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EXPERIMENTAL BIOLOGY

Dynamics of Hematological Indexes in Alcoholic Intoxication in Relation to Ecological Factors

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UDC 616.89-008.441.13:616.152:614.7

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 116, № 8, pp. 195-197, August, 1993
Original article submitted February 19, 1993

Key Words: *ecological narcology; erythrocytes; disruption of reactivity*

In recent years investigations in the field of narcology have increasingly had to take into account the steadily deteriorating ecological situation. This has led to the emergence of a new area of research: ecological narcology [9]. A model for the development of diseases under the impact of intensive anthropogenic activity is provided by the alcoholization of those working in hydrolysis-based ethyl alcohol production. Some workers take to imbibing the alcohol produced, and the effect is often compounded by the effect of inhaling furfural and methanol, the chemical by-products, in elevated concentrations and under unstable temperature conditions.

Obviously, a detailed study of alcoholization under ecologically unfavorable and at times highly detrimental conditions calls for experimental confirmation of the results by using laboratory animals. As is well known, the clinical picture of alcoholic intoxication does not depend on the paths by which alcohol enters the body [4]. Ethanol, methanol, and furfural are substances of a resorptive nature, exerting their influence after they have been absorbed into the blood-stream. Thus, the purpose of our investigation was to compare the reaction of the blood system in the case of ordinary alcoholic intoxication with that in the case of the imbibing of ethanol combined with the inhaling of methanol and furfural in surroundings with elevated temperatures.

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MATERIALS AND METHODS

In our experiments we used 8 groups of 80 conventional male white rats with an initial weight of 170-180 g. The first four groups (1a-4a) took part in experiments lasting 14 days, while the other four groups (1b-4b) participated in experiments lasting 28 days. The animals in group 1 were sub-

0007-4888/93/0008-1002$12.50

jected to daily intragastric injection of 40% ethanol in doses of 1/2 LD_{50} [10]. Group 2 rats were placed for four hours every day in a chamber sprayed with furfural and methanol in concentrations corresponding to 3-4 times the maximum permissible concentration (extrapolating the data concerning humans and animals), the temperature being raised from 22-23 to 26-27°C. Group 3 animals were given a daily intragastric injection of ethanol in a dose of 1/4 LD_{50} and were simultaneously exposed to the inhalational effect. Group 4 consisted of control animals that were given an intragastric injection of distilled water in the same amount. Blood was taken from the caudal vein. The erythrocyte count, the hematocrit, and the mean volume of the erythrocyte (MVE) were determined with the aid of a Picoscole instrument (Hungary), and the total amount of hemoglobin was determined photometrically by the hemoglobin-cyanide method. The color index (CI), the mean amount and concentration of hemoglobin in the erythrocytes (MHE and MCHE), and the mean saturation of erythrocytes with hemoglobin (MSEH) were determined by the Kassirskii method [5]. The number of reticulocytes, the daily erythropoiesis (DE), and the mean life span of the erythrocytes (MLSE) were determined by the Mosyagina method [7]. The pH and the partial pressure of oxygen in the blood were determined with the aid of an MK-2 microanalyzer.

RESULTS

The blood of the animals of groups 1 and 2 showed a tendency toward an increase in the number of erythrocytes (Table 1). The total amount of hemoglobin increased only in group 1a (by 11.9%), while that in group 1b and group 2 decreased somewhat. On the other hand, in group 3 there was a considerable decrease in the amount of erythrocytes and hemoglobin. According to some researchers [12], a reduction in the number of erythrocytes in the periferal blood is related to the setting in of alcoholism.

The most stable parameters were the hematocrit, MHE, and CI; a definite increase in these parameters was observed only in group 1. As is known, CI and MSEH depend only on the amount of hemoglobin and the number of erythrocytes, but also on the size of the erythrocytes. The stability of CI in group 2 was also due to the relative stability of the experimental results. However, relying on CI alone we do not get a clear picture of the absolute saturation of erythrocytes with hemoglobin. This can be seen from the cor-

TABLE 1. Parameters for Erythrocytes of Rats under Different Types of Alcohol–Toxic Influence ($M \pm m$)

Group	DE, $\times 10^7$	Reticulocytes, %	Hematocrit	Hemoglobin, g/liter	Erythrocytes, $\times 10^{12}$	CI	MVE, μ^3	MHE, picograms	MCHE, picograms/μ^3	MSEH	MLSE, days
Observations over a 14-day period											
1a	306.03±23.18	2.07±0.08*	0.53±0.03*	155.74±6.61*	6.69±0.09	0.69±0.02*	79.90±3.75*	23.23±0.79*	0.29±0.01	0.77±0.03	22.20±1.41
2a	314.51±16.49	2.11±0.11*	0.45±0.02	137.32±3.99	6.49±0.11	0.63±0.01	69.56±1.84	21.13±0.47	0.30±0.003	0.80±0.008	20.92±1.21
3a	329.02±22.48*	2.21±0.09*	0.48±0.03	122.66±9.96	5.54±0.30*	0.66±0.03	87.70±2.04*	22.09±0.96	0.25±0.006*	0.66±0.02*	17.15±1.86*
4a	262.48±22.38	1.82±0.09	0.46±0.01	139.16±2.88	6.61±0.09	0.63±0.01	69.42±1.78	21.04±0.30	0.30±0.004	0.80±0.01	25.52±2.03
Observations over a 28-day period											
1b	320.78±38.23	2.09±0.13*	0.53±0.04	141.20±18.6	6.57±0.13	0.64±0.02	81.19±7.57	21.47±0.82	0.27±0.03	0.72±0.08	19.27±1.20*
2b	369.27±10.68*	2.15±0.08*	0.51±0.01	136.66±6.91	6.66±0.11	0.61±0.02	76.29±1.92	20.49±0.77	0.27±0.01	0.71±0.03	18.12±0.77*
3b	214.75±18.26*	2.03±0.13	0.49±0.009	114.0±7.36*	5.56±0.35*	0.62±0.03	89.45±6.59*	20.56±0.86	0.23±0.02*	0.61±0.04*	–
4b	265.54±6.64	1.83±0.07	0.53±0.02	145.14±5.21	6.53±0.11	0.66±0.02	75.69±1.52	22.22±0.59	0.29±0.006	0.77±0.02	24.27±0.67

Note. * $p<0.05$.

relation between MCHE, MSEH, and MVE in group 1 and, especially, in group 3, where a definite increase in MVE only masked the overall decrease in MCHE and MSEH. This indicated a change in the rate of synthesis of pigment in the erythroid cells and showed the intensity of intracellular metabolism. Moreover, a decrease in MCHE also hampers the transmembrane exchange of oxygen in the tissues, leading to tissue hypoxia. This is corroborated by our experiments, in which a definite decrease in the partial pressure of oxygen in the blood was observed in the animals of group 3b by 56.4% (49.3±14.5 mm Hg; in the control 113.1+3.2 mm Hg; $p<0.001$).

Macrocytosis has been observed in the case of chronic alcoholic intoxication [1,14]. This is due both to the deterioration of the internal medium of the cell, accompanied by losses of K^+ and the extensive penetration of Na^+ and water [2], and to persistent pathological changes in the ration between the groups of plasma lipoproteins affecting the erythrocyte membrane, with the ration increasing steadily as the disease develops [1]. We had noted an increase in the size of the erythrocytes even in the case of alcoholic intoxication of relatively short duration. It can therefore be assumed that macrocytosis, as an important warning sign, probably appeared already at the prenosological stage, reflecting a growing imbalance between the intracellular mechanisms.

Data have been published [12] showing that the degree of macrocytosis depends directly on the daily dose of alcohol and on prolonged excessive use of alcoholic beverages. However, we cannot consider these to be the sole criteria of macrocytosis. We noted a considerably greater degree of macrocytosis in the animals of group 3, despite a twofold decrease in the dose of ethanol. While the inhaling of furfural-methanol (group 2) did not cause macrocytosis, the appearance of the phenomenon in group 3 was probably due to more than the additive effect of furfural-methanol, which quickly led to pathological humoral changes.

The functional reaction of the erythrocyte system can be better understood by carrying out an analysis of two interrelated processes: erythropoiesis and erythrodieresis. Under normal conditions the regeneration of erythrocytes is in dynamic balance with their physiological destruction. Our previous investigations [3] showed that there were substantial changes in the function of erythrocytes in chronic alcoholism. And according to Eschwege [13], the suppression of reticulocytosis due to the effect of alcohol on hemopoiesis is characteristic of severe forms of alcoholism.

The relative decrease in the number of reticulocytes in the blood of the animals of group 3b, as compared with all the other groups, like the decrease in DE, once more indicates that the protective-compensatory mechanisms in the given case are clearly insufficient for supporting physiological homeostasis. This was also confirmed by an investigation of MLSE. A definite decrease in the duration of the circulation of erythrocytes in the blood was observed in the animals of groups 1 and 2 only in the experiment that lasted 28 days. In the animals of group 3a, on the other hand, such a decrease was noted within two weeks, while in the animals of group 3b the maximum divergence in the parameters was observed (from 13.46 to 28.79 days), attesting to the desynchronization of erythrokinetic equilibrium. The increased degree of hemolysis may be due to the direct influence of toxic factors on the erythrocyte membrane [8], especially as ethanol can, under ecologically unfavorable conditions, act as a universal solvent capable of entrapping to an excessive degree toxic agents in the body [6]. The extensive destruction of erythrocytes was undoubtedly facilitated also by a shift in the acid-base equilibrium in the blood of the animals of group 3 towards pH 7.291±0.01 (in the control animals the pH was 7.340±0.01; $p<0.05$). This shift was probably due to a change in the buffer system of hemoglobin; the system accounted for up to 75% of the buffer capacity of the blood [11].

Thus, our investigation show that the change in a number of hematological parameters depends not only on the duration, but also on the nature of the alcohol-toxic influence. The influence of ethanol or of a furfural-methanol mixture by itself led initially to stimulation of the protective-compensatory mechanisms, followed (after one month) by an insignificant lowering of the effectiveness of these mechanisms. As for ethanol intoxication, even in the case of relatively small doses, but under ecologically unfavorable conditions, it led to a substantial breakdown in the compartmentalization of interrelated processes. The number of erythrocytes, like MSEH and the total concentration of hemoglobin, decreased noticeably. This led to a still greater deficiency of hemoglobin (the mechanism of the disruption of reactivity). We observed a considerable increase in erythropoiesis and erythrodieresis, which on the whole is due to an insufficient compensatory reaction to severe hypoxia. The suppression of the regulatory mechanisms for the erythron system took place simultaneously with an increase in MVE. This indicates, in the case of relatively short-term al-

cohol intoxication, the disruption of intracellul0ar autoregulation.

A comparison of the experimental results with clinical data and data contained in the literature permits us to conclude that the hematological state in the case of short-term alcohol intoxication under ecologically unfavorable conditions tends to resemble that in the case of long-term excessive alcohol intoxication. The resultant changes in the erythrocyte system can undoubtedly be regarded as one of the contributing factors in ecological-narcological diseases.

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Chronobiological Regularities of Amphibian Metamorphosis

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UDC 612.018:[577.175.444+577.175.328].019.08

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 116, № 8, pp. 197-199, August, 1993
Original article submitted February 19, 1993

Key Words: *amphibians; metamorphosis; thyroxine; prolactin; chronobiology*

The metamorphosis of amphibians, one of the most important biological processes, has been fairly thoroughly studied. The main controlling mechanisms of this process have also been described [1,4-6,10]. However, there is little information on the chronobiological regularities of metamorphosis and on the relationship between the biological action of such metamorphogenic hormones as thyroxine and prolactin and the photoperiodic conditions [3,7,9,11].

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The purpose of this work was to make a chronobiological study of the effect of thyroxine and prolactin on certain temporal characteristics of metamorphosis of tailless amphibians in relation to the phase of the light-dark cycle.

MATERIALS AND METHODS

In our experiments we used *Rana temporaria* larvae in the state of prometamorphosis, and also in the state of metamorphosis proper from the 23rd to the 29th stage of development [8]. The light-

0007-4888/93/0008-1005$12.50