SIMULTANEOUS RECORDING OF TWO FUNCTIONAL PARAMETERS OF PLATELETS

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The functional properties of platelets and their relations with each other can be correctly studied only by simultaneous recording of several parameters and their changes in response to various specific factors. Systems capable of studying two parameters simultaneously – the release reaction together with redistribution of intracellular Ca^{++} [1] and three parameters simultaneously – aggregation, shape, and release reaction of platelets (the lumiaggregometer) [2] already exist.

In this paper a method enabling changes in four functional parameters – aggregation and shape of the cells, release of adenine nucleotides, and redistribution of intracellular Ca^{++} – to be recorded simultaneously in one sample of platelet suspension is suggested. This method combined for the first time parallel measurement of aggregation and shape together with fluorescence of a dye bound with intracellular Ca^{++}.

EXPERIMENTAL METHOD

To make this method possible an apparatus was constructed on the basis of the principle of time division of channels (Fig. 1). The apparatus consists of a cuvette 1 containing the test specimen, a thermostat 2, magnetic mixer 3, diaphragm 5, light filters 6, 8, 13, 17, and 21, shutters 7 and 20, a source of UV light 4, a photodiode 12, a semitransparent mirror 11, a mirror 16, photoelectronic multipliers 14 and 18, phase detectors 10, 15, 19, and 23, and a multichannel recorder (not shown on the scheme). The apparatus works as follows: UV radiation from the light source 4, after passing through the diaphragm 5, filter 6 (transmission band 380-400 nm) and shutter 7, enters the cuvette with the specimen and excites fluorescence of the Ca^{++}-sensitive probe chlortetracycline (CTC) which, passing through the narrow band filter 8 (transmission band 525-540 nm), falls on the photoelectronic multiplier (PhEM) 9. The signal from the PhEM is led to a phase detector and recorded on an automatic writer. Infrared (IR) radiation (0.94 μ) from the photodiode 12 is directed by the semitransparent mirror 11 through the screen 7 into the cuvette 1. IR-radiation scattered by platelets passes through the filter 13 (transmission band 0.8-1.4 μ) and falls on the photodiode 14. The signal from the photodiode is led to the phase detector 15 and recorded on the automatic writer. Changes in the intensity of scattered radiation are a measure of aggregation of the platelets. The geometric dimensions of the aggregation channel described above are chosen so that a change in the orientation of the platelets and in their spherulation has no effect on the intensity of scattered radiation. Having passed through the cuvette the IR radiation is directed by mirror 16 through the filter 17 (transmission band 0.8-1.4 μ) on to the photodiode 18. The signal from the photodiode is led to phase detector 19 and recorded on the automatic writer. The intensity of transmitted light depends on platelet orientation, so that the shape of the cells and changes in their shape can be determined. The optical channels for measurement of fluorescence, aggregation, and changes in shape described above lie in one plane. The bioluminescent radiation of a luciferin–luciferase system, characterizing the extracellular ATP concentration, passes through the screen 20, filter 21 (transmission band 560-750 nm), and falls on the PhEM 22, the signal from which is led to phase detector 23 and recorded on the automatic writer. The optical axis of the bioluminescence channel lies above the plane of the other optical channels. Shut-

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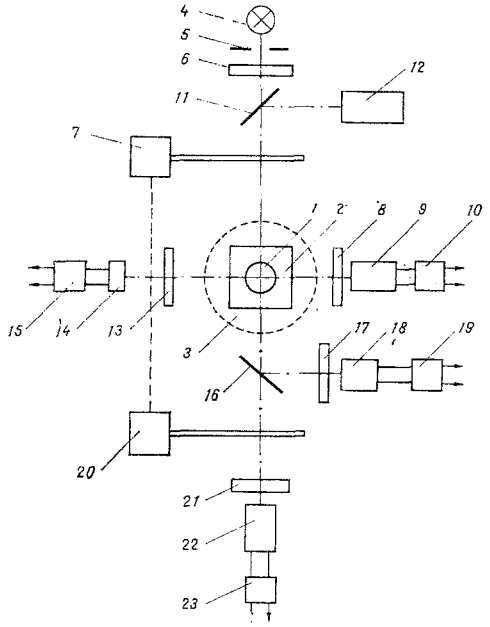

Fig. 1

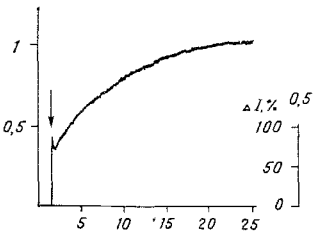

Fig. 2

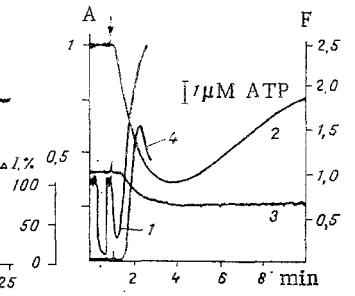

Fig. 3

Fig. 1. Block diagram of experimental arrangements (description in text).

Fig. 2. Changes in fluorescence of CTC during incubation of platelets with probe. Abscissa, time (in min); ordinate, intensity of fluorescence (in relative units). Arrow indicates time of introduction of probe (25 μM).

Fig. 3. Thrombin-induced changes in functional properties of platelets. ΔI) Changes in light transmission characterizing shape of platelets (1): A) intensity of scatter of light (in relative units), characterizing platelet aggregation (2); F) intensity of fluorescence of CTC (in relative units), characterizing distribution of intracellular calcium (3); 4) ATP release. Arrow indicates time of addition of thrombin (0.17 unit/ml).

ters 7 and 20 are fixed on the same axis so that when shutter 7 does not transmit UV- and IR-radiation, shutter 20 transmits bioluminescent radiation and vice versa.

Blood was obtained from a rabbit's heart by direct puncture and stabilized with acid citrate-dextrose solution in the ratio of 6:1. The platelets were washed by the standard method [3]. The thrombin, CTC, and luciferase used in the experiments were obtained from Sigma, USA; the remaining reagents were of the chemically pure grade.

EXPERIMENTAL RESULTS

The platelet suspension was incubated for 30 min at 22°C with CTC in a final concentration of 25 μM. After addition of CTC a rapid increase in fluorescence was observed (Fig. 2), due to penetration of the probe into the cell and the formation of a CTC–Ca^{++}–membrane complex, the quantum yield of which was significantly greater than the quantum yield of the free probe. During the incubation period, the intensity of fluorescence reached a constant level. Incubation of platelets in a calcium-free medium with CTC led to a similar increase in fluorescence. Consequently, that increase could not be connected essentially with the transfer of exogenous Ca^{++} inside the platelets. No appreciable change in concentration of the extracellular probe occurred. Before addition of thrombin, the mixer was switched on and off; under these circumstances changes were observed in the transmission of light by the platelet suspension (Fig. 3), indicating that the CTC-labeled platelets were discoid in shape. After addition of thrombin in a final concentration of 0.17 unit/ml to the platelet suspension, changes in the shape of the platelets from discoid to spherical, aggregation of the cells (partially reversible), an ATP release reaction, and an irreversible decrease in fluorescence of the probe were observed (Fig. 3). It must be presumed that the thrombin-induced change of fluorescence of the probe was connected with redistribution of intracellular Ca^{++} in places where it is stored on platelet membranes, and also with its release from the platelets.

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