean year-to-year correlation coefficient of BILI barely met this criterion of statistical significance, and CK did not.

Longitudinal analysis: From results of this analysis, we selected three representative blood biochemical analytes for graphic display in Fig. 2. The panels in the right-hand column indicate for each chimpanzee the best-fitting straight lines representing longitudinal data of ALKPHOS, POTAS, and GLOB. The reader can compare the cross-sectional (left column) with the longitudinal results for the three analytes. Thus, for the ALKPHOS cross-sectional data, the best linear unbiased estimates for females (red line) and males (blue line) were congruent, reflecting lack of a significant sex difference, with significant linear (age) and curvilinear (age^2) terms, as reported in Table 8. (The upward curvature away from the cloud of data points after about 40 years of age could be eliminated with more thorough modeling [e.g., inclusion of a cubic term in the model]; however, such more precise modeling was beyond the scope of this analysis.)

The right-hand panel for ALKPHOS indicates graphically that values for this analyte decreased rapidly for most females and males during the first decade of life, as confirmed by the significantly negative slopes for females and males in the first decade, and continuing into the second decade for females (Table 10). In the cross-sectional data for POTAS, females and males did not differ significantly from each other in the cross-sectional data, but both had significant linear decreases with age (Fig. 2, left-hand center panel; Table 8). The right-hand center panel for POTAS indicates graphically that the individual slopes were not uniform in direction or magnitude. Since the average slopes for this variable did not differ significantly from zero, POTAS was not included in Table 10.

The cross-sectional data for GLOB indicated no sex effect, as revealed by the congruence of the red and blue lines, but had significant linear and curvilinear changes with age (Fig. 2, left-hand bottom panel; Table 8). The average slopes for GLOB were significantly greater than zero for males and females for the first two decades of life (Table 10). However, the right-hand bottom panel of Fig. 2 clearly indicates presence of a strong secular increase in this variable. (A secular increase is an upward change as a function of sidereal time, rather than as a function of age.)

Table 10 lists the 11 biochemical analytes (GLUC, BUN, CREAT, PHOS, AST, ALKPHOS, BILI, TRIGLYC, PROT, GLOB, A/G RATIO) mean slopes (unit per year) of which differed significantly

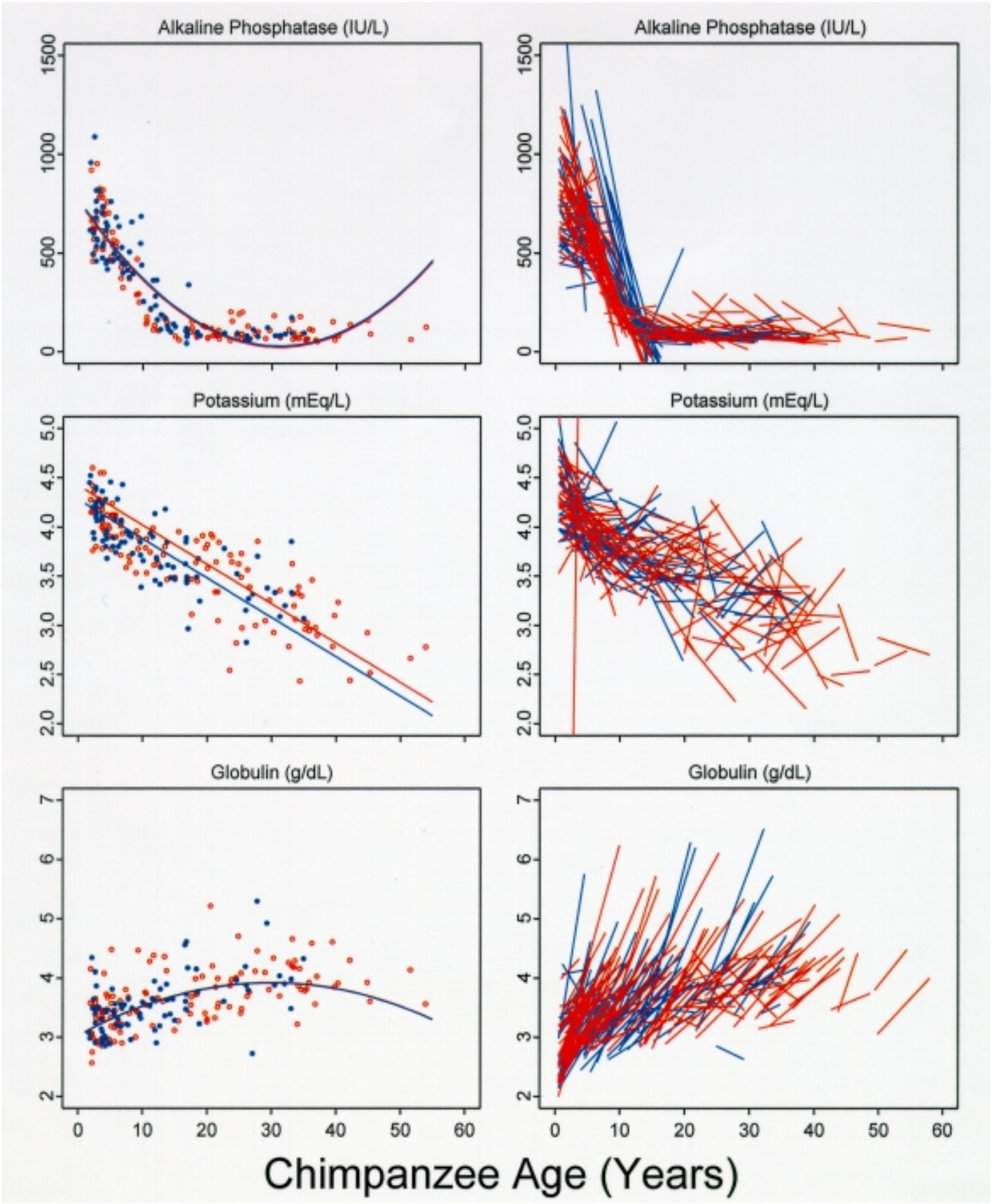

Figure 2. Results of three blood biochemical analytes with different, but representative patterns of results. Lines in the left-hand column were drawn from parameters given in Table 8. See Fig. 1 for key.

Table 8. Parameters (intercepts and coefficients) and significance levels for regression models of cross-sectional measures of 20 blood biochemical variables

Variable	Intercept	Sex	Age	Age^2
GLUC	93.62	4.48	0.46	—
	0.0000	0.0119	0.0000	—
BUN	12.44	0.93	—	—
	0.0000	0.0627	—	—
CREAT	0.88	0.19	0.02	-0.001
	0.0000	0.0000	0.0000	0.0000
CALC	9.34	0.12	-0.02	0.001
	0.0000	0.0204	0.0000	0.0008
PHOS	3.47	-0.10	-0.10	0.003
	0.0000	0.2438	0.0000	0.0000
SOD	139.27	0.90	0.11	-0.002
	0.0000	0.0001	0.0000	0.0012
POTAS	3.82	-0.14	-0.04	—
	0.0000	0.1333	0.0000	—
CHLOR	99.47	-1.12	-0.13	—
	0.0000	0.0000	0.0000	—
CK	273.05	62.48	3.77	-0.16
	0.0000	0.0003	0.0000	0.0031
AST	22.76	8.62	0.43	-0.01
	0.0000	0.0000	0.0000	0.004
ALKPHOS	218.69	6.93	-24.52	0.76
	0.0000	0.6819	0.0000	0.0000
ALT	34.63	7.12	—	—
	0.0000	0.0000	—	—
BILI	0.26	0.05	0.001	-0.0001
	0.0000	0.0000	0.0322	0.0335
TRIGLYC	75.94	0.54	0.81	—
	0.0000	0.8888	0.0000	—
CHOLEST	220.27	-12.67	-0.12	0.04
	0.0000	0.0328	0.6870	0.0347
PROT	7.20	0.12	0.02	-0.001
	0.0000	0.0322	0.0000	0.0007
ALBU	3.50	0.13	-0.01	—
	0.0000	0.0001	0.0000	—
GLOB	3.70	-0.01	0.03	-0.001
	0.0000	0.8133	0.0000	0.0030
A/G RATIO	0.98	0.04	-0.01	0.0002
	0.0000	0.0377	0.0000	0.0034
B/C RATIO	14.27	-1.29	-0.42	0.02
	0.0000	0.0167	0.0000	0.0000

— = not calculated.

from zero ($P < 0.0001$) during the first decade of life for one or both sexes. For seven of these analytes, changes occurred only in the first decade of life, whereas BILI, PROT, GLOB, and A/G RATIO also changed in later decades for at least one of the sexes.

Several analytes had significant patterns that did not easily fit into the succinct format of Table 10, which we, therefore, describe here separately. For example, ALBU had significant ($P < 0.0001$) mean decreases in slope in the second and third (but not the first) decades for males only; ALT had a significant decrease in the second decade in females only. In both of these cases, inspection of graphs of the data (not shown) revealed presence of secular changes similar to those seen in GLOB (Fig. 2, right-hand bottom panel). Several longitudinal slopes achieved borderline levels of significance ($P < 0.05$). For example, there was a weak decrease in ALKPHOS ($P < 0.005$) in the second decade for both sexes. There also were marginal decreases in POTAS ($P < 0.005$) and CHLOR ($P < 0.005$) in the first decade for females, and decreases in CALC ($P < 0.05$) and SOD ($P < 0.05$) for males. Because of their marginal statistical significance in an analysis in which many *P*-values were computed, the suggested trends in these analytes must be regarded cautiously.

Discussion

Our results supply reference values for clinically important hematologic and blood biochemical variables of the chimpanzee. To the authors' knowledge, this is the first cross- sectional presentation of these analytes covering both sexes throughout the entire life span of the chimpanzee. Furthermore, it is the first longitudinal study on individual chimpanzees over a period of 9 years.

Table 9. Consistency of 20 blood biochemical variables: mean year-to-year correlations, retransformed from Fisher Z statistics

	Females (n = 87.25)		Males (n = 66.25)	
Variable	*r*	*P*-value	*r*	*P*-value
GLUC	0.48	0.000	0.47	0.000
BUN	0.48	0.000	0.63	0.000
CREAT	0.73	0.000	0.77	0.000
CALC	0.45	0.000	0.57	0.000
PHOS	0.66	0.000	0.70	0.000
SOD	0.27	0.011	0.40	0.000
POTAS	0.56	0.000	0.62	0.000
CHLOR	0.43	0.000	0.48	0.000
CK	0.14	0.162	0.17	0.172
AST	0.45	0.000	0.53	0.000
ALKPHOS	0.87	0.000	0.86	0.000
ALT	0.53	0.000	0.59	0.000
BILI	0.22	0.039	0.27	0.028
TRIGLYC	0.58	0.000	0.59	0.000
CHOLEST	0.74	0.000	0.76	0.000
PROT	0.60	0.000	0.70	0.000
ALBU	0.57	0.000	0.72	0.000
GLOB	0.70	0.000	0.80	0.000
A/G RATIO	0.74	0.000	0.77	0.000
B/C RATIO	0.52	0.000	0.75	0.000

Mean number of chimpanzees in the 8 separate year-to-year comparisons is indicated by n.

In general, our cross-sectional hematologic data were in accord with those reported (2, 6, 9). One of those studies (6) calculated statistical models to examine changes in values across the age range from 1.2 to 15.0 years, and found some trends that differ from our results. For example, Hodson and colleagues (6) reported a decrease in RBC (in males) lasting to the age of 5 years, followed by a subsequent increase. Although they did not specifically analyze sex differences, it appeared from their figures that this pattern in RBC was found in males only, with that in females being constant. In contrast, we did not see a trend with age in RBC, but did note that males had significantly higher mean RBC values than did females. We found that HCT, HGB, MCV, and MCH increased with age, and appeared to flatten at about the age of 10 years, whereas Hodson and co-workers (6) reported a complex curvilinear pattern in HCT and HGB, with a decrease up to about 5 years of age and an increase thereafter.

Hodson and co-workers (6) also observed decreases in WBC in males and females, with a curvilinear pattern in females. We did not confirm the gradual decrease in WBC across age groups, but detected an overall sex difference in this variable, as indicated by a significant coefficient for sex. Our PLAT data decreased with age in both sexes; Hodson's group (6) reported a similar decrease only for females. To our knowledge, the remaining hematologic analytes of our study had not been not analyzed by others for possible age- or sex-related trends.

Mean values obtained for BUN, PROT, SOD, POTAS, CHLOR, and CALC in the first two decades of life were closely comparable to reported values (5, 7, 9). However, Hodson and colleagues (7) found a linear decrease in BUN with age for females, whereas we saw no change with age. We observed significant age-related increases in CALC, SOD, and PROT, and decreases in POTAS and CHLOR, whereas Hodson's group (7) found no linear change in these variables.

Most of the blood biochemical variables we measured were related to age in cross-sectional data, either in linear or curvilinear manner. Only BUN and ALT were entirely unrelated to

Table 10. Mean longitudinal slopes of 7 hematology and 11 blood chemistry variables for which the mean slope in the first decade of life differed significantly ($P < 0.0001$) from zero

	Females		Males	
Hematology	Mean	SEM	Mean	SEM
RBC	-0.05	0.010	NS	—
HCT	0.31	0.072	0.77	0.128
HGB	0.16	0.034	0.33	0.051
MCV	1.29	0.138	1.41	0.211
MCH	0.56	0.064	0.6	0.093
MCHC	0.19	0.043	NS	—
PLAT	-10.82	2.905	-17.75	2.323
Blood Chemistry				
GLUC	NS	—	2.17	0.549
BUN	0.69	0.321	1.02	0.193
CREAT	0.05	0.004	0.06	0.012
PHOS	-0.22	0.025	-0.19	0.029
AST	-0.79	0.018	NS	—
ALKPHOS	-56.41	7.300	-54.63	23.140
BILI	0.02[a,b,c]	0.003	0.03[a]	0.003
TRIGLYC	2.94	0.801	3.96	0.623
PROT	0.15[a,b]	0.016	0.17	0.022
GLOB	0.15[a]	0.015	0.17[a]	0.023
A/G RATIO	-0.06[a]	0.007	-0.06	0.010

[a] Second decade also differed significantly from zero.
[b] Third decade also differed significantly from zero.
[c] Fourth decade also differed significantly from zero.
NS = nonsignificant ($P > 0.0001$).
— = not computed.

age. The patterns of the relationship with age varied widely, and are best considered in light of the longitudinal data.

In an ideal study, cross-sectional and longitudinal data obtained from the same individuals should yield closely similar results: variables that change in cross-sectional data should change in the same direction and magnitude as do those for longitudinal data. Some of our hematologic and blood biochemical variables followed this ideal pattern; for example ALKPHOS decreased significantly in the cross-sectional and longitudinal data (Fig. 2, top row).

A second pattern of results was change in values across the age range, but without longitudinal consistency. This pattern was evident for SOD (increase), POTAS (decrease), and CHLOR (decrease), all of which changed in cross-sectional levels, but there was no tendency for individuals to have a uniform direction of change. The center row of Fig. 2 illustrates the downward trend in POTAS. The cross-sectional decrease seen in the left-hand panel was reflected by location of the individual animals' regression lines in the right-hand panel, but the directions of the lines were not consistent among individuals. This inconsistency between cross-sectional and longitudinal data may indicate that the underlying physiologic changes were too slow to be seen in an individual in the period under study.

A third pattern, seen in BILI, PROT, GLOB, and A/G RATIO, was that of a consistent longitudinal change (i.e., all lines change in the same direction), but with, at best, small changes in the cross-sectional data. The bottom row of Fig. 2 depicts this type of pattern for GLOB. The scatterplot on the left indicates a small but significant increase (indicated by the significant age effect in Table 8), with a significant curvilinear component (age^2 effect in Table 8). The right-hand bottom panel of Fig. 2 indicates the uniformity of increases (positive slopes) in many individuals. This pattern means that the values changed consistently from sample to sample for individuals (i.e., across calendar years [3]). A time-associated effect of this type is deemed a secular effect, and may reflect, changes in diet, assay characteristics, husbandry conditions, or other factors. Regardless of the cause of this secular effect, it was confounded with any effect due to chronologic age. One way to deal with this would be to estimate the size of the apparent year-to-year increase in GLOB in age-specific cohorts, then simply subtract the secular effect from the data. Since the subjects obviously increase in chronologic age as the secular (calendar-year) effect is occurring, the effect of age is distorted. Secular effects can introduce artifacts into cross-sectional analyses of age, so that age falsely appears to be a causal factor (10-12).

Some authors have suggested that similarity of changes in cross-sectional and longitudinal data is a prerequisite for considering a biological variable to be a biomarker of adult aging (13, 14). Several variables in our study were consistent between longitudinal and cross-sectional trends, but all had a longitudinal effect during the maturational period, rather than during the period from early adulthood to senescence. It is possible that some variables, (e.g., SOD, POTAS, CHLOR) that had a uniform change with age in the cross-sectional data, but that had an inconsistent pattern in the longitudinal analysis, may prove, in longer follow-up studies, to conform to the trends suggested by our cross-sectional analysis.

Our main goals were to establish normative values for hematologic and blood biochemical variables across the life span of the chimpanzee, and to undertake a longitudinal study following individuals for a period of several years. The latter goal was achieved by means of two-stage random effects modeling. This statistical approach, to our knowledge, not previously applied to studies of chimpanzee blood analytes, detected significant average trends for several variables during the first decades of life. Our results, therefore, highlight developmental and age-related changes. In addition, the tables and graphs we have provided should be of use to veterinarians with clinical responsibility for chimpanzees. Indeed, some patterns well known to clinicians appear in the data, such as the distinct sex differences in analytes related to red blood cells, and the marked decrease in alkaline phosphatase activity during maturation.

Most, but not all of the analytes considered, were quantitatively consistent from year to year, indicating that hematologic and blood biochemical results could be considered idiopathic traits of individuals. The regression models of the cross-sectional data suggested that many characteristics changed consistently with age, during maturation and the postmaturational progression toward senescence. In the longitudinal analyses, however, there were no consistent within-individual changes in the later portion of the life span. Instead, the most robust and reliable changes in several variables were restricted to the first few years of life. A notable difference was seen between males and females in hematologic analytes, with males having higher values and rates of maturational increase for several variables associated with erythrocytes.

Because life-long records are often available for chimpanzees, a study of their hematologic and blood biochemical variables should be undertaken, in which individuals are followed throughout their entire lifetime. Such studies would make it possible to determine whether consistent within-individual changes occur during adult aging. Additional studies of chimpanzees and other species of primates should address several important questions. They might clarify whether age-related changes in serum proteins (e.g., GLOB) are detectable in epochs when no secular effect is evident. They could test the hypothesis that the significant sex differences in variables associated with erythrocytes (e.g., RBC, HGB) in chimpanzees account for the large

difference in life expectancy in the sexes. Finally, they could determine whether the monotonic changes that appeared in our cross-sectional data for some electrolytes (SOD, POTAS, CALC, CHLOR) can actually be observed over time in the same individuals. If this were possible, these chemicals could serve as useful biomarkers of adult aging in the chimpanzee.

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