11. L. Lewan and T. Ynger, Int. J. Biochem., 8, 477 (1977).
12. T. F. Slater, B. Sawyer, and U. Strauli, Arch. Int. Physiol. Biochem., 72, 427 (1964).

STRUCTURAL CHANGES IN ERYTHROCYTE MEMBRANES IN NEPHROPATHY

O. M. Panasenko, R. I. Shalina, and O. A. Azizova

UDC 616.61-07:616.155.1-018.1:576.314]-07

KEY WORDS: erthyrocyte membranes; membrane structure; spin probes; nephropathy.

One of the main problems in modern scientific and practical obstetrics is the study of mechanisms of the pathogenesis of late toxemia of pregnancy (LTP). It is now known that nephropathy is accompanied as a rule by disturbance of the circulation [3]. This is manifested in particular by changes in the properties of the blood cells [3, 5, 6, 8]. One of the most important links in the chain of pathogenesis of this polyetiologic disease is disturbance of the respiratory function of the erythrocytes, specifically, depression of their ability to give up oxygen to the tissues [6, 8], with consequent hypoxia [3, 8]. With the development of LTP the lipid composition of the erythrocyte plasma membranes is also known to change: The content of phospholipids (PL) and cholesterol (Ch) increases, while at the same time the content of free fatty acids and of triglycerides decreases [13]. In nephropathy the aggregating power of the erythrocytes has been shown to be increased, whereas their electrophoretic mobility is reduced [5]. The causes of disturbance of erythrocyte function have not been established. It can be tentatively suggested that these disturbances are connected with structural modifications of erythrocyte plasma membranes.

The aim of this investigation was to study structural changes in the erythrocyte membrane of patients with nephropathy of different degrees of severity. The spin probe method, capable of determining precise changes in the structure of biomembranes [9], was used for this purpose.

EXPERIMENTAL METHOD

Erythrocytes from the blood of 15 healthy pregnant women and also of 39 women with a mild (20) and a severe (19) degree of nephropathy (according to the Wittlinger scale) were studied. Blood from nine healthy nonpregnant women was used as the control. Erythrocytes were isolated by triple centrifugation in NaCl solution (150 mM) containing 10 mM Tris-HCl, pH 7.3, for 10 min at 1000g. EPR spectra were recorded on an E-4 radiospectrometer (Varian, USA) at 37°C. The spin probes used were 2-(3-carboxypropyl)-4,4-dimethyl-2-tridecyl-3-oxazolidinyloxyl (probe I, from Syva, USA), 2,2,6,6-tetramethyl-4-dodecyl-Δ^3-dihydropiperidine-1-oxyl (probe II, synthesized at the Research Institute for Biological Testing of Chemical Compounds), and 2,2,6,6-tetramethyl-4-(4-phenylbutinyl)-piperidine-1-oxyl (probe III, synthesized at the Institute of Chemical Physics, Academy of Sciences of the USSR). EPR spectra and structural formulas of the probes are given in Fig. 1. The probes were added to the erythrocyte suspension in the form of a solution in ethanol. The concentration of the probe in the specimen was 5×10^{-5} M, of ethanol 2%, and of erythrocytes 10^{10} cells/ml. To characterize behavior of the probes in the membrane the following parameters were used: the degree of order S, describing the mobility of the fatty-acid chains of PL, and the isotropic constant of hyperfine interaction a, from the values of which the parameter of hydrophobicity h was determined, giving an estimate of the degree of polarity of the environment of the nitroxyl fragment of the probe [9].

$$h = (a_w - a)/(a_w - a_o),$$

where a_w and a_o were determined for the probe in an aqueous solution of NaCl (150 mM) con-

Laboratory of Biophysical Principles of Membrane Pathology, Research Institute of Physicochemical Medicine, N. I. Pirogov Second Moscow Medical Institute. Department of Obstetrics and Gynecology, Pediatric Faculty, N. I. Pirogov Second Moscow Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR Yu. M. Lopukhin.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 99, No. 4, pp. 434-437, April, 1985. Original article submitted April 21, 1984.

0007-4888/85/9904-0453$09.50

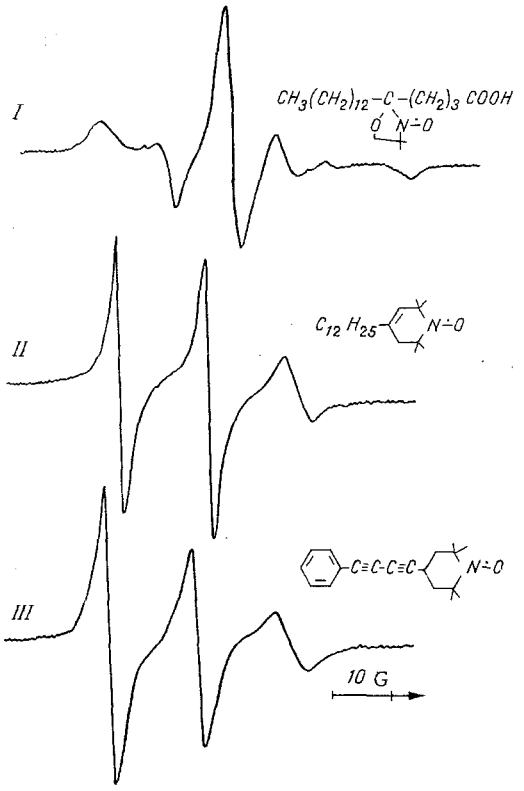

Fig. 1. Structural formulas and EPR spectra of spin probes I, II, and III located in erythrocyte suspension. Measuring medium: 150 mM NaCl, 10 mM Tris-HCl, pH 7.3; probe 5×10^{-5} M, erythrocytes 10^{10} cells/ml, ethanol 2%.

taining 10 mM Tris-HCl, pH 7.3, and in octanol respectively. For probe I a_w = 15.76 G and a_o = 14.75 G. The results were subjected to statistical analysis by programmed EMG-666/B microcomputer (Hungary), using Student's test. Arithmetic mean values and their standard errors [11] are given in Figs. 1-3.

EXPERIMENTAL RESULTS

The probes used were located in the erythrocytes in different parts of the lipid bilayer of the plasma membrane. For instance, the paramagnetic fragment of probe I was buried into the lipid bilayer to a depth of 6-8 Å from the membrane surface. Probes II and III are virtually insoluble in water and were located in all probability in hydrophobic lipid regions. Values of parameters S and h, calculated from EPR spectra of probe I, located in a suspension of erythrocytes from healthy nonpregnant and pregnant women, and also from patients with nephropathy, are given in Fig. 2: S in healthy pregnant women was essentially unchanged compared with the control, but in patients with nephropathy it was considerably increased, whether the disease was in a mild or severe form. The increase in S points to changes in structure of the erythrocyte membrane, leading to immobilization of the microenvironment of the probe. The fact that the parameter S was significantly higher in patients with mild nephropathy than in healthy blood donors and that it was unchanged with an increase in severity of the disease is evidence of modification of the lipid bilayer of the erythrocyte membranes even in the early stages of the disease. The parameter of hydrophobicity was shown to be reduced in healthy pregnant women compared with the control, but its values for patients with nephropathy were smaller still and, moreover, they correlated with the severity of the disease (Fig. 2b). This indicates that modification of the structure of the erythrocyte membrane in nephropathy consists not only of a reduction of the degree of mobility of the acyl chains of PL, but also an increase in polarity of the lipid bilayer of the membrane.

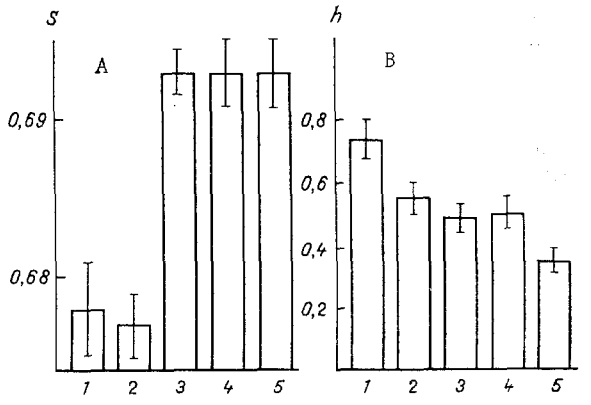

Fig. 2. Values of degree of order (A) and hydrophobicity (B) of probe I in erythrocyte suspension. 1) Healthy nonpregnant women, 2) healthy pregnant women, 3) nephropathy (all cases), 4) mild nephropathy, 5) severe nephropathy.

Similar investigations were carried out with probes II and III. However, no significant differences were found in the behavior of these probes in erythrocyte membranes of healthy pregnant women and patients with nephropathy. Development of the pathological process was evidently accompanied by modification, not of the whole membrane, but only of certain regions of it, in which (albeit partially) probe I is distributed, but in which probes II and III are evidently not present.

The next step was to discover whether correlation exists between the calculated values of S and h for probe I, on the one hand, and blood pressure, severity of edema, and obesity of the patients, on the other hand. It was found that S for the patients was significantly higher than for healthy blood donors (Fig. 3). However, it did not depend on the degree of edema. Meanwhile parameter h decreased significantly with the development of edema, evidence of an increase in the degree of polarity of the environment of the radical center of the probe (Fig. 3). No regular dependence could be established between the degree of obesity and arterial pressure, on the one hand, and parameters S and h on the other hand.

The results thus indicate that, first, in nephropathy the structure of the erythrocyte membrane is modified: Mobility of the acyl chains of PL is reduced and polarity of the lipid bilayer is increased; second, with an increase in severity of the disease and the degree of edema, parameter h decreases. It can be tentatively suggested that the increase in the parameter S was due to a change in the lipid composition of the membrane: an increase in the content of Ch [13], which limits mobility of the acyl chains of PL [10]. However, under these circumstances an increase in the PL content also is observed in the erythrocyte membrane [13], and this must cancel out the condensing effect of Ch. As regards the pathogenesis of the edema in LTP, at present a role is ascribed to increased capillary permeability and hydrophilicity of the tissues as a result of an increase in their hyaluronidase activity which, in turn, leads to retention of Na^+ and, correspondingly, of fluid, in the body [3]. Meanwhile, during the development of nephropathy, "spasm of vessels at the periphery leads to hypoxia of the tissues, accumulation of incompletely oxidized products in them, elevation of the osmotic pressure and, consequently, an increase in hydrophilicity, i.e., to the development of edema [3]." The decrease which we found in the parameter h makes it possible to add that the above-mentioned causes probably lead not only to retention of water in the body and hydrophilicity of the tissues, but also to increased permeability of the erythrocyte membrane and an increase in the quantity of membrane-bound water, i.e., to hydrophilicity of particular regions of the lipid bilayer.

Attention must be drawn to the fact that an increase in the degree of order of the membrane lipids and of polarity of the bilayer takes place during intensification of membrane lipid peroxidation (LPO) [1, 7]. The decrease in mobility of the acyl chains of PL is probably connected with a decrease in cis-double bonds as a result of their oxidation and the formation of "cross-linkages" between proteins and PL [1, 12]. The increase in polarity of the bilayer is evidently due both to an increase in permeability for water molecules and to accu-

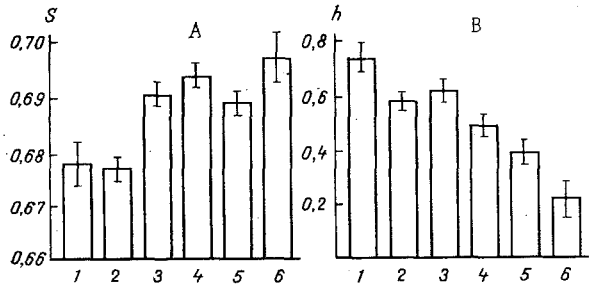

Fig. 3. Degree of order (A) and hydrophobicity (B) of probe I in erythrocyte suspension from patients with nephropathy and different degrees of edema. 1) Healthy nonpregnant women, 2) healthy pregnant women, 3) nephropathy with absence of edema, 4) with mild degree of edema, 5) with moderate degree of edema, 6) with severe edema.

mulation of polar products of LPO, with the possible formation of hydrophilic inclusions in the hydrophobic bilayer of the membrane [1, 12]. It can therefore be suggested that one cause of the structural changes observed in the erythrocyte membrane in nephropathy is intensification of LPO.

There is experimental evidence in support of this hypothesis. In particular, we know that nephropathy is accompanied by a fall in the level of endogenous antioxidants such as vitamin B_6, B_{12}, C and, in particular, E in the plasma [3], leading to inhibition of antioxidative activity in the blood [1, 12]. The creation of hypovitaminosis E, together with hypoxia and other factors, leads to the development of a pathological condition with a course similar to nephropathy in animals [3]. It must also be pointed out that in LTP the concentrations of substances participating directly in the regulation of the LPO level in the plasma change [1]: The number of reduced SH-groups and, in particular, the content of reduced glutathione is reduced [3]. At the same time the content of PL, the main substrate of LPO [1], in the erythrocyte membranes increases [13]. Finally, it has been shown [4] that in nephropathy accompanied by hypoxia of the maternal and fetal tissues, induced chemiluminescence in the serum rises considerably. This is evidence of intensification of LPO processes and (or) a decrease in antioxidant activity of the serum [1].

The results thus suggest that activation of LPO may be at least one of the causes of modification of the erythrocytes and their plasma membranes in nephropathy.

The authors are grateful to Professor Yu. A. Vladimirov for discussing the results.

LITERATURE CITED

1. Yu. A. Vladimirov and A. I. Archakov, Lipid Peroxidation in Biological Membranes [in Russian], Moscow (1972).
2. O. Griffith and P. Jost, in: The Spin-Label Method. Theory and Application [Russian translation], Moscow (1979), p. 489.
3. V. I. Grishchenko, Modern Methods of Diagnosis and Treatment of Late Toxemia of Pregnancy [in Russian], Moscow (1977).
4. V. I. Grishchenko, Yu. S. Parashchuk, and V. S. Lupoyad, in: Conference of Obstetricians and Gynecologists on the Subject of Toxemias of Pregnancy [in Russian], Khmel'nitskii (1981), p. 179.
5. G. D. Dzhivelegova, S. Ya. Malinovskaya, and R. I. Shalina, in: Abstracts of Proceedings of the 5th Congress of Obstetricians and Gynecologists of the RSFSR [in Russian], Moscow (1982), p. 77.
6. V. A. Zaliznyak, in: Problems in Theoretical and Clinical Medicine [in Russian], Moscow (1979), p. 22.
7. V. E. Kagan, O. A. Azizova, Yu. V. Arkhipenko, et al., Biofizika, 22, 625 (1977).
8. A. G. Kolomiitseva, G. I. Makarenko, and L. E. Tumanova, in: Conference of Obstetricians and Gynecologists on the Subject of Toxemias of Pregnancy [in Russian], Khmel'nitskii (1981), p. 72.
9. A. N. Kuznetsov, The Spin Probe Method [in Russian], Moscow (1976).

10. Yu. M. Lopukhin, A. I. Archakov, Yu. A. Vladimirov, et al., Cholesterolosis [in Russian], Moscow (1983).
11. Yu. S. Maleta and V. V. Tarasov, Mathematical Methods of Statistical Analysis in Biology and Medicine [in Russian], Moscow (1982).
12. F. Z. Meerson, V. E. Kagan, Yu. P. Kozlov, et al., Kardiologiya, No. 2, 81 (1982).
13. T. S. Chernenko, in: Conference of Obstetricians and Gynecologists on the Subject of Toxemias of Pregnancy [in Russian], Khmel'nitskii (1981), p. 137.

COMPARATIVE STUDY OF ZIXORYN AND PHENOBARBITAL AS INDUCERS OF ENZYMES OF THE LIVER MONO-OXYGENASE SYSTEM

A. S. Loginov, B. N. Matyushin, L. Kh. Ausheva, and E. A. Bendikov

UDC 615.214.24.015.42:612.-351.11:577.152. 1

KEY WORDS: inducers of microsomal oxidases; zixoryn; phenobarbital; liver mono-oxygenase system.

Enzymic induction is nowadays regarded as an adaptive response associated with an increase in the number of molecules of a specific enzyme, to the inducing substance [7]. Cytochrome P-450-dependent enzymes of microsomal oxidation, which are responsible for detoxication of many substrates and for homeostasis of the chemical environment of the organism [1, 6], are induced by various compounds. Common properties of inducers of enzymes of the mono-oxygenase system of the hepatocyte are their lipophilicity, ability to bind with cytochrome P-450, and a high half-elimination period. The most widespread and best studied compound of this group is phenobarbital, whose effect is connected with selective proliferation of the smooth endoplasmic reticulum and hypertrophy of the liver. It considerably accelerates the biotransformation of several drugs and thereby reduces their therapeutic activity, and it also stimulates metabolism of endogenous compounds [9]. From this point of view of matter of clinical interest is an increase in bilirubin clearance in unconjugated hyperbilirubinemia, in the Gilbert, Crigler–Najjar, and Dubin–Johnson syndromes and also, probably, in intrahepatic cholestasis. However, the use of phenobarbital in liver pathology is restricted because of its central action, its low therapeutic index, and the presence of several side effects. There is no doubt that drugs with a selective action on biosynthesis (and, or activity) of enzymes of the mono-oxygenase system of the hepatocyte endoplasmic reticulum are needed for clinical practice for the pharmacological regulation and correction of biotransformation of physiologically active substances.

TABLE 1. Effect of Phenobarbital in a Dose of 80 mg/kg (5-day course) and of Z in Doses of 80 and 40 mg/kg (5- and 2-day courses) on Concentrations of Cytochromes P-450 and b_5 and on Aniline Hydroxylase Activity (AHA) of Rat Liver (M ± m)

Group of animals	Experimental conditions	number of animals	Cytochrome P-450		Cytochrome b_5		AHA, nanomoles/mg protein
			nanomoles/g tissue	nanomoles/mg protein	nanomoles/g tissue	nanomoles/mg protein	
1	Control	7	20,2±1,9	0,390±0,061	12,40±1,46	0,260±0,023	0,177±0,021
2	Phenobarbital, 80 mg/kg (5-day course)	6	66,3±6.8	1,45±0,15	17,80±1,95	0,388±0,039	—
3	Z, 80 mg/kg (5-day course)	5	22,8±1,4	0,522±0,046	25,10±2,64	0,579±0,080	0,228±0,021
4	Z, 80 mg/kg (4-day course)	6	15,4±1,4	0,654±0,048	10,60±1,55	0,224±0,030	—
5	Z, 20 mg/kg (4-day course)	6	23,9±2,9	0,370±0,040	12,4±1,06	0,273±0,017	—
	P_{1-2}		<0,01	<0,001	<0,05	<0,05	
	P_{1-3}				<0,01	<0,01	
	P_{1-4}			<0,05			

Central Research Institute of Gastroenterology, Moscow. Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 99, No. 4, pp. 437-439, April, 1985. Original article submitted September 7, 1984.

