Gels containing human BJ, obtained by electrophoresis and revealed by counterflow IB, are shown in Fig. 3a. The absence of background staining of the NCM will be noted. The reaction with L_K in the control zone is evidence that the developing reagents were used in sufficient excess and, consequently, that all components of the L_K family present in the preparation were detected. The sensitivity of the method in this case was about 2 ng protein per strip of NCM. Since about 100 µl of sample may be used in the experiment, it is possible by this method to analyze solutions even of a heterogeneous protein present in a concentration of 20-30 ng/ml.

Simultaneous demonstration of L_K and L_α in the urine of three normal individuals in the same strip of NCM is illustrated in Fig. 3b, where their heterogeneity can be clearly seen.

The counterflow IB method thus possesses high sensitivity and specificity and can be used to detect proteins on imprints of electrophoretic gels quickly and automatically. It is equally suitable for work with monoclonal and polyclonal antibodies and also with electrophoretic records obtained both on CAM and on PAG. The field of application of the method is very wide. It can evidently be used also for simultaneous determination of antibodies to different virus antigens in carriers of virus infections.

LITERATURE CITED

1. G. I. Abelev and É. R. Karamova, Progress in Science and Technology. Series: Immunology [in Russian], Vol. 17, Moscow (1986), pp. 177-198.
2. G. I. Abelev and E. R. Karamova, Zh. Vsesoyuz, Khim. Obshch. im. D. I Mendeleeva, 27, No. 4, 429 (1987).
3. A. I. Faerman, A. V. Chervonskii, T. A. Chipysheva, et al., Byull. Éksp. Biol. Med., No. 5, 632 (1987).
4. M. J. Escribano, J. Cordier, M. Nap, et al., Int. J. Cancer, 38, 155 (1986).
5. H. Haglund, Sci. Tools, 17, 2 (1970).
6. H. Towbin and J. Gordon, J. Immunol. Meth., 72, 313 (1984).

METHOD OF DETERMINATION OF LIPID PEROXIDATION PRODUCTS IN BLOOD SERUM

L. A. Mamedov, T. I. Gudz', A. V. Nikolaev, V. V. Zakharov, E. B. Goncharenko, Yu. B. Kudryashov, D. N. Motorin, and A. V. Rubin

UDC 616.153.915-39-074

KEY WORDS: lipid peroxidation products; thermoluminescence; serum; wounds

The high specificity of biological membranes, the complexity and multiplicity of their lipid composition suggest that systems maintaining the lability of their composition and ensuring the rapidity of response of the cell to changes taking place in the body and also systems responsible for the strictly definite structural organization of membrane lipids, ensuring the effective performance of their biological functions, must coexist in membranes [2, 8]. One of the mechanisms of disturbance of this process and of injury to cells and intracellular organelles is lipid peroxidation (LPO) [2], which lies at the basis of many pathological states: burns [1], atherosclerosis and ischemic heart disease [5], aseptic inflammation [3], neoplastic diseases [2], etc.

Various methods are used to investigate LPO processes in biological systems: titrometric [6], spectrophotometric [10, 12], chemiluminescence [7, 9], biochemical [11, 13], etc. The main disadvantages of these methods are their laboriousness, the complexity and time-consuming nature of the investigations, their low level of accuracy, and their inadequate sensitivity.

The authors have developed a new, easier, and more sensitive thermoluminescence method of determining LPO products in blood serum, and a description of it is given below.

I. M. Sechenov First Moscow Medical Institute.

Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 105, No. 5, pp. 628-630, May, 1988. Original article submitted July 17, 1987.

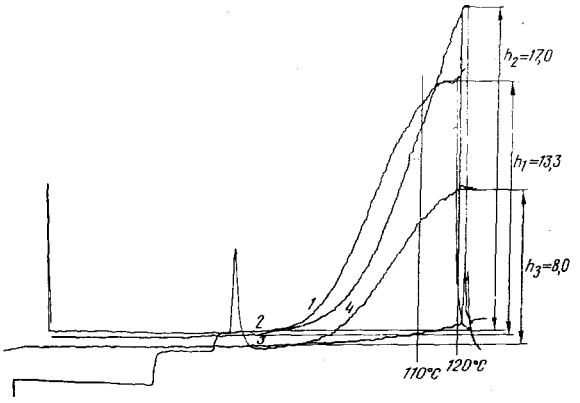

Fig. 1. Intensity of luminescence of LPO products determined by the suggested method 1 day after operation: h_1) intact rat No. 9; h_2) rat with aseptic wound (No. 2); h_3) rat with infected wound (No. 4).

EXPERIMENTAL METHOD

LPO products were determined in the blood serum of 15 intact rats and 30 rats with models of aseptic and infected superficial wounds with an area of 400 mm^2. A model of aseptic wounds was created in 15 rats by the method described previously [4]. In the remaining 15 rats the edges and floor of the wound were subjected to additional trauma with toothed forceps and infected with a suspension of a 24-h culture of a pathogenic staphylococcus. Blood (1 ml) was taken from a vein of the experimental and intact animals 24 h after the operation and serum was obtained from it by the standard method. Next, 0.02 ml of serum was applied to a circle of filter paper 8 mm in diameter and placed on the cuvette attachment of an FÉU-38 photoelectric multiplier with heating element and thermocouple. At the same time as the thermocouple was connected the screen of the photoelectric multiplier was opened and the specimen heated to 120°C at the rate of 60°C/min. The kinetics of luminescence was recorded for 2 min on the LKD4-003 automatic writer. The intensity of luminescence of the LPO products was expressed in relative units (1 rel. U = 1 cm height of the peak on the resulting curve). The end of the 2nd minute after the beginning of recording, incidentally, corresponds to a temperature of 120°C and to the peak on the curve which is followed by a straight line (plateau). If the recording is stopped before this time, for example, at a temperature of 110°C, the resulting curve will not gain its true height (Fig. 1), and this may be the cause of unreliable information about the intensity of luminescence of LPO products.

To compare the sensitivity of the suggested method, LPO products were also determined in the same specimen of test serum by a spectrophotometric method [10]. To a centrifuge tube containing 0.1 ml of the test serum 0.1 ml of $0.1 \cdot 10^{-2}$ M solution of $FeSO_4 \cdot 7H_2O$ was added, and the mixture shaken for 2 min, after which 0.1 ml of a $0.1 \cdot 10^{-2}$ M solution of ionol, 1.5 ml of glycine buffer (pH 3.6), 1.5 ml of 0.5% 2-thiobarbituric acid (TBA), and 1.5 ml of an 8% solution of sodium dodecylsulfate were added in that order. The contents of the tube were mixed and boiled on a waterbath for 15 min, then cooled in ice to 4°C for 10 min. To the obtained sample were added 1 ml of concentrated acetic acid and 2 ml of chloroform and the solution was centrifuged at 4°C and at 8000 rpm for 15 min. Next, 2 ml of the resulting supernatant was transferred into the cuvette of a Hitachi-556 spectrophotometer (Japan) its density was determined on the two-wave mode λ_1/λ_2 = 650/532 nm, and the intensity of fluorescence of LPO products (in ng/mg protein) was calculated by the formula:

$$\frac{DV}{E \cdot 1000 \cdot C}$$

where D denotes the optical density of the test sample at a wavelength of 532 nm, C the protein concentration in the test sample, determined by the method in [14], and E the molar extinction of malonic dialdehyde, namely $1.56 \cdot 10^5$ M^{-1} cm^{-1}; V denotes the volume of the sample (2 ml).

EXPERIMENTAL RESULTS

When determined by the spectrophotometric method the intensity of luminescence of LPO products in intact animals was 0.059 ± 0.004 nm/mg, in rats with aseptic wounds it was 0.072 ± 0.001 nm/mg, and in rats with infected wounds 0.056 ± 0.004 nm/mg. When the same parameter was determined by our suggested method the following results were obtained: intact rats 11.5 ± 0.7 rel. U, rats with aseptic wounds 18.8 ± 0.7 rel. U, and rats with infected wound 7.8 ± 0.8 rel. U (Fig. 1). When expressed as a percentage the intensity of luminescence of LPO products in the serum 1 day after the operation in rats with aseptic wounds

was increased by 37.2%, and in rats with infected wounds it was reduced by 32.2% compared with that in intact rats.

Comparison of the results indicates that our suggested method is more sensitive and more informative. For instance, the increase in the intensity of luminescence of LPO products in the blood serum of rats with aseptic wounds 1 day after the operation compared with intact animals, when determined by the spectrophotometric method, was 22.4% and when determined by our suggested method it was 37.2%. The decrease in this parameter in the case of infected wounds was 5.1 and 32.2%, respectively.

Moreover, if the spectrophotometric method is used, the volume of serum required is 0.1 ml, compared with only 0.02 ml for our suggested method.

The method we have developed can thus be used to determine LPO products in blood serum with five times higher sensitivity and three times faster. The method is simple and convenient and it can be widely used in all branches of medicine and biology for various purposes.

LITERATURE CITED

1. E. B. Burlakova, Kardiologiya, No. 8, 48 (1980).
2. V. E. Kagan, O. N. Orlov, and P. L. Prilipko, Analysis of Endogenous Lipid Peroxidation Products (Progress in Science and Technology, Series: Biophysics, Vol. 18) [in Russian], Msocow (1986).
3. V. I. Kalmykova, Bioantioxidants in the Regulation of Metabolism under Normal and Pathological Conditions [in Russian], Moscow (1982), pp. 181-194.
4. L. A. Mamedov, N. Yu. Kosaganova, G. T. Rikhereva, et al., Vorp. Med. Khimii, 32, No. 6, 82 (1986).
5. V. G. Mkhitaryan, M. I. Agadzhanov, D. M. Gevorkyan, and É. M. Mikaelyan, Vest. Akad. Med. Nauk SSSR, No. 9, 15 (1982).
6. E. A. Neifakh and V. E. Kagan, Biokhimiya, 34, No. 3, 511 (1969).
7. T. B. Suslova, V. I. Olenev, and Yu. A. Vladimirov, Biofizika, 14, No. 3, 510 (1969).
8. N. G. Khrapova, Biochemistry of Lipids and Their Role in Metabolism [in Russian], Moscow (1981), pp. 147-155.
9. V. N. Shilov, The Chemiluminescence Method in Biology and Medicine [in Russian], Kiev (1978), pp. 111-113.
10. T. Asakawa and S. Maisushita, J. Lipids, 5, No. 3, 137 (1980).
11. G. Braght, Agents and Actions, 10, 536 (1980).
12. J. Bunyan, E. A. Murrell, and J. Creen, Br. J. Nutr., 21, 475 (1967).
13. L. Placez, Nahrung, 12, 679 (1968).
14. G. R. Schasterle and R. L. Pollack, Anal. Biochem., 51, No. 2, 654 (1973).