CORRELATION BETWEEN HEMATOLOGIC AND BIOCHEMICAL PARAMETERS IN *Testudo horsfieldi*

L. A. Tiunov, V. A. Ivanova,
A. I. Kliorin, A. A. Turdyev,
and V. P. Timofeev

UDC 612.111/112:612.015.1/.3]-019:598.13

KEY WORDS: turtles; blood morphology and biochemistry; correlation; adaptation.

Maintenance of optimal relations between the components of the cell during continuous changes in its functional activity, linked with changes in external environmental conditions, is of great importance in the maintenance of intracellular homeostasis [6]. It can accordingly be postulated that during adaptation of organisms to the action of external environmental factors optimal relations have developed between cell components and, as a result of this, optimal relations have arisen between biochemical and physiological processes, expressed as correlations of various kinds between them.

It was shown previously that definite correlations exist in healthy animals and man between individual variations in some hematologic and biochemical indices [4, 8]. Such correlations have been found in albino mice and rats, rabbits, monkeys, and man. It has been suggested that individual variations in indices linked by such correlations may determine the formation of individual responses to external factors and may play a role in adaptation of the organism to changing external environmental conditions. Similar data also have been obtained by studies of correlations between interspecific variations in hematologic and biochemical indices [4].

Investigation of such correlations in animals of other classes, differing in their responses to external factors from mammals, is of considerable interest. Among such animals may be mentioned turtles, which possess well-marked radioresistance and which are also extremely resistant to hypoxia [9].

EXPERIMENTAL METHOD

The work was done on turtles (*Testudo horsfieldi*) in November at the Institute of Zoology and Parasitology, Academy of Sciences of the Uzbek SSR. Mature individuals with a body weight of 701 ± 16 g were studied. Erythrocytes and leukocytes were counted and the blood formula calculated by the usual method.

To determine the biochemical indices the blood was preserved by drying on paper. The level of sulfhydryl groups in blood thus preserved was determined spectrophotometrically. Glucose-6-phosphate dehydrogenase (G6PDH) activity was determined by the method in [10] and lactate dehydrogenase (LDH) by the method in [12]. Blood levels of glucose, cholesterol, lactic and pyruvic acids, nonprotein nitrogen, and urea were determined in accordance with the technical recommendations given in [7]. The numerical results were subjected to statistical analysis by Student's t test. The rank correlation coefficient was calculated by Spearman's formula and its significance estimated from tables [2].

EXPERIMENTAL RESULTS

The study of hematological and biochemical indices of the blood in *Testudo horsfieldi* ($n = 6$) gave the following results: erythrocytes 1.1 ± 0.08 million/mm^3, hemoglobin 5.43 ± 0.47 g%, leukocytes 13,342 ± 600/mm^3, polymorphs 6200 ± 400/mm^3, lymphocytes 5900 ± 500/mm^3, basophils 460 ± 60/mm^3, eosinophils 590 ± 90/mm^3, monocytes 410 ± 40/mm^3, G6PDH 148 ± 4.07 pmoles NADP$^+$/ml/min, LDH 7.2 ± 0.8 pmoles NADH/ml/min, sulfhydryl groups 145 ± 36 mg %, glucose 50.3 ± 5.07 mg %, lactic acid 5.4 ± 0.32 mg %, pyruvic acid 0.49 ± 0.04 mg %, cholesterol 161 ± 11.3 mg %, nonprotein nitrogen 57.3 ± 9.6 mg %, urea 46 ± 4.98 mg %. Analysis

(Presented by Academician of the Academy of Medical Sciences of the USSR M. I. Kuzin.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 92, No. 7, pp. 47-49, July, 1981. Original article submitted March 25, 1981.

 0007-4888/81/9207-0910$07.50

TABLE 1. Correlations between Hematologic and Biochemical Indices (rank correlation coefficients) in *Testudo horsfieldi*

Index	Erythrocytes	Leukocytes	Lymphocytes	Polymorphs	G6PDH	Cholesterol
Hemoglobin	+0,62	+0,48	−0,68	−0,24	−0,35	+0,53
P	>0,05	>0,05	>0,05	>0,1	>0,05	>0,05
Sulfhydryl groups	−0,60	−0,85	−0,90	+0,72	+0,78	+0,90
P	>0,05	>0,1	0,01	0,05	0,05	0,01
Glucose	+0,30	−0,35	−0,20	+0,43	−0,20	−0,24
P	>0,05	>0,05	>0,1	>0,05	>0,1	>0,1
Lactic acid	+0,74	+0,70	+0,63	−0,32	−0,50	+0,33
P	0,05	0,05	>0,05	>0,1	>0,05	>0,05
Pyruvic acid	+0,40	+0,08	+0,58	+0,24	+0,56	+0,88
P	>0,05	>0,1	>0,05	>0,05	>0,05	0,01
G6PDH	−0,92	−0,42	−0,74	+0,60	—	+0,80
P	0,01	>0,05	0,05	>0,05	—	0,01
LDH	−0,60	−0,25	−0,57	+0,43	+0,89	−0,50
P	>0,05	>0,05	>0,05	>0,05	0,01	>0,05
Cholesterol	+0,80	−0,45	+0,67	+0,08	−0,20	—
P	0,01	>0,05	>0,05	>0,01	>0,1	—
Urea	+0,33	−0,18	−0,17	−0,01	−0,20	+0,13
P	>0,1	>0,1	>0,1	>0,1	>0,1	>0,1

of these data reveals a number of particular features characteristic of turtles' blood. The low erythrocyte count and the relatively high hemoglobin content per cell will be noted. These observations agree with data in the literature, according to which the hemoglobin content per erythrocyte in turtles is 85×10^{-12} g, compared with 28×10^{-12} g in man. The erythrocyte volume is correspondingly greater in turtles (300 μ^3) than in man (72 μ^3) [5]. The relatively low erythrocyte count in turtles' blood is thus compensated by the high saturation of these blood cells with hemoglobin.

Meanwhile the leukocyte count in turtles' blood is relatively high. This fact is in agreement with the results of the writers' previous investigations which showed that interspecific variations in the erythrocyte level correlate negatively with interspecific variations in the leukocyte count [4].

Another noteworthy feature is that the levels of sulfhydryl groups, glucose, and lactic and pyruvic acids, and also G6PDH and LDH activity are lower in turtles than in mammals. These results agree with those obtained by other workers who found that in reptiles the overall level of metabolism is about seven times lower than in mammals of the same size kept at the same temperature [11]. Meanwhile the blood level of products of nitrogen metabolism (urea and nonprotein nitrogen) is relatively higher in turtles. In this connection it is worth noting that during adaptation of albino rats to hypoxia a high blood urea concentration is observed, and the increase is more marked in animals with high resistance to hypoxia (up to 22 mg %) [3]. In turtles, on the other hand, the blood urea is 46 ± 4.98%.

However, the most important factor determining ability of an animal to adapt itself to changing external environmental conditions is the character and strength of correlations between the various indices, which reflect the state of regulatory mechanisms and the degree of synchronization of physiological and biochemical processes.

Analysis of correlations between hematologic and biochemical indices of turtles' blood (Table 1) reveals a definite pattern just as is found in mammals, evidence of the general biological importance of this phenomenon. However, the number of significant correlations is comparatively low in turtles, not more than 25% of the number tested. For comparison it is worth noting that the percentage of significant values of coefficients of correlation between hematologic and biochemical blood indices in monkeys reached 80 [8].

The absence of close correlations, it can be tentatively suggested, allows the course of metabolic processes to be changed more rapidly and the animal to adapt itself more easily to changing external environmental conditions. In this respect some interesting observations were made by Baevskii [1], who found that the presence of weak correlation between physiological indices reflects their ability to switch rapidly to a new optimal level of function, when active mobilization of functional systems is required. Meanwhile the predominance of strong correlations between physiological indices results in inertia of physiological systems: a decrease in the "freedom" of individual elements of the system to become incorporated into new functional assemblages, the appearance of which is necessitated by changes in external environmental conditions.

Analysis of the data in Table 1 reveals a much stronger correlation between individual variations in the erythrocyte count and biochemical indices than is the case with leukocytes. The white blood system in turtles is probably more labile and adapts itself more rapidly to meet the conditions of existence.

Examination of particular combinations of indices in turtles shows that correlation between variations in the erythrocyte count and the hemoglobin content per erythrocyte is weaker than in mammals. Meanwhile, just as in mammals, strong negative correlation exists between the number of erythrocytes and their G6PDH activity: The lower the erythrocyte count the higher the enzyme activity, i.e., the fewer the erythrocytes in an animal, the higher their resistance and functional activity.

Positive correlation between variations in the erythrocyte count and the cholesterol concentration is combined with a high cholesterol level in turtles' red blood cells.

The correlations found between the cholesterol level and some indices of carbohydrate metabolism are interesting. For instance, the relatively high cholesterol level is the result of low activity of the sulfhydryl enzyme G6PDH and a correspondingly low overall level of SH groups. At the same time the pyruvic acid concentration is relatively high. These results agree with views on the mechanism of endogenous cholesterol formation. The correlations discovered can be used to predict the blood cholesterol level.

It may be that the particular pattern of quantitative relations found between the hematologic and biochemical indices and also in the character and strength of correlations between them reflects particular features of functional activity of the cells and the specific nature of metabolic processes in turtles, a factor which probably plays an important role in the mechanism of the high resistance of these animals to hypoxia and radiation.

LITERATURE CITED

1. R. M. Baevskii, Prediction of States on the Borderline between Normal and Pathological [in Russian], Moscow (1979).
2. N. Bailey, Statistical Methods in Biology [Russian translation], Moscow (1962).
3. V. A. Berezovskii, K. N. Boiko, K. S. Klimenko, et al., Hypoxia and Individual Differences in Reactivity [in Russian], Kiev (1978).
4. A. I. Kliorin and L. A. Tiunov, Functional Inequality of Erythrocytes [in Russian], Leningrad (1974).
5. P. A. Korzhuev, Hemoglobin [in Russian], Moscow (1964).
6. D. S. Sarkisov, in: Homeostasis [in Russian], Moscow (1976), pp. 133-178.
7. V. P. Timofeev, Technical Recommendations for the Use of a Method of Combined Microchemical Analysis of Blood [in Russian], Leningrad (1979).
8. L. A. Tiunov, V. A. Voronin, V. A. Ivanova, et al., Fiziol. Zh. SSSR, No. 2, 235 (1975).
9. A. A. Turdyev, in: Radiosensitivity and Repair Processes in Animals and Plants [in Russian], Tashkent (1979), pp. 35, 48, and 81.
10. H. Bergmeyer, Methoden der enzymatischen Analyse, Weinheim (1962).
11. W. R. Dawson and G. A. Bartholomew, Physiol. Zool., 31, 100 (1958).
12. B. R. Hill and C. Levi, Cancer Res., 14, 513 (1954).