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Biometry Research Unit, Indian Statistical Institute, Calcutta

Age-Related Trends in the Blood Chemistry and Hematology of the Indian Carp *(Catla catla)**

By B. C. Das

Catla catla (Ham.) is one of the major species of Indian carp and was first classified by *Hamilton* in 1822. It is a soft-rayed teleost fish (Class *Osteichthyes*, Subclass *Actinopterygii*, family *Cyprinidae*), and is characterized by three median fins, dorsal, anal, and caudal, and two sets of paired fins, pectoral and ventral. The head is scaleless and the body is covered with cycloid scales; in color it is grayish on the back and silvery underneath and on the sides, with dark colored fins (*Adams* and *Eddy*, 1949; *Day*, 1958; *Hyman*, 1944; *Lagler*, 1956). It possesses a large air-vessel, and in common with other fishes, is poikilothermous or cold-blooded. *Catla catla* resides in fresh or brakish water, both in rivers and ponds, and is a non-predatory surface feeder, subsisting mainly on zooplankton and phytoplankton. Its maximum rate of growth is during the first two years of life, after which it becomes sexually mature. It reaches asymptotic growth at 127.5 cm length, corresponding to an age of more than six years (*Natarajan* and *Jhingran*, 1963).

Hematological studies of temperate zone teleost fishes have shown that the red blood corpuscles are nucleated, oval shaped, and larger than those of man. Concentration of hemoglobin has been found to vary among different species, and generally to be lower than that of mammals (*Mott*, 1957). There is a general absence of data on the blood chemistry of temperate zone teleost fishes. In a previous report from this laboratory, blood chemistry data on glucose and

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total protein were given for three major species of Indian carp, *Catla catla* (Ham.), *Cirrhina mrigala* (Ham.), and *Labeo rohita* (Ham.) (*Das*, 1961b). Aside from this report, the literature does not reveal any data on the blood chemistry or hematology of the Indian carp nor of other tropical teleost fishes.

Among the normal physiological functions of the organism with which the blood is concerned are respiration, nutrition, excretion, maintenance of water content, hormone regulation, and protection against disease and injury. Data are gradually becoming available which suggest that changes in the blood and blood supply occur with growth and aging in human beings (eg., *Goodale*, 1955; *Macy* and *Kelly*, 1957; *Das*, 1964). These changes must be related to or must affect the normal physiological functions carried out by the blood. The demonstration of age-related trends in blood chemistry and hematology in comparative investigations, across species, may provide insight into the fundamental processes of growth from youth to old age.

Catla catla was chosen as the subject of the present investigation of blood chemistry and hematology in relation to age because it is one of the fastest growing species of Indian carp and reaches asymptotic growth at a relatively large size. For the study of age-related trends in blood chemistry and hematology, the findings on a cold-blooded species might be of interest when compared to data collected on mammals. At the same time the data will fill in some of the gaps in knowledge on the blood of tropical zone teleost fishes, and particularly, characterize the species *Catla catla*. To permit comparison with data collected on human subjects, standard clinical methods of blood chemistry and hematology have been employed.

Methods

Three hundred and twenty-seven fish of the *Catla catla* (Ham.) species (abbreviated as *Catla*) were randomly drawn from a natural pond adjacent to the laboratory (in the northern industrial suburbs of Calcutta, India). The pond was 27 m wide, 46 m long, and 4.5 m deep along the central line. It had been stocked mainly with *Catla* at the postembryonic stage following hatching, but also with immature *Catla* from other sources. As a result, the *Catla* population of the pond had a mixed age distribution. Two months before the initial stocking of the pond with postembryonic *Catla* took place, all the fish present were removed, and the pond was thoroughly cleaned and fertilized with the oil-cake residue from the extraction of oil from mustard seeds. No other artificial food was added. When the initially stocked fish were 210 days old, random samples of 6 to 10 fish were caught daily between 8.00 and 9.00 a.m. for blood chemistry and hematology determinations. After being caught, the sample fish

were kept in a container in which the water was continually aerated. Between 9.30 and 10.00 a.m., blood was removed directly from the heart using a hypodermic syringe with a 20 bore needle. Eight to ten ml of blood were taken, depending on the amount available. Care was taken to avoid contamination of the blood with surface mucus and water. All laboratory tests were begun within 30 min after removal of the blood from the heart. The blood of each fish was kept separately for purposes of chemical and hematological analysis. EDTA (ethylene diamine tetra acetic acid) was used as the anticoagulant for blood to be processed directly without clotting as oxalate and heparin were found to be unsuitable as anticoagulants for *Catla* blood. After removal of blood, weight, length and height were recorded, and the fish was placed temporarily in a container of water. It was then transferred to another natural pond so that no fish would be sampled twice. More than 95% of the fish survived this procedure.

Measurements of 3 physical variables, 14 blood chemistry variables, and 5 hematological variables were planned for each fish in the sample. Due to an insufficient amount of blood in some cases, not all variables could be measured for all fish in the sample. The variables are numbered consecutively from 0 to 21 [see columns (1) and (2) of Table II]; variables 0, 1, and 2 are length, weight, and height respectively. After removal of blood, each fish was weighed on a standardized scale in grams. Length, defined as fork length in cm, was measured from the tip of the snout to the end of rays in the center of the caudal fin (*Carlander*, 1953). Height, defined as the maximum midsagittal distance in cm, was measured at a point immediately anterior to the dorsal fin. Variables 3 to 16 were indices of blood chemistry, and standard clinical methods were employed for their measurement [column (3) of Table II]. Variables 17 and 18 were hematological indices which were measured by standard clinical methods [column (3) of Table II], while variables 19 to 21 were hematological in character requiring the application of a new procedure. To ensure that the chemical and hematological methods were giving acceptable values, it was necessary to use human blood as a control in all determinations. The references for the methods used for variables 3 to 18 are as follows: *King* and *Wooten* (1956) for variables 3, 4, 6, 11, 12, 13 and 14; *Hawk et al.* (1954) for variables 5, 9, and 16; *Gradwohl* (1956) for variables 7 and 8; *Tirnsky* (1958) for variable 10; *Varley* (1962) for variable 15; and *Hepler* (1955) for variables 17 and 18. Column (4) of Table II gives the units of measurement for all of the variables.

Attempts to carry out a differential count as for human leukocytes were unsuccessful with *Catla* blood. Instead, the following procedure was developed to differentiate morphological types of *Catla* blood corpuscles:

(i) 2 ml of blood are treated with 5 mg EDTA anticoagulant;
(ii) a drop of the EDTA treated blood is placed on a slide, a film drawn, and fixed in methyl alcohol (3 min);
(iii) the slide is dipped in eosin (10 sec);
(iv) the slide is washed in distilled water and then dipped in Stevenel's blue (15 sec);
(v) the slide is washed in distilled water and dipped in eosin (5 sec);
(vi) the slide is washed in distilled water and dried.

Examination of the slide under an oil immersion objective has revealed three types of blood corpuscles. Type A corpuscles are oval shaped with a centrally located oval shaped nucleus; their cytoplasm is stained light bluish violet while the nucleus is stained dark violet; no granules are observed. Type B corpuscles are round shaped with an eccentrically located irregularly shaped nucleus; their cytoplasm is stained blue while the nucleus is stained dark violet; fine granules are observed. Type C corpuscles are sickle shaped with a centrally located oval shaped nucleus; their cytoplasm is stained light pink while the nucleus is stained dark violet; no granules are observed. For each fish, five slides have been prepared by the above procedure, and the mean number of each type of corpuscle has been recorded. These numbers have been converted to number 10^{-6} mm^3.

Results

Seven length-at-age groups have been defined in terms of length and weight, and the age-length relationship determined for *Catla* using Petersen's length-frequency method by *Natarajan* and *Jhingran* (1963). Table I gives the minimum, mean, and maximum values of length and weight for each of the length-at-age groups, and the corresponding minimum, mean, and maximum values of estimated age in days. Photographs of two specimens of *Catla* are given in Fig. 1. The estimated ages are 350 days for A and 450 days for B. Between the two specimens there is a difference in estimated age

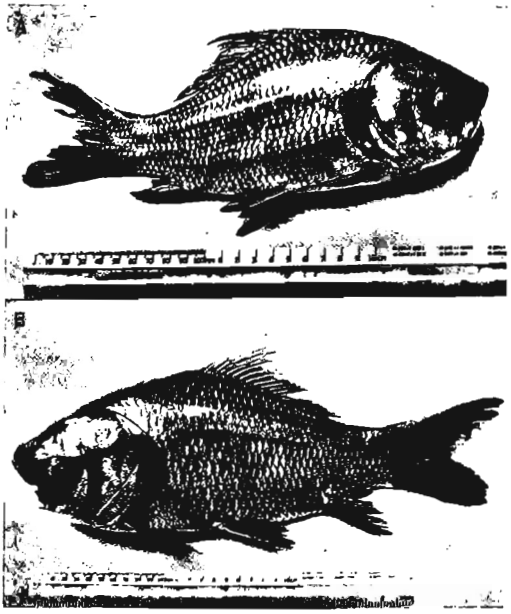

Fig. 1. Photographs of two fish of the species *Catla catla* (Ham.):
A, length 27 cm, weight 336 g, estimated age 350 days;
B, length 35 cm, weight 1012 g, estimated age 450 days.

Table I

Minimum, Mean, and Maximum Values of Estimated Age, Length, and Weight of *Catla catla* According to Length-at-Age

Length-at-age group (1)	Estimated age (days)			Length (cm)			Weight (g)			Number in group (11)
	Minimum (2)	Mean (3)	Maximum (4)	Minimum (5)	Mean (6)	Maximum (7)	Minimum (8)	Mean (9)	Maximum (10)	
1	210	228	273	16	17.67	20	0	102.38	174	21
2	274	301	337	21	23.06	25	175	274.17	349	47
3	338	347	373	26	27.06	28	350	422.04	499	51
4	374	392	401	29	29.89	30	500	540.64	599	44
5	402	411	428	31	31.82	32	600	660.11	699	38
6	429	438	455	33	33.52	34	700	764.15	849	65
7	456	475	529	35	36.13	39	850	997.34	1249	61

of 100 days, in length of 8 cm, and in weight of 676 g. These differences illustrate the cube law, i.e., the rate of increase in weight is the cube of the rate of increase in length. For the entire set of data, the equilibrium constant or exponential power of weight increase, in relation to length, is actually 3.09. This value, for pond *Catla*, is slightly lower than the equilibrium constant of 3.28 reported by *Natarajan* and *Jhingran* (1963) for river *Catla*.

Table II presents a statistical summary of the measurements obtained for the seven length-at-age groups on the 22 physical, chemical, and hematological variables. Four statistics are reported [column (5)]: mean (M), standard deviation (s), coefficient of variation (CV), and number (n). While the mean and standard deviation are expressed in the units of measurement specified in column (4), the coefficient of variation is a pure number obtained by computing 100 ($\frac{s}{M}$). The seven length-at-age groups are identified by their estimated mean ages, in days, given above columns (6) to (12), and the four summary statistics are reported by variable for all groups.

The best fitting linear equation has been computed by the least-squares procedure to express, quantitatively, the relationship between a given variable and length-at-age (*Lewis*, 1960). The value of the variable, Y, can then be estimated by the fitted equation,

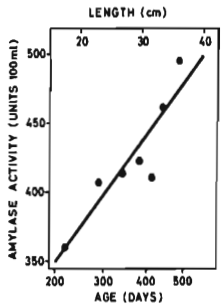

Fig. 2. Amylase activity of *Catla catla* serum in relation to length-at-age. The ordinate gives amylase activity in units/100 ml. The upper abscissa gives length in cm and the lower abscissa gives the corresponding age in days. The straight line exhibits the fitted relationship of amylase activity to length and the points represent the observed mean values of amylase activity for the length-at-age groups.

Table II

Means, Standard Deviations, and Coefficients of Variation of Physical, Blood Chemistry, and Hematological Variables for *Catla catla* According to Length-at-Age

Variable Number (1)	Name (2)	Method and material analyzed (3)	Unit of measurement (4)	Statistic* (5)	Length-at-age group (estimated mean age in days) 228 (6)	301 (7)	347 (8)	392 (9)	411 (10)	438 (11)	475 (12)
0	Length	Fork length	cm	M	17.67	23.06	27.06	29.89	31.82	33.52	36.13
				s	1.43	3.60	0.71	0.32	0.39	0.50	1.05
				CV	8.07	15.59	2.62	1.06	1.22	1.49	2.90
				n	21	47	51	44	38	65	61
1	Weight	Standardized scale	g	M	102.38	274.17	422.04	540.64	660.11	764.15	997.34
				s	34.80	57.77	35.80	29.75	24.80	37.68	117.32
				CV	33.99	21.07	8.48	5.50	3.76	4.93	11.76
				n	21	47	51	44	38	65	61
2	Height	Maximum midsagittal distance	cm	M	6.05	7.70	8.80	9.57	10.21	10.88	12.45
				s	0.79	1.22	1.07	0.62	0.52	0.85	0.88
				CV	12.99	15.77	12.12	6.46	5.10	7.82	7.06
				n	21	47	51	44	38	65	61
3	Amylase activity	*Somogyi*, serum	units/100 ml	M	360.00	408.00	413.86	423.44	411.43	463.58	494.60
				s	0	0	56.88	75.04	105.86	67.55	85.61
				CV	0	0	13.74	17.72	25.73	14.57	17.31
				n	1	1	7	9	7	12	10
4	Calcium	Modified EDTA, serum	mg/100 ml	M	9.00	5.12	7.93	7.71	8.17	7.71	7.58
				s	0	2.61	1.82	1.31	1.24	1.32	1.53
				CV	0	51.04	22.97	16.94	15.15	17.18	20.22
				n	1	12	15	14	21	31	26
5	Chlorides	*Whitehorn*, blood	mg/100 ml	M	448.88	519.14	483.34	481.56	517.65	516.45	521.10
				s	54.84	60.54	75.71	55.74	56.26	66.22	77.88
				CV	12.22	11.66	15.67	11.57	10.87	12.63	14.99
				n	8	29	28	32	34	59	50
6	Cholesterol, total	*Zak*, serum	mg/100 ml	M	159.07	124.76	116.63	125.86	135.34	151.72	178.02
				s	33.47	58.62	55.92	50.52	39.90	68.20	84.26
				CV	21.04	46.99	47.94	40.14	29.48	44.95	47.33
				n	9	28	42	35	33	59	55

* M = mean, s = standard deviation, CV = coefficient of variation, n = number.

Table II (continued)

Variable Number (1)	Name (2)	Method and material analyzed (3)	Unit of measurement (4)	Statistic* (5)	Length-at-age group (estimated mean age in days) 228 (6)	301 (7)	347 (8)	392 (9)	411 (10)	438 (11)	473 (12)
7	Cholesterol, free	*Zak*, serum	mg/100 ml	M	63.82	51.62	46.76	49.73	53.65	61.06	71.01
				s	13.27	26.02	22.37	20.89	16.20	27.38	30.04
				CV	20.80	50.40	47.85	42.01	30.19	44.84	42.31
				n	9	28	42	35	33	59	55
8	Cholesterol ester	*Zak*, serum	mg/100 ml	M	95.24	73.14	69.86	76.13	81.68	90.32	107.01
				s	20.22	35.44	30.47	29.80	23.75	41.15	51.03
				CV	21.23	48.45	43.61	39.14	29.08	45.56	47.69
				n	9	28	42	35	33	59	55
9	Creatinine	*Folin* and *Wu*, blood	mg/100 ml	M	1.00	0.92	1.56	1.26	1.37	1.50	1.43
				s	0.14	0.63	0.94	0.47	0.63	0.82	0.65
				CV	14.14	68.75	60.30	37.08	45.87	54.79	45.59
				n	3	10	20	17	13	22	24
10	Glucose	*Asatoor* and *King*, blood	mg/100 ml	M	62.88	76.48	71.50	79.04	76.28	81.06	80.33
				s	21.00	28.32	32.96	26.58	25.84	21.04	25.88
				CV	33.40	37.03	46.10	33.63	33.88	25.96	32.22
				n	8	25	24	24	32	49	43
11	Nonprotein nitrogen	*King*, blood	mg/100 ml	M	74.47	58.10	48.65	43.58	56.09	53.94	59.11
				s	28.04	16.17	23.59	14.78	20.74	27.96	25.02
				CV	37.65	27.84	48.48	33.92	37.04	51.84	42.32
				n	13	29	46	40	35	61	52
12	Phosphatase, acid	*King* and *Armstrong*, serum	KA units/100 ml	M	252.83	182.13	192.33	192.78	195.28	176.53	183.64
				s	88.36	54.59	28.99	46.83	55.40	65.08	60.27
				CV	34.95	29.97	15.07	24.29	28.37	36.87	32.82
				n	13	7	21	20	20	42	34
13	Phosphatase, alkaline	*King* and *Armstrong*, serum	KA units/100 ml	M	1.75	3.81	2.97	3.45	2.60	2.19	2.47
				s	0.75	3.04	3.60	2.39	2.08	1.71	1.71
				CV	42.86	79.84	121.18	69.09	80.02	78.04	69.10
				n	2	8	20	21	22	44	34
14	Protein, total	micro-Kjeldahl, serum	g/100 ml	M	2.67	2.50	2.74	2.61	2.91	2.75	2.91
				s	0.29	0.83	0.48	0.42	0.64	0.53	1.04
				CV	10.75	33.09	17.69	16.12	22.15	19.09	35.69
				n	3	19	26	29	31	46	39

* M = mean, s = standard deviation, CV = coefficient of variation, n = number.

Table II (continued)

Variable Number (1)	Name (2)	Method and material analyzed (3)	Unit of measurement (4)	Statistic * (5)	Length-at-age group (estimated mean age in days) 228 (6)	301 (7)	347 (8)	392 (9)	411 (10)	438 (11)	475 (12)
15	Urea	Urease Nesslerization, blood	mg/100 ml	M	8.81	4.79	4.32	4.86	4.81	5.66	5.56
				s	5.51	3.62	2.21	2.48	3.09	3.46	3.37
				CV	62.57	75.51	51.21	50.96	64.25	61.23	60.70
				n	12	30	40	37	36	61	52
16	Uric acid	*Brown*, blood	mg/100 ml	M	1.58	1.81	1.38	1.43	1.54	1.91	1.83
				s	0.75	0.85	0.76	0.65	0.26	0.64	0.66
				CV	47.62	46.71	45.38	45.38	16.86	33.52	32.27
				n	8	21	31	32	30	46	47
17	Blood corpuscles	standard erythrocyte count	million/mm³	M	2.60	2.32	2.73	2.66	2.92	2.86	2.88
				s	0	0.99	0.44	0.39	0.31	0.31	0.52
				CV	0	42.69	15.98	14.60	10.50	10.88	18.06
				n	1	6	15	27	21	37	27
18	Hemoglobin	*Sahli-Hellige*, blood	g/100 ml	M	7.29	8.18	7.44	7.58	8.48	8.61	8.37
				s	0.88	0.28	1.29	1.19	1.54	1.44	1.69
				CV	12.09	3.45	17.28	15.63	18.18	16.70	20.21
				n	7	28	27	31	31	59	54
19	Corpuscles (type A)	counting **	number/10^{-2} mm³	M	37.14	40.56	41.29	46.66	63.75	49.94	49.94
				s	5.94	19.07	12.69	17.87	16.21	16.70	11.32
				CV	15.99	47.01	30.73	38.30	25.43	33.44	22.66
				n	3	7	6	8	7	10	7
20	Corpuscles (type B)	counting **	number/10^{-2} mm²	M	1.13	1.13	1.60	1.34	1.13	1.06	1.21
				s	0.46	0.60	0.51	0.69	0.43	0.44	0.36
				CV	40.82	53.45	31.68	51.30	37.79	41.63	29.82
				n	3	7	6	8	7	8	7
21	Corpuscles (type C)	counting **	number/10^{-2} mm²	M	1.32	0.75	1.70	0.57	0.57	0.85	0.79
				s	0.27	0.27	0	0	0	0.28	0.20
				CV	20.20	35.35	0	0	0	33.33	24.99
				n	3	3	1	3	6	4	5

* M = mean, s = standard deviation, CV = coefficient of variation, n = number.
** Stained cells under oil immersion objective of microscope.

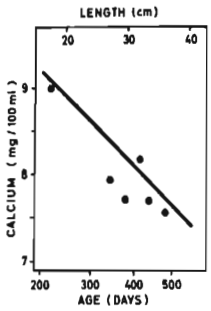

Fig. 3

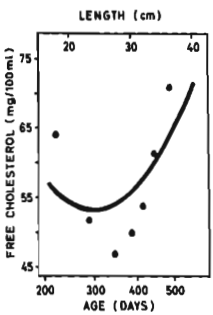

Fig. 4

Fig. 3. Calcium of *Calla calla* serum in relation to length-at-age. The ordinate gives calcium in mg/100 ml. The upper abscissa gives length in cm and the lower abscissa gives the corresponding age in days. The straight line exhibits the fitted relationship of calcium to length and the points represent observed mean values of calcium for the length-at-age groups.

Fig. 4. Free cholesterol of *Calla calla* serum in relation to length-at-age. The ordinate gives free cholesterol in mg/100 ml. The upper abscissa gives length in cm and the lower abscissa gives the corresponding age in days. The parabolic curve exhibits the fitted relationship of free cholesterol to length and the points represent observed mean values of free cholesterol for the length-at-age groups.

$Y = a+b\,X$, in which a and b are the fitted coefficients and X is length in cm. For the following variables, a linear equation is appropriate: amylase activity (see Fig. 2), calcium, chlorides, creatinine, acid phosphatase, alkaline phosphatase, total protein, blood corpuscles, hemoglobin, and type A corpuscles. Values of all these variables, except three, tend to increase with length-at-age. The three variables decreasing in value as length-at-age increases are calcium (see Fig. 3), acid phosphatase, and alkaline phosphatase. For six other variables, a parabolic equation is more appropriate, as the value of the variable decreases and then increases with length-at-age. The value, Y, of these variables, is estimated by the fitted equation, $Y = a+b\,X+c\,X^2$, in which a, b, and c are the coefficients fitted by the least-squares procedure, and X is length in cm. The six variables are total cholesterol, free cholesterol (see Fig. 4), cholesterol ester, nonprotein nitrogen, urea, and uric acid. No equation has been fitted to type B or C corpuscles as no

relationship with length-at-age has been observed. Table III gives both the observed mean (M_o), from Table II, and the fitted mean (M_f) obtained by using the fitted coefficients in the appropriate equation. The fitted coefficients are reported in column (4), the statistics are specified in column (5), and values for the seven length-at-age groups are presented in columns (6) to (12). The second length-at-age group has been ignored in fitting a linear equation to the values of calcium, and the first length-at-age group has been ignored in fitting a linear equation to the values of alkaline phosphatase.

The hypothesis that the fitted equation expresses the relationship between a variable and length-at-age is tested statistically by t tests (*Lewis*, 1960). For each length-at-age group, M_f serves as the hypothesized mean, and M_o serves as its sample estimate. To test the hypothesis, the difference between M_o and M_f is divided by the standard error of M_o. The result is t, which is evaluated for significance by comparing it with the tabled value of $t_{0.975}$ ($P < 0.05$, two-tailed) and $t_{0.995}$ ($P < 0.01$, two-tailed). The values of t are reported in Table III, along with their levels of significance.

Each t test of the set computed for any variable provides a statistical test of the significance of the difference between a pair of observed and fitted means. The significance of the set of t tests for a variable can also be evaluated using the partial or cumulative sums of the binomial expansion. If only one t is significant out of the seven t tests computed for the seven length-at-age groups, the null hypothesis cannot be rejected for the set as a whole, i.e., the probability of one $t \geq t_{0.975}$ is 0.30 and the probability of one $t \geq t_{0.995}$ is 0.07. If two out of seven t tests are significant, then the null hypothesis can be rejected, e.g., for $t_{0.975}$ the probability is 0.04 (*Edwards*, 1954; *National Bureau of Standards*, 1949). In the present context, the null hypothesis for a set of t tests states that there is no difference between observed and fitted values. This hypothesis can be accepted for all of the chemical and hematological variables except chlorides, nonprotein nitrogen and uric acid. For these three variables, the fitted equation cannot be accepted as the best expression of the relationship between the variable and length-at-age.

Discussion

The blood chemistry and hematology of *Catla* are described in terms of mean values on nineteen indices in Table II. Characteri-

Table III

Observed and Fitted Means, Coefficients of Equations Fitted to Length, and t Tests for Blood Chemistry and Hematological Variables for *Catla catla* According to Length-at-Age

Variable Number (1)	Name (2)	Unit of measurement (3)	Coefficients of fitted equation (4)	Statistic* (5)	Length-at-age group (estimated mean age in days) 228 (6)	301 (7)	347 (8)	392 (9)	411 (10)	438 (11)	475 (12)
3	Amylase activity	units/100 ml	a = 251.90	M_o	360.00	408.00	413.86	423.44	411.43	463.58	494.60
			b = 6.08	M_t	359.37	392.20	416.68	433.70	445.44	455.82	471.69
				t	**	**	0.12	0.39	0.79	0.38	0.80
4	Calcium	mg/100 ml	a = 10.43	M_o	9.00	–	7.93	7.71	8.17	7.71	7.58
			b = −0.08	M_t	9.09	–	8.37	8.16	8.01	7.88	7.68
				t	**	–	0.90	1.24	0.57	0.70	0.33
5	Chlorides	mg/100 ml	a = 411.39	M_o	448.88	519.14	483.34	481.56	517.65	516.45	521.10
			b = 3.06	M_t	465.37	481.84	494.06	502.70	508.60	513.79	521.77
				t	0.80	4.56++	0.74	2.05+	0.92	0.31	0.06
6	Cholesterol, total	mg/100 ml	a = 216.57	M_o	159.07	124.76	116.63	125.86	135.34	151.72	178.02
			b = −7.47	M_t	135.66	131.41	134.42	139.65	144.66	150.14	160.36
			c = 0.16	t	1.98	0.59	2.04+	1.59	1.32	0.18	1.54
7	Cholesterol, free	mg/100 ml	a = 93.24	M_o	63.82	51.62	46.76	49.73	53.65	61.06	71.01
			b = −3.40	M_t	55.43	52.81	53.54	55.42	57.33	59.48	63.56
			c = 0.07	t	1.79	0.30	1.94	1.59	1.28	0.44	1.82
8	Cholesterol ester	mg/100 ml	a = 122.19	M_o	95.24	73.14	69.86	76.13	81.68	90.32	107.01
			b = −3.97	M_t	79.86	78.09	80.15	83.29	86.22	89.38	95.23
			c = 0.09	t	2.15	0.73	2.16+	1.40	1.08	0.17	1.70
9	Creatinine	mg/100 ml	a = 0.46	M_o	1.00	0.92	1.56	1.26	1.37	1.50	1.43
			b = 0.03	M_t	0.98	1.14	1.25	1.33	1.39	1.44	1.52
				t	0.21	1.02	1.42	0.59	0.12	0.31	0.61
10	Glucose	mg/100 ml	a = 51.03	M_o	62.88	76.48	71.50	79.04	76.28	81.06	80.33
			b = 0.86	M_t	66.14	70.76	74.20	76.59	78.24	79.70	81.93
				t	0.41	0.99	0.39	0.44	0.42	0.45	0.40

* M_o = observed mean, M_t = fitted mean; ** M_o = based on one observation; + $P < 0.05$; ++ $P < 0.01$.

Table III (continued)

Variable Number (1)	Name (2)	Unit of measurement (3)	Coefficients of fitted equation (4)	Statistic* (5)	Length-at-age group (estimated mean age in days) 228 (6)	301 (7)	347 (8)	392 (9)	411 (10)	438 (11)	475 (12)
11	Nonprotein nitrogen	mg/100 ml	a = 96.98	M_o	74.47	58.10	48.65	43.58	56.09	53.94	59.11
			b = −2.38	M_f	65.23	59.30	56.04	54.33	53.44	52.82	52.22
			c = 0.03	t	1.14	0.39	2.10+	4.54++	0.75	0.31	1.97
12	Phosphatase, acid	KA units/100 ml	a = 281.55	M_o	252.83	182.13	192.33	192.78	195.28	176.53	183.64
			b = −2.99	M_f	228.74	212.61	200.58	192.22	186.45	181.35	173.55
				t	0.39	1.37	1.27	0.05	0.69	0.47	0.96
13	Phosphatase, alkaline	KA units/100 ml	a = 6.31	M_o	–	3.81	2.97	3.45	2.60	2.19	2.47
			b = −0.11	M_f	–	3.72	3.27	2.96	2.74	2.55	2.26
				t	–	0.08	0.37	0.95	0.32	1.39	0.73
14	Protein, total	g/100 ml	a = 2.27	M_o	2.67	2.50	2.74	2.61	2.91	2.75	2.91
			b = 0.02	M_f	2.55	2.64	2.71	2.75	2.78	2.81	2.85
				t	0.55	0.73	0.37	1.78	1.07	0.73	0.34
15	Urea	mg/100 ml	a = 15.89	M_o	8.81	4.79	4.32	4.86	4.81	5.66	5.56
			b = −0.67	M_f	7.28	5.95	5.35	5.14	5.08	5.10	5.23
			c = 0.01	t	0.92	1.72	2.93++	0.67	0.53	1.25	0.70
16	Uric acid	mg/100 ml	a = 2.12	M_o	1.58	1.81	1.38	1.43	1.54	1.91	1.83
			b = −0.05	M_f	1.59	1.56	1.59	1.62	1.66	1.69	1.76
			c = 0.001	t	0.05	1.31	1.45	1.63	2.42+	2.25+	0.99
17	Blood corpuscles	million/mm^3	a = 1.78	M_o	2.60	2.32	2.73	2.66	2.92	2.86	2.88
			b = 0.03	M_f	2.33	2.50	2.63	2.71	2.77	2.83	2.91
				t	••	0.42	0.85	0.67	2.18+	0.72	0.30
18	Hemoglobin	g/100 ml	a = 6.30	M_o	7.29	8.18	7.44	7.58	8.48	8.61	8.37
			b = 0.06	M_f	7.35	7.67	7.91	8.08	8.19	8.30	8.45
				t	0.18	1.24	1.86	2.31+	1.03	1.66	0.35
19	Corpuscles (type A)	number/10^{-3} mm^3	a = 93.46	M_o	65.67	71.71	73.00	82.50	112.71	88.30	88.29
			b = 1.75	M_f	64.33	73.75	80.79	85.67	89.04	92.03	96.58
				t	0.18	0.15	0.78	0.27	2.02	0.38	1.02

* M_o = observed mean, M_f = fitted mean; •• M_o = based on one observation; + $P < 0.05$; ++ $P < 0.01$.

zation of this species may be conveniently carried out by comparing *Catla* values with normal human values. A comparison is feasible because this investigation employed standard clinical methods customarily used to test human blood in the same laboratory, and samples of human blood were tested as controls throughout the experimental period. *Catla* values and human values are similar for total cholesterol, free cholesterol, cholesterol ester, creatinine, and glucose. In view of its size and aquatic habitat, the similarity between *Catla* and human subjects in cholesterol is noteworthy. *Catla* values tend to be slightly lower than human values for calcium, chlorides, and alkaline phosphatase. Amylase activity, nonprotein nitrogen, and acid phosphatase reach high levels in *Catla*; values typical for *Catla* would be regarded as abnormal in human subjects. Total protein, urea and uric acid are low in *Catla*. Total protein has also been observed to be low in *Cirrhina mrigala* and *Labeo rohita*, two other species of Indian carp (*Das*, 1961b). Low values of urea and uric acid are consistent with the hypothesis that aquatic organisms can readily remove excretory products from their systems. Hematological comparison reveals relatively low hemoglobin, and a standard erythrocyte count of *Catla's* nucleated blood corpuscles also reveals low values. These hematological findings are consistent with those reported for temperate zone teleost fish (*Mott*, 1957). These similarities and differences suggest that blood chemistry and hematological indices can serve as species and family markers. For *Catla* high values on amylase activity, nonprotein nitrogen, and acid phosphatase may be uniquely characteristic; low total protein may typify Indian carp (family *Cyprinidae*). Habitat, nutrition, and other environmental factors may, on investigation, also be shown to affect blood chemistry and hematology.

The period of most rapid growth in *Catla* takes place during the first two years of life (*Natarajan* and *Jhingran*, 1963). At the end of this period, which is prepuberal, the fish reach a length of 50 cm, which is approximately 40% of the length at asymptotic growth, 127.5 cm. Changes in blood chemistry and hematology, if any, which are associated with changes in age may be amplified during this period of accelerated growth. Examining fish according to length-at-age (which is more directly measurable than age itself in fish) the present results show that a number of blood chemistry and hematological variables do exhibit such changes. Values of the following variables tend to increase linearly with length-at-age:

amylase activity, chlorides, creatinine, glucose, total protein, blood corpuscles, hemoglobin, and type A corpuscles. The coefficients a and b of the linear equations fitted by the least-squares procedure are given in column (4) of Table III. Fitted mean values computed using these coefficients are presented in columns (6) to (12) of Table III for the seven length-at-age groups. To test the goodness of the fit, t tests have been computed for each of the length-at-age groups and the significance of the set of t tests for each variable has been evaluated. The hypothesis of a linear increase with age, during the prepuberal period, appears tenable for amylase activity (see Fig. 2), creatinine, glucose, total protein, blood corpuscles, hemoglobin, and type A corpuscles. These indices may reflect synthesis or growth of the organism. From this set of variables, glucose, blood corpuscles (as erythrocytes), and hemoglobin have been investigated for adult human subjects in this laboratory. Glucose showed a significant increase with age, erythrocytes decreased significantly with age, and hemoglobin was not significantly related to age (*Das*, 1964). An increase in chlorides for *Catla* with length-at-age has been noted, but the hypothesis of a linear fit is not tenable; in the human subjects, chlorides showed a significant increase with age (*Das*, 1964).

As length-at-age increases, calcium (see Fig. 3), acid phosphatase, and alkaline phosphatase decrease in value for *Catla* (Table II), and the hypothesis of a linear decrease with length-at-age appears tenable (Table III). Only the first of these three variables has been investigated in this laboratory for human subjects, and it showed a significant increase, rather than decrease, with age (*Das*, 1964). Children have been reported to exhibit higher values of alkaline phosphatase than adults (*Goodale*, 1955); this report is consistent with the present finding.

For six variables, an initially high value tends to decrease, and then subsequently to increase, with length-at-age during the prepuberal period of *Catla*. This relationship may be described as parabolic. The hypothesis of a parabolic relationship may be regarded as tenable for total cholesterol, free cholesterol (see Fig. 4), cholesterol ester, and urea (Table III). For nonprotein nitrogen and uric acid the fitted equation is apparently not adequate although the general trend appears to be parabolic. A parabolic relationship may result from the interaction of a number of factors. Initially active factors may have an inhibitory effect on a particular variable, and sub-

sequently active factors may have a facilitative effect. Weight may be considered as an example of the latter group of factors. It has been observed to increase at a rate cubic to that of length in *Catla*, so its effect will increase more rapidly than age itself. Data from this laboratory have shown that weight influences cholesterol level in adult human subjects (*Das*, 1959, 1960, 1961a, 1964). The operation of two factors, age and weight, could possibly explain the observed parabolic relationship of cholesterol to length-at-age.

These data have suggested that values of blood chemistry and hematological variables do change with age in *Catla*. Data have been reported elsewhere which suggest that age may influence these variables in human beings (*Goodale*, 1955; *Macy* and *Kelly*, 1957; *Das*, 1964). The confirmation of relationships between age and blood chemistry and hematological variables in future studies, both within and between species, may guide detailed investigations of the chemistry of growth and aging as well as of age-related changes in the functioning of the organ systems.

Summary

Blood chemistry and hematological variables were measured in 327 fish of the *Catla catla* (Ham.) species of Indian freshwater carp. Using length-at-age determined by Petersen's method, the fish were found to vary from 210 to 529 days of age. They were allocated to seven length-at-age groups, having mean ages of 228, 301, 347, 392, 411, 438 and 475 days.

Twenty two variables were measured: three physical variables of length, weight, and height; fourteen chemical variables for blood or serum, i.e., amylase activity, calcium, chlorides, cholesterol (total, ester, and free), creatinine, glucose, nonprotein nitrogen, acid and alkaline phosphatase, total protein, urea, and uric acid; and five hematological variables, i.e., hemoglobin and three types of blood cells (separately and combined).

For each of the variables, mean, standard deviation, and coefficient of variation have been computed, separately by length-at-age group, and equations have been fitted to length-at-age for each variable using the least squares procedure.

Values of the following variables tended to increase linearly with length-at-age: amylase activity, chlorides, creatinine, glucose, total protein, hemoglobin, and blood cells. Values of calcium and

of acid and alkaline phosphatase tended to decrease linearly with length-at-age. A parabolic relationship with length-at-age was observed for total cholesterol, free cholesterol, cholesterol ester, non-protein nitrogen, urea, and uric acid.

The implications of these results for the physiology of growth and aging are discussed.

Zusammenfassung

Bei 327 indischen Frischwasserkarpfen *Catla catla* (Ham.) wurden chemische und hämatologische Blutuntersuchungen gemacht. Die Fische waren nach der Methode von Petersen, wobei das Alter nach der Länge bestimmt wird, 210–529 Tage alt. Sie wurden in 7 Längenaltersgruppen mit durchschnittlichem Alter von 228, 301, 347, 392, 411, 438 und 475 Tagen eingeteilt.

22 Variable wurden bestimmt: drei physikalische betreffend Länge, Gewicht und Höhe, 14 chemische im Blut oder im Serum, nämlich Amylaseaktivität, Calcium, Chloride, Cholesterin (Total, Ester und freies), Kreatinin, Glucose, Nichtproteinstickstoff, saure und alkalische Phosphatase, Gesamtstickstoff, Harnstoff, Harnsäure, sowie 5 hämatologische Bestimmungen, und zwar Hämoglobinmessung und Bestimmung von 3 Arten von Blutzellen (einzeln und kombiniert). Für jede dieser Bestimmungen wurde der Durchschnitt, die Standardabweichung und der Variationskoeffizient bei jeder Längenaltersgruppe bestimmt. Für jede Altersgruppe wurden nach der «least squares»-Methode Gleichungen für jede Variable aufgestellt.

Die folgenden Werte nehmen mit dem Alter linear zu: Amylaseaktivität, Chloride, Kreatinin, Glucose, Gesamtstickstoff, Hämoglobin und Blutzellen. Calcium, saure und alkalische Phosphatase nehmen linear ab mit dem Alter. Ein parabolischer Zusammenhang mit dem Alter wurde für Totalcholesterin, Cholesterinester, freies Cholesterin, Nichtproteinstickstoff, Harnstoff und Harnsäure gefunden.

Die Bedeutung der Resultate für die Wachstums- und Altersphysiologie wird diskutiert.

Résumé

Un certain nombre de caractéristiques sanguines et hématologiques de *Catla catla*, poisson d'eau douce Indien, ont été étudiées sur 327 animaux. L'âge de ces derniers a été estimé d'après leur taille, suivant la méthode de Petersen; il variait de 210 à 529 jours. Les poissons étudiés ont été répartis en 7 catégories d'âge, correspondant respectivement à 228, 301, 347, 392, 411, 438 et 475 jours.

22 variables ont été mesurées: longueur, poids et hauteur du corps, activité de l'amylase sérique, calcium, chlorures, cholestérol (total, estérifié et libre); créatinine, glucose, azote non protéique, phosphatases acide et alcaline, protéines totales, urée, acide urique, teneur en hémoglobine et nombre de cellules sanguines.

Dans chaque cas, on a calculé la moyenne, l'écart type et le coefficient de variation et étudié leurs variations en fonction de l'âge.

L'activité de l'amylase sérique, les teneurs du sang en chlorures, créatinine, glucose, protéines totales et en hémoglobine, ainsi que le nombre de cellules sanguines, tendent à augmenter linéairement avec l'âge. Inversement le calcium sanguin et les phosphatases acide et alcaline diminuent au fur et à mesure que le poisson augmente en taille. Un rapport parabolique a été observé entre l'âge et le cholestérol (total, libre et estérifié), l'azote non protéique, l'urée et l'acide urique.

L'auteur discute les conséquences de ces résultats pour la physiologie de la croissance et du vieillissement des poissons.

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Author's address: Dr. B. C. Das, Biometry Research Unit, Indian Statistical Institute, 203 Barrackpore Trunk Road, *Calcutta* (India).