CHANGES IN SERUM LYSOZYME ACTIVITY IN TERMINAL STATES CAUSED BY BLOOD LOSS

S. K. Anan'eva

UDC 616-005.1-036.882-07:616.153.1:577.152.321

The dynamics of the serum lysozyme activity was investigated in dogs after surviving clinical death for a period of 1 min as a result of blood loss giving rise to hypovolemic hypotension lasting 2 h. A progressive increase in the serum lysozyme activity in the course of hypotension and during the first 30 min of the postresuscitation period was found. Increased lysozyme activity of the serum was detected during the 4 days after resuscitation. The importance of the serum lysozyme activity as an indicator of hypoxic damage to the internal organs in terminal states is discussed.

KEY WORDS: terminal state; lysozyme activity.

In a previous investigation during the study of natural immunologic responses in dogs surviving after cardiac arrest produced by electric shock and lasting for 10-12 min, a regular increase in the serum lysozyme activity was discovered. The dynamics of the serum lysozyme activity under these circumstances differed essentially from the dynamics of other indices of natural immunity [1].

In the investigation described below an attempt was made to discover whether the increase in the serum lysozyme activity is characteristic of the postresuscitation period in general and to determine its role under those circumstances.

EXPERIMENTAL METHOD

The dynamics of the serum lysozyme activity was investigated in animals resuscitated after clinical death lasting 1 min and associated with prolonged hypotension resulting from blood loss. In this type of terminal state more severe damage to the internal organs is observed than after prolonged clinical death of rapid onset from electric shock [3].

Experiments were carried out on dogs. Pantopon (8 mg/kg) was injected subcutaneously into the animals 40-80 min before the beginning of the experiments. To prevent the blood from clotting, 0.4% heparin solution (100 units/kg) was injected intravenously before the experiment. Bleeding was carried out from the femoral artery until the arterial pressure fell to 40 mm Hg. It was maintained at this level for 2 h by additional bleedings and injections of small volumes of blood (10-15 ml). Free exsanguination was carried out 2 h later, with the result that clinical death ensued after 8.16 ± 1.18 min. Resuscitation began 1 min after the last inspiration, by means of artificial respiration and intraarterial centripetal injection of the previously removed blood under the pressure of 180-220 mm Hg [3]. In the control experiments the animals were prepared in the same way, including dissection of the blood vessels, but thereafter they were kept in a state of immobilization for 3 h. The serum lysozyme activity was determined by a turbidimetric method based on the degree of clarification of a suspension of an acetone powder of Micrococcus lysodeikticus in phosphate buffer, pH 6.5, after incubation for 1 h with the test serum at 37°C [2].

Laboratory of Experimental Physiology of Resuscitation, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR P. D. Gorizontov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 80, No. 11, pp. 32-34, November, 1975. Original article submitted October 29, 1974.

TABLE 1. Dynamics of Serum Lysozyme Activity in Dogs Surviving Clinical Death for 1 min Caused by Blood Loss Associated with Hypovolemic Hypotension Lasting 2 h

Statistical index	Initial value	Hypotension of 40 mm Hg			Postresuscitation period				
		1–3 min	1 h	2 h	restoration of respiration	1/2 h	2 h	1 day	4 days
M	13,4	+2,3	+15,7	+28,3	+26,5	+32,7	+30,6	+6,0	+8,2
$\pm m$	0,6	0,7	2,4	2,1	2,6	2,8	3,0	2,4	1,3
P	—	<0,01	<0,001	<0,001	<0,001	<0,001	<0,001	<0,001	<0,01

TABLE 2. Dynamics of Serum Lysozyme Activity in Control Dogs

Statistical index	Initial value	Period of observation after dissection of blood vessels					
		1/2 h	2 h	3 h	1 day	5–6 days	8–9 days
M	16,1	+0,2	+0,5	+1,7	+2,2	+1,2	+0,5
$\pm m$	1,5	1,2	1,0	2,0	0,7	1,7	4,2
P	—	>0,5	>0,5	>0,5	<0,05	>0,5	>0,5

EXPERIMENTAL RESULTS

Of the 13 experimental dogs 5 survived, but 8 died at various times in the course of 4 days. Cardiac activity was restored after 2.02 ± 0.34 min, respiration after 3.07 ± 0.28 min, and the corneal reflexes 7.44 ± 0.97 min after the beginning of resuscitation.

Although heparin is considered to be an inhibitor of lysozyme [8, 9], its injection in the above dosage caused no appreciable decrease of serum lysozyme activity. During hypovolemic hypotension the serum lysozyme activity increased considerably (Table 1), the degree of increase depending on the duration of hypotension ($P < 0.001$). At the time of appearance of spontaneous restoration the serum lysozyme activity still remained high, and for the first 30 min thereafter it increased still more ($P < 0.01$). After 2 h a small decrease in its activity ($P < 0.01$) was observed, with a considerable decrease by the end of 24 h, although even at this period and for the next 4 days it was higher than initially in the surviving dogs.

In the control experiments (Table 2) no regular changes in serum lysozyme activity were found during 3 h of observation after dissection of the vessels; however, after 24 h a very small but significant increase was detected, evidently in connection with the development of inflammation at the site of dissection of the vessels. Later it was almost indistinguishable from the initial level.

The character of dynamics of the serum lysozyme activity in animals resuscitated after clinical death caused by blood loss was thus the same as after clinical death caused by electric shock [1]. In the former, however, the serum lysozyme activity was considerably higher at the beginning of resuscitation. For instance, 30 min after resuscitation in animals surviving circulatory arrest for 12 min following electric shock, it was 6.4 ± 1.5 units above the initial level whereas in animals dying from blood loss it was 32.7 ± 2.8 units higher ($P < 0.001$). Since the greater part of the serum lysozyme arises from fragmented leukocytes (monocytes and neutrophils) [5], most workers consider that its level reflects the kinetics of the leukocytes [6, 7]. One cause of increased leukocyte destruction in terminal states may be the appearance of toxic substances caused by hypoxic damage to the internal organs [10], more especially because the dynamics of serum toxicity in the postresuscitation period corresponds in its general features to the dynamics of lysozyme activity: It is highest 30 min after the beginning of resuscitation, relatively lower after 1-2 h, and continues for 2 days [4].

It can be concluded from the facts described above that the level of the serum lysozyme activity in terminal states can characterize to some degree the severity of hypoxic injury to the internal organs.

LITERATURE CITED

1. S. K. Anan'eva, Byull. Éksp. Biol. Med., No. 2, 80 (1972).
2. K. A. Kagramanova and Z. V. Ermol'eva, Antiobiotiki, No. 10, 917 (1966).
3. V. A. Negovskii, Pathophysiology and Treatment of Agony and Clinical Death [in Russian], Moscow (1954).
4. L. G. Shikunova and R. V. Nedoshivina, Byull. Éksp. Biol. Med., No. 2, 21 (1972).
5. S. Finch, J. Lamphere, and S. Jablon, Yale J. Biol. Med., 35, 350 (1964).

6. M. Fink and S. Finch, Proc. Soc. Exp. Biol. (New York), 127, 365 (1968).
7. N. Hansen and H. Karle, Brit. J. Haemat., 21, 261 (1971).
8. E. Kaiser, Nature, 171, 607 (1953).
9. G. Kerby and G. Eadie, Proc. Soc. Exp. Biol. (New York), 83, 111 (1953).
10. A. Lefer, Circulat. Res., 32, 129 (1973).