4. J. W. Eaton, J. R. Eckman, et al., Nature, 264, 758 (1976).
5. J. R. Eckman and J. W. Eaton, Nature, 278, 754 (1979).
6. N. Etkin and J. W. Eaton, in: Erythrocyte Structure and Function, New York (1975), p. 219.
7. J. Fridovich, Adv. Enzymol., 41, 35 (1974).
8. Y. O. Kobayashi, S. Okahata, and T. Usui, Biochem. Biophys. Res. Commun., 91, 1288 (1979).
9. G. Perona, G. C. Guidi, et al., Br. J. Haematol., 39, 399 (1978).
10. W. Trager, Bull. World Health Org. (Russian edition), 55, 295 (1978).

EXTRUSION OF IONIZED CALCIUM AS A MARKER OF PLATELET RELEASE

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The platelet release reaction (PRR) of biologically active substances is unchanged by the action of a critical concentration of aggregating substances on the platelets, of which thrombin gives the most complete natural effect. The study of PRR is interesting in order to understand the mechanisms of prevention of formation of platelet aggregates in the circulation and the development of vascular thrombosis.

Aggregating platelets release a number of pharmacologically active substances [1], simultaneously with which they also release ionized calcium (Ca^{++}). Quantitative determination of extruded ATP, ADP, serotonin, various enzymes, and other substances requires the use of complex techniques, whereas no great difficulty arises during precise measurement of Ca^{++} by the use of an ion-selective technique [7].

The method of atomic absorptiometry has shown [3] that the total calcium content in human platelets may reach 260 ± 62 nmoles/10^9 cells. Under the influence of thrombin, depending on the functional activity of the cells, up to 58-85% of the Ca^{++} is released from the platelets [8]. The exceptional importance of this ion in the activity of all cells of the body is confirmed by the latest convincing evidence [2, 4, 6]. Accordingly the possibility of monitoring PRR by Ca-selective electrodes can facilitate the study of this phenomenon in pathological states associated with the risk of disturbances of blood coagulation, and also during treatment with various drugs.

This paper gives the results of an investigation of the release of Ca^{++} from platelets under the influence of thrombin, using an ion-selective electrode.

EXPERIMENTAL METHOD

Platelet-enriched plasma was obtained from citrated human plasma in the usual way. Platelets were isolated by repeated centrifugation for 15 min at 1000 g, then washed three times with buffer consisting of 0.9% NaCl, 0.5% glucose, and 0.02 M Tris-HCl, pH 7.4. The platelets were then resuspended in the same buffer up to a concentration of $2.2 \cdot 10^8$ cells/ml. To unify the conditions of measurement the calcium concentration in the suspension was adjusted to 10^{-5} M.

Commercial thrombin, manufactured by the Kaunas Bacterial Preparations Factory, was dialyzed for 48 h against Tris-HCl, pH 7.0, to remove as much as possible of the calcium contained in it. Thrombin with activity of 100 units/ml and with a residual calcium content of 0.1 mmole/liter was used in the experiment.

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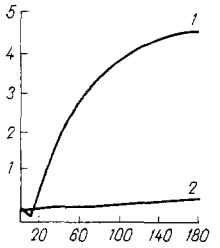

Fig. 1

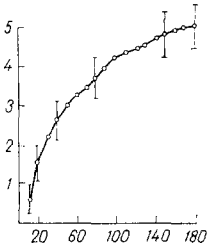

Fig. 2

Fig. 1. Dynamics of Ca^{++} release from platelets under the influence of thrombin. 1) After addition of thrombin in final concentration of 1.5 units/ml to platelet suspension containing $2.2 \cdot 10^8$ cells/ml; 2) the same, but after preliminary addition of verapamil up to 8.0 mg/ml to the same suspension before addition of the same dose of thrombin. Abscissa, time (in sec); ordinate, Ca^{++} content (in nmoles/10^8 platelets).

Fig. 2. Dynamics of Ca^{++} release from platelets under the influence of 1.5 units/ml thrombin (data of 20 measurements). Legend as to Fig. 1.

The concentration of extracellular Ca^{++} and the kinetics of its liberation from the platelets were recorded by measuring system containing a Selelectrode F $2^1/_{12}$ Ca (from Radiometer, Denmark) Ca-selective electrode, consisting of an ion-exchange membrane electrode with a sensitive element consisting of a polyvinyl chloride membrane [5]. Before measurement the system was calibrated against standard solutions in which the calcium concentration was determined by amperimetric titration of the basic solution.

To record the kinetics (for 3-4 min) of Ca^{++} release from the platelets, thrombin was added to 2 ml of the suspension in a final concentration of 1-1.5 units/ml.

EXPERIMENTAL RESULTS

The kinetics of liberation of Ca^{++} from platelets under the influence of thrombin is illustrated in Fig. 1. On the addition of more than the critical quantity of thrombin, gradual liberation of Ca^{++} from the platelets took place. The most intensive release of Ca^{++} took place in the first minute, but after 2-3 min the process slowed down considerably and by the 4th minute it was completely stabilized.

The presence of verapamil, which blocks the calcium current, in the system in a final concentration of 8.0 mg/ml completely inhibited PRR (Fig. 1).

A curve of the kinetics of Ca^{++} release from platelets plotted from data of 20 measurements is given in Fig. 2. It shows that the quantity of Ca^{++} released differed significantly after 20, 40, 80, and 160 sec of PRR.

Changes in the quantity of Ca^{++} liberated with time can be represented by the following equation:

$$K_{Ca} = 11.39 \cdot 0.14^{(0,80T)}$$
$$\ln K_{Ca} = 2.43 - 1.97^{(0,80T)},$$

where KCa is the quantity of Ca^{++} (in nmoles/ml suspension containing $2.2 \cdot 10^8$ platelets/ml; T is the time of action of thrombin, in sec$\cdot 10^{-1}$).

After transformation for a concentration of 10^8 platelets/ml the equation assumed the form:

$$K_{Ca} = 5.18 \cdot 0.14^{(0,80T)} \text{ or}$$
$$\ln K_{Ca} = 1.64 - 1.97^{(0,80T)}.$$

In the course of the PRR, 5.04 ± 0.58 nmoles Ca^{++} ions was thus released from 10^8 platelets. If this is converted to the platelet concentration usually adopted in the literature, namely 10^9 cells/ml, the figure is 50.4 ± 5.8 nmoles Ca^{++}, or about 20% of the total content in human platelets.

The results for the kinetics of Ca^{++} release under the influence of thrombin thus obtained indicate that this process can be recorded by means of ion-selective electrodes and they open up definite prospects for the study of PRR and the mechanism of aggregation of platelets and their activity in various pathological states and under the influence of certain drugs.

LITERATURE CITED

1. T. C. Detwiler and R. D. Feinman, Biochemistry (Washington), 12, 2462 (1973).
2. T. C. Detwiler, I. P. Charo, and R. D. Feinman, Thromb. Hemostas., 40, 207 (1978).
3. M. Doni and G. Fantin, Haematologica, 60, 286 (1975).
4. M. B. Feinstein, Bibl. Haemat., 45, 1 (1978).
5. L. B. Kornstein, L. S. Robblee, and D. Shepro, Thromb. Res., 11, 471 (1977).
6. P. Massini, in: Platelets and Thrombosis, London (1977), pp. 33-34.
7. L. S. Robblee, L. B. Kornstein, and D. Shepro, Thromb. Hemostas., 37, 407 (1977).
8. R. J. Skaer, P. D. Peters, and J. P. Emmines, J. Cell. Sci., 15 679 (1974).

AGE CHANGES IN SUPEROXIDE DISMUTASE AND GLUTATHIONE PEROXIDASE ACTIVITY IN CYTOSOL AND MITOCHONDRIA OF RAT LIVER

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Processes of free-radical lipid peroxidation (LPO) may play an essential role in disturbance of the function of cell systems [1, 2]. The writers previously found a considerable increase in activity of enzymic and nonenzymic LPO liver membranes in various pathological states, including experimental malignant growth [3, 4], chemical carcinogenesis [4, 5], and experimental atherosclerosis [6, 7]. It was shown that activation of LPO systems in the liver is observed not only in pathological states, but also during normal physiological processes and, in particular in the early stage of postnatal development [7, 8]. This finding was confirmed in investigations by various workers in recent years, both *in vitro* [9-12] and *in vivo* [13]. The intensity of LPO in the subcellular organelles of the rat liver falls with age [7-10], simultaneously with an increase in NADPH and in the SH-dependent antioxidant action of the cytosol [10]. It can be postulated on the basis of results so far obtained [7, 8, 10] that LPO in the cell at various stages of development and aging does not lead to induction of pathological changes, for it is under the effective control of "antioxidant" enzymes [2, 7], namely superoxide dismutase (SOD) and glutathione peroxidase (GP). The investigation described below was devoted to an experimental test of this hypothesis.

EXPERIMENTAL METHOD

Male Wistar rats of different ages (newborn, aged 1 and 2 weeks and 1, 2, 3, 6, 12, 18, and 24 months), kept under standard conditions, were used. Embryos were obtained by autopsy of the pregnant females of the same line one week before the expected date of birth. The rats were killed by decapitation and, after perfusion with cold 0.154 M KCl, pH 7.4 (the liver of the embryos and of the newborn and week-old rats was not perfused) the liver was homogenized

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