# PERMEABILITY OF ERYTHROCYTE MEMBRANES FOR MONOVALENT CATIONS ($Na^+$, $K^+$) AND THEIR TRANSFORMATION IN ESSENTIAL SYMPTOMATIC (RENAL) HYPERTENSION

V. G. Kavtaradze, N. G. Aleksidze,
N. I. Nadiradze, and L. D. Cheishvili

UDC 616.12-008.331.1-07:[616.155.1-018.1:576.314]-008.6

KEY WORDS: hypertension; erythrocyte membrane permeability; electron microscopy; erythrocyte transformation

The writers showed previously [1] that erythrocytes of patients with essential hypertension (EH), preloaded with $Na^+$ and deprived of $K^+$ by means of the sodium salt of *p*-chloromercuribenzoic acid (PCMB) [9] have a membrane defect, which is expressed as retention of $Na^+$ and accelerated reuptake of $K^+$. In symptomatic hypertension (SH) no such changes were observed [2, 9]. Different pathological processes are reflected in the functional and morphological properties of the erythrocytes. Changes in shape of the cells can provide definite information on the intensity and nature of the pathological process [7]. Since differences in the velocity of transport of $Na^+$ and $K^+$ ions through the erythrocyte membrane of patients with EH and SH are found after exposure to PCMB, it was interesting to discover to what extent the erythrocytes change their shape under these circumstances.

The electrolyte composition, the permeability of erythrocyte membrane for $Na^+$ and $K^+$ ions, and the morphology of the erythrocytes of patients with EH and SH before and after treatment with PCMB (in vitro) were studied simultaneously.

## EXPERIMENTAL METHOD

Erythrocytes of 37 individuals were studied: 18 adolescents and men aged from 13 to 42 years with stage I of EH (essential hypertension according to the WHO classification); seven men with SH aged from 32 to 45 years; 12 clinically healthy individuals (five women and seven men aged from 15 to 41 years) formed the control group. The presence of EH in all patients was confirmed by appropriate clinical and instrumental tests. Pharmacotherapy was withheld for 3 days before the beginning of the investigation. Permeability of the erythrocyte membranes for monovalent cations was studied by the method in [9]. Erythrocytes, preloaded with $Na^+$ and deprived of $K^+$ by treatment with PCMB were added to Ringer's solution, where they were able to restore the steady-state $Na^+$ and $K^+$ concentrations. Outflow of $Na^+$ and reuptake of $K^+$ in the erythrocytes were recorded by measuring the concentration of the cations in the erythrocytes, which is linear in character and forms the basis for estimation of permeability of the erythrocyte membrane. The shape and surface relief of the erythrocytes were studied in a scanning electron microscope (SEM) (Nanolab, Japan). Erythrocytes were prepared for SEM by the method in [8]. The morphological terminology of the erythrocytes was taken from [6]. Altered forms of erythrocytes were counted per 100 erythrocytes. Concentrations of $Na^+$ and $K^+$ in the erythrocyte hemolysate were determined by flame photometry (FP).

## EXPERIMENTAL RESULTS

The study of the shape and surface architectonics of erythrocytes from clinically healthy individuals (before treatment with PCMB) showed that the basic circulating form of erythrocytes consists of biconcave cells (discocytes), typical of the normal human subject, with a smooth surface (95.2%). Other cell forms accounted for not more than 4.8% of cases (Fig. 1a, Table 1).

Changes in the shape and surface relief of the erythrocytes were found in cells from patients with EH in stage I. A statistically significant decrease in the number of discocytes

Academician M. D. Tsinamdzgvrishvili Research Institute of Clinical and Experimental Cardiology, Ministry of Health of the Georgian SSR, Tbilisi. (Presented by Academician of the Academy of Medical Sciences of the USSR M. M. Khananashvili.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 107, No. 5, pp. 554-557, May, 1989. Original article submitted December 14, 1988.

 0007-4888/89/0005-0632$12.50 

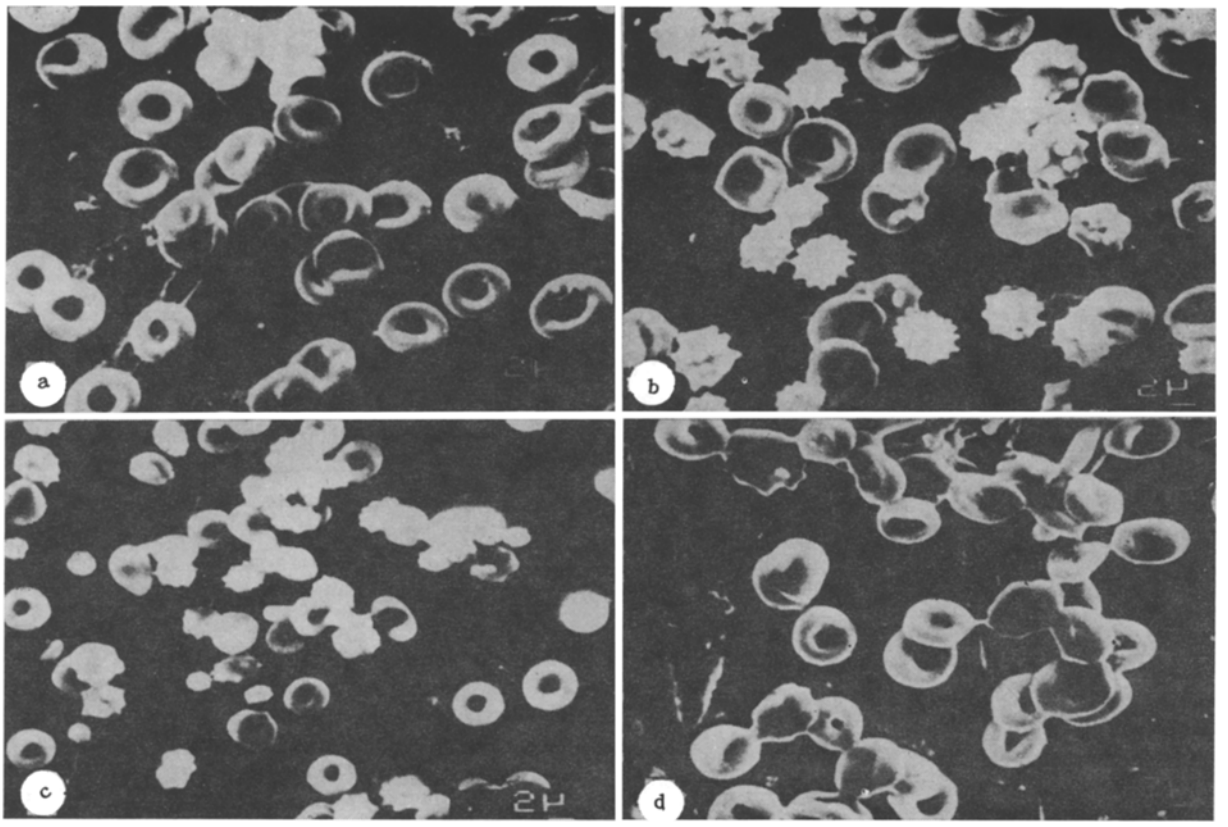


Fig. 1. Microrelief of erythrocytes from normal persons and patients with EH and SH before and after exposure to PCMB. a) Healthy human erythrocytes; b) healthy human erythrocytes after treatment with PCMB (an increase in the number of echinocytes); c) erythrocytes from patient with EH (increase in number of echinocytes and decrease in their volume after treatment with PCMB); d) erythrocytes of patient with SH (increased erythrocyte aggregation after treatment with PCMB and glutaraldehyde). SEM; magnification: a, b, d) 2500 ×, c) 2000 ×.

and an increase in the number of echinocytes were seen compared with the erythrocytes of healthy subjects ($p < 0.001$). At the same time there was increase in the number of other modified erythrocytes: stomatocytes and spherocytes. In essential hypertension a decrease in the mean volume of erythrocytes and a change in their shape also have been observed by other workers [4].

Similar changes in the blood picture, but less marked, were found in the erythrocytes of patients with SH.

As already noted, the rate of flow of $Na^+$ and $K^+$ was studied after a change in the concentration of $Na^+$ and $K^+$ ions in the erythrocytes as a result of exposure to PCMB, which has a twofold action on transport and concentration of monovalent cations in erythrocytes: it increases passive permeability and inhibits active transport, as a result of which the $Na^+$ concentration in the erythrocytes is increased whereas the $K^+$ concentration is reduced [10].

Treatment of erythrocytes with PCMB led to sharp changes in the relative proportions of the morphological types of erythrocytes in both normal subjects and patients. The number of nondiscocyte forms, namely echinocytes, stomatocytes, and others, was increased (Fig. 1b).

Changes in the concentration of monovalent cations in healthy human erythrocytes caused an increase in transformation to echinocytes and stomatocytes in about 50% of cases.

A decrease in the number of discocytes also was observed in the morphological picture of the blood patients with EH on account of an increase in the number of other forms of erythrocytes compared with the initial level (before treatment with PCMB). Unlike in healthy subjects, after treatment with PCMB a decrease in the number of echinocytes and an increase in the number of stomatocytes, spherocytes, and spheroechinocytes were observed. Stomatocytic and echinocytic cell transformation can, as we know, be reversible, and is of compensatory and adaptive importance [7], whereas spherocytes and spheroechinocytes are characterized

TABLE 1. Effect of Cations in Erythrocytes and Their Morphological Characteristics in Patients with EH and SH (absolute number of corresponding types of erythrocytes, concentrations of $Na^+$ and $K^+$ ions, and ratio between flow rates of $Na^+$ and $K^+$ in erythrocytes; M ± m)

| Group of subjects | $Na^+$, mmoles/liter erythrocytes | $K^+$, mmoles/liter erythrocytes | Morphology of erythrocytes | | | | | Ratio of flow rates of $Na^+$ and $K^+$ |
|---|---|---|---|---|---|---|---|---|
| | | | discocytes | echinocytes | stomatocytes | spherocytes | spheroechinocytes | |
| Healthy individuals ($n$=12) | 18,42±1,01 | 84,89±3,23 | 95,2±1,33 | 3,2±0,20 | 1,0±0,085 | 0,2±0,08 | 0,4±0,035 | |
| Patients with EH ($n$=18) | 20,42±0,94 | 88,57±2,42 | 78,25±2,84** | 16,77±0,72*** | 2,30±0,16** | 2,0±0,12** | 0,68±0,039 | |
| Patients with SH ($n$=17) | 19,37±0,85 | 91,00±3,79 | 86,33±2,29* | 9,52±1,12** | 2,33±0,18** | 1,16±0,09* | 0,66±0,06 | |
| | | | After treatment with 0.1 mM PCMB | | | | | |
| Healthy individuals ($n$=12) | 96,61±3,23 | 10,12±0,97 | 45,16±1,47 | 46,81±1,43 | 5,71±0,16 | 1,32±0,81 | 1,0±0,16 | 3,41±0,47 |
| Patients with EH ($n$=18) | 98,50±2,20 | 11,94±1,04 | 40,25±1,72* | 35,81±1,58*** | 15,48±0,41*** | 4,96±0,33** | 3,5±0,17** | 1,74±0,34** |
| Patients with SH ($n$=7) | 103,6±3,50 | 12,07±0,21 | 38,63±1,87* | 47,13±1,81 | 10,68±0,57** | 2,33±0,28 | 1,23±0,26 | 3,05±0,48 |

Legend. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ compared with control.

by irreversible ultrastructural changes [3]. Since the ratio of the flow rates of $Na^+$ and $K^+$ was lower in the patients with EH than in the control group (1.74 ± 3.34 and 3.41 ± 0.47 respectively), it can be tentatively suggested that the formation of erythrocytes with irreversible transformation in patients with EH after treatment with PCMB is probably due to changes existing initially in erythrocyte structure.

In some patients with EH the volume of eichinocytes was significantly lower than in the control, and this also confirms the hypothesis regarding the existence of changes in erythrocyte structure (Fig. 1c).

The study of flow rates of $Na^+$ and $K^+$ and of erythrocyte morphology of patients with EH showed that the ratio between flow rates of $Na^+$ and $K^+$ in the erythrocytes (3.05 ± 0.48) and the number of echinocytes, spherocytes, and spheroechinocytes did not differ from the corresponding values in erythrocytes in the control group. Transformation into echinocytes, incidentally, was more marked in erythrocytes of healthy subjects and patients with SH. The formation of the echinocytic form is linked with a number of internal and external factors [5].

In the groups tested, the concentrations of $Na^+$ and $K^+$ ions in erythrocytes before and after treatment with PCMB did not differ significantly, but changes in shape of the erythrocytes were more distinct.

To prepare erythrocytes for electron microscopy, a 2% solution of glutaraldehyde was used.

It is interesting to note that addition of glutaraldehyde to blood from patients with SH, after treatment with PCMB, was accompanied in nearly every case by partial aggregation of the erythrocytes (Fig. 1d). This phenomenon also was observed in blood from patients with EH. Aggregation of erythrocytes is probably the result of essential biochemical differences in their membranes, which respond in this fashion to the action of PCMB and glutaraldehyde.

The facts described above are evidence of a disturbed structural organization of the plasma membrane of erythrocytes in essential hypertension, and this may be a sufficient condition for changes to take place in their functional activity.

## LITERATURE CITED


1. V. G. Kavtaradze, N. G. Aleksidze, N. K. Kvirikadze, and N. I. Nadiradze, Byull. Éksp. Biol. Med., No. 11, 542 (1986).
2. I. A. Kassirskii and G. A. Alekseev, Clinical Hematology [in Russian], 4th edition, Moscow (1970).
3. G. M. Kravtsov, Yu. V. Kotelevtsev, N. I. Pokudnyi, et al., Third European Conference on Hypertension [in Russian], Milan (1987).
4. V. A. Lyusov, S. N. Orlov, I. Yu. Postnov, and V. I. Kharchenko, Kardiologiya, No. 3, 76 (1983).

5. M. Bessis and R. I. Weed, Adv. Biol. Med. Phys., 14, 35 (1973).
6. M. Bessis, Corpuscles, New York (1974).
7. M. Bessis and N. Mochendas, Blood Cells, 1, 315 (1975).
8. I. A. Clarke and A. I. Salsbury, Nature, 215, 402 (1967).
9. R. P. Garay and P. C. Meyer, Lancet, 1, 349 (1979).
10. R. M. Sutherland, A. Rothstein, and R. I. Weed, J. Cell. Physiol., 69, 185 (1967).

# ROLE OF ACTIVATION OF LIPID PEROXIDATION IN THE PATHOGENESIS OF EXPERIMENTAL PERITONITIS

S. G. Konyukhova, A. Yu. Dubikaitis,
L. V. Shabunevich, V. I. Strashnov,
and M. V. Belotserkovskii

UDC 616.381-002-092.9-07:
616.153.915-39

KEY WORDS: lipid peroxidation; peritonitis

The aim of this investigation was to study the role of lipid peroxidation (LPO) in the pathogenesis of development of destructive lesions in the abdomen, accompanied by peritonitis and endotoxicosis. This approach is justified by the fact that these pathological processes are based on damage to membrane structures, whose integrity and functional activity is determined by LPO activity [6].

## EXPERIMENTAL METHOD

Experiments were carried out on 38 noninbred dogs weighing 20-25 kg, of which eight formed the control group, undergoing mock operations. The destructive lesion and peritonitis were induced by ligation of the vermiform appendix at its base [7]. Under hexobarbital anesthesia (8-10 mg/kg) a midline laparotomy was performed. During the subsequent days and until death of the animals, the clinical picture of the disease was kept under observation and the following biochemical tests were carried out after 6, 24, 48, and 72 h: 1) biochemical blood analysis (bilirubin, creatinine, urea, sugar, total protein and protein fractions, electrolytes $K^+$, $Na^+$, $Ca^{++}$, alanine-transaminase (ALT) and asparagine-transaminase (AST) activity; 2) determination of the steady-state level of LPO metabolites: diene conjugates (DC) [2], and malonic dialdehyde (MDA), by the reaction with 2-thiobarbituric acid (TBA) in our modification [0.8 ml $H_2O$ and 1.0 ml of 0.6% TBA solution in glacial acetic acid were added to 0.2 ml of plasma; the mixture was boiled for 30 min and, after cooling, 1 ml of 5N KOH and 2 ml isopropanol were added; this was followed by centrifugation at 12,000 rpm for 20 min; the supernatant was subjected to spectrophotometry at 520 nm against a control containing water instead of plasma; the total antioxidant status of the blood plasma was estimated by measuring induced in vitro production of DC ($DC_a$) and MDA ($MDA_a$) after activation of blood plasma LPO by $Fe^{++}$ ions (final concentration 0.54 mM) in the presence of $KMnO_4$ (final concentration 0.1 mM), a chaotropic agent]; 3) determination of ceruloplasmin (CP) activity [11], and of superoxide dismutase (SOD) activity [12] and of total blood plasma proteolytic activity (TPPA) [1]; 4) investigation of the level of medium mass molecules (MMM) in the blood plasma [5].

## EXPERIMENTAL RESULTS

During the first 6-8 h after the operation the animals were in a state of postanesthetic sleep. After 24 h the general condition of animals of the main group was determined by the presence of the pathological focus in the abdominal cavity, reflected in the typical clinical picture of diffuse peritonitis. After 48 h, 11 animals of the main group had died and the rest were in a serious condition, some showing cerebral coma and decerebrate rigidity. After

Academician I. P. Pavlov First Leningrad Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR F. G. Uglov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 107, No. 5, pp. 557-559, May, 1989. Original article submitted July 20, 1988.

  