EFFECT OF DIFFERENT METHODS OF ISOLATION OF PLATELETS ON SURFACE STRUCTURE AND BIOCHEMICAL PARAMETERS OF THEIR SEROTONIN SYSTEM

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The high sensitivity of platelets to various physical and chemical factors is associated with the specific functions of these cells [2, 7]. The presence of a contractile apparatus, similar to the actomyosin complex in muscles, in platelets enables them to change the shape of their surface actively – from a smooth disc to a contoured sphere, with the formation of pseudopodia [3]. This properties makes it difficult to isolate platelets in an inactivated form, i.e., in the morphological and functional state in which they circulate in the blood stream. The investigator often needs to know in what morphological and functional state the platelets isolated by a given method will be found. In order to study platelets, therefore, the comprehensive investigation of their structure by the use of biochemical methods is essential. Such a study can enable the morphological assessment of structure to be switched from the field of passive observation into the study of the "structural physiology" of the cell [8].

In the investigation described below the method of scanning electron microscopy was used to obtain a three-dimensional image of the object when studying surface properties of platelets isolated by different methods – by centrifugation and by gel-filtration. The effect of these methods Or isolation on the basic biochemical parameters of the serotonin system of the platelets, specifically on serotonin uptake by human peripheral blood platelets and binding of imipramine by these cells, also was evaluated.

EXPERIMENTAL METHOD

Blood from ten physically and mentally healthy women aged from 30 to 41 years was studied. To isolate platelets by centrifugation, blood samples (40 ml) were collected in plastic test tubes containing anticoagulant (citric acid 1.37 g, sodium citrate 2.5 g, glucose 2 g to 100 ml water, pH 5.7). Platelet-enriched plasma (PEP) was obtained by centifuging blood at 200 g for 15 min at room temperature. The plasma thus obtained was centrifuged at 14,600*g* for 10 min at 4°C. To the residue were added 4 ml of buffer 1 (50 mM Tris-HCl, 120 mM NaCl, 5 mM KCl, 7 mM citric acid, 8 mM sodium citrate, 11 mM glucose, pH 7.5) [5], and the contents were mixed. This procedure was repeated. Buffer (4-5 ml) was added to the residue, which was used for future work. Platelets were isolated by gel-filtration by passing PEP through sepharose CL-2B ("Pharmacia Fine Chemicals," Sweden) in a column (2.6 × 15 μm) previously equilibrated in buffer 1 (pH 7.5, 20°C) [5]. To study the surface structure of the platelets by scanning electron microscopy a platelet suspension was fixed for 30 min in 2.5% glutaraldehyde, made up in buffer 1. The platelets were washed with buffer and sedimented by centrifugation at 1500*g* on aluminum foil and dehydrated in a series of ethanols of increasing concentration: 30, 50, 70, 80, 90, and 100°.

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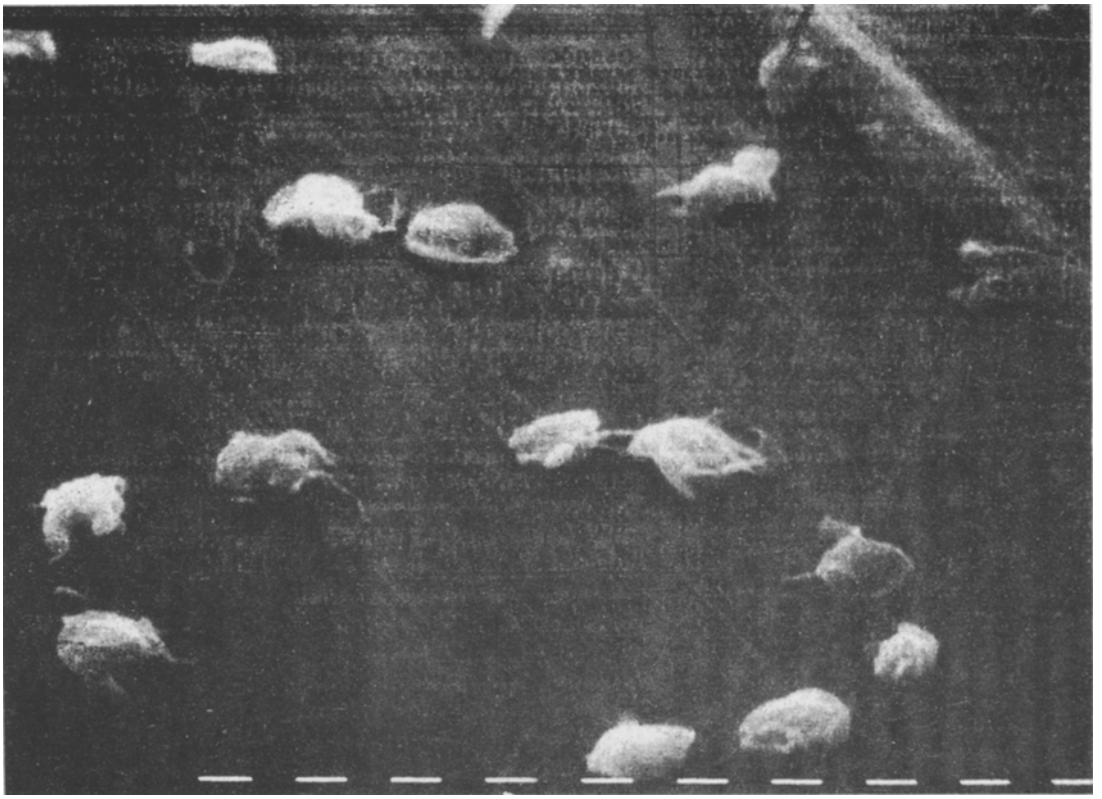

Fig. 1. Platelets isolated by gel-filtration. 5000×.

After dehydration, the platelets, together with the aluminum foil, were fixed to stages by means of special conducting glue ("Polaron," U.K.), dried in air, coated with gold by means of an "Emscope 500" spray (U.K.). A "Philips 501 SEM" (The Netherlands) scanning electron microscope was used for the investigation. To determine reuptake of ^{3}H-serotonin (SRU) by platelets (the parameter V400) aliquots of the platelet suspension (0.1 ml) were added to a solution of ^{3}H-serotonin (400 nM) ("Amersham," 15 Ci/mmole) and incubated at 37°C for 1 min in the absence and in the presence of 50 μM zimelidine (a selective inhibitor of SRU [4]). Values of SRU in the presence of zimelidine gave the nonspecific uptake of ^{3}H-serotonin. After incubation for 1 min the platelets were quickly filtered through GF/B filters ("Whatman," England), using a multiple filtration system in an adaptation of the "Microplate Cell Harvester" type ("Flow Laboratories," U.K.). The filters were washed with buffer of special composition: to buffer 1 was added 6 mM bovine serum albumin, pH 7.5, at 2°C. The dried filters were immersed in ZHS-8 scintillator and radioactivity measured on an LS-1801 counter ("Beckman") with an efficiency of 54%. Binding of ^{3}H-imipramine by platelets was carried out as follows. To a solution of ^{3}H-imipramine (20 nM) was added 200 μl of platelet suspension. Incubation was carried out for 2 h at 4°C with mixing. Nonspecific binding was assessed in the presence of 100 μM unlabeled imipramine. The procedure of filtration and counting the radioactivity followed that described above for determination of SRU.

EXPERIMENTAL RESULTS

The study of the surface structure of platelets by scanning electron microscopy showed that platelets isolated by gel-filtration differ in their morphological properties from those isolated by centrifugation. The former characteristically had an inactivated shape, namely a regular disc, but unlike erythrocytes, the disc was flat. The diameter of the platelets was about 2.5 μ. No invaginations or pseudopodia could be observed and the surface of these cells was virtually smooth (Fig. 1).

Platelets isolated by centrifugation showed marked evidence of activation. They differed from inactivated platelets in a number of characteristic properties. First, the tendency of these cells to form conglomerates of various sizes. The surface of the platelets when activated was irregular, and the cells themselves had from 1 to 3 pseudopodia, longer than the diameter of the platelets themselves (Fig. 2).

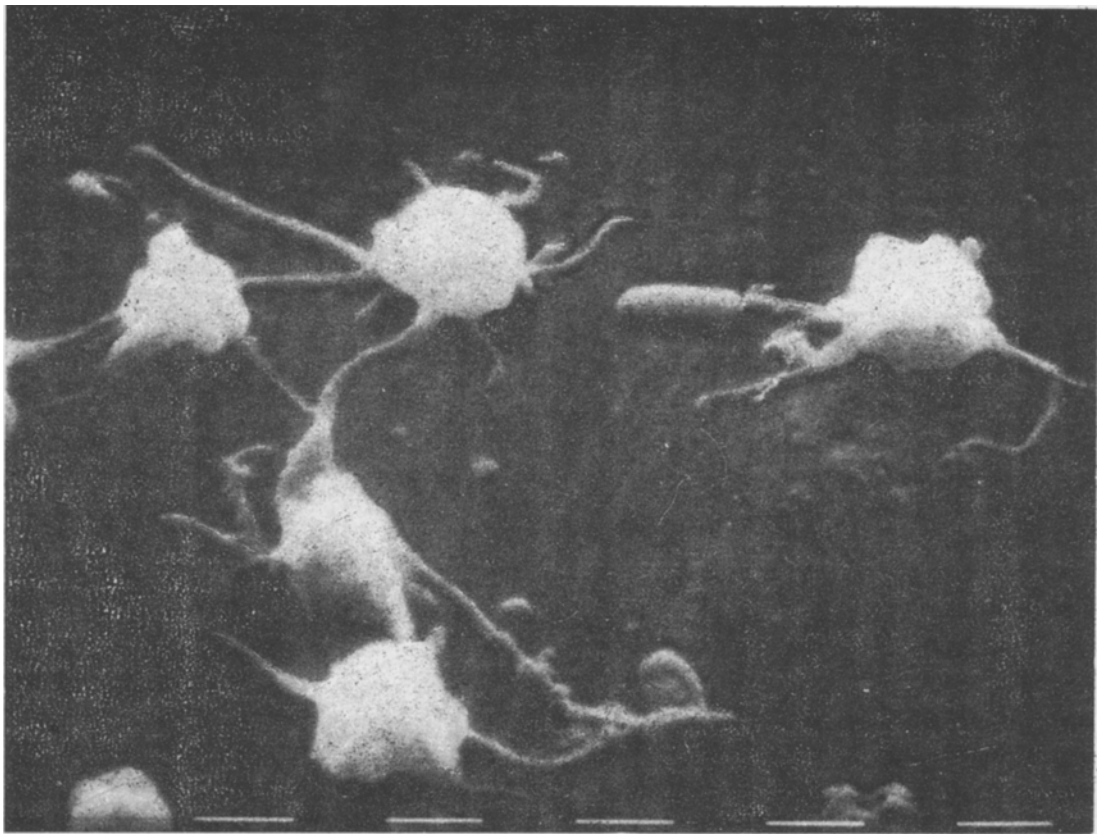

Fig. 2. Platelets isolated by centrifugation. 10,000×.

A platelet residue isolated by centrifugation could contain proteins, unlike platelets isolated by gel-filtration, and this, in turn, could distort the values of specific binding of ^{3}H-imipramine and ^{3}H-serotonin uptake by these cells if the differences were considerable. Accordingly, the protein concentration in the incubation medium containing the platelets was determined. For this purpose, after filtration, filters with cells were cut into two equal parts, and one half of the filter was extracted in a solution of 1 ml of 10% sodium dodecylsulfate at 37°C for 3 h. Protein in the extracts was determined as in [1]. The protein content was found to be equal in platelets isolated by gel-filtration and by centrifugation (1.25 ± 0.13 mg/ml).

Comparison of the density of specific binding sites of ^{3}H-imipramine by platelets isolated by centrifugation and by gel-filtration showed that the number of binding sites for platelets isolated by centrifugation was about twice that for platelets isolated by gel-filtration, namely 1.12 ± 0.10 and 0.54 ± 0.09 pmole/10^9 platelets respectively ($p < 0.01$).

Determination of the rate of SRU by the platelets (V_{400}) also showed a significant increase in the value of V_{400} for platelets isolated by centrifugation: 140 ± 10 and 205 ± 15 pmoles/min/10^9 platelets for those isolated by gel-filtration and centrifugation respectively ($p < 0.01$).

Thus centrifugation, used to isolate platelets, causes their activation. Gel-filtration is a much more sparing method of isolating platelets. During elution with buffer, platelets are the first fraction to be eluted from the column, and are followed a short time later by proteins of PEP, which can be seen on the chromatogram as two peaks of optical density, which are quite separate from one another. By this method it is possible to obtain platelets similar in their structure to platelets in PEP. This conclusion is in agreement with those obtained in [6], the authors of which used transmission microscopy to compare the degree of activation of platelets in PEP and platelets isolated by gel-filtration and centrifugation, and showed that platelets close in structure to platelets in PEP can be obtained by gel-filtration.

Activation of platelets, manifested as the formation of pseudopodia, causes activation of the serotonin system: an increase in specific binding of imipramine and of SRU. During the formation of pseudopodia, the platelet membrane becomes deformed, stretched, and it can be tentatively suggested that as a result of this process changes take place in the conformation of the receptor complex, and/or in its accessibility for binding with the ligand, and this in turn brings about activation of the serotonin system.

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EFFECT OF SEROTONIN ON HEMATOPOIETIC STEM CELLS IN BONE MARROW

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Serotonin (5-HT), which performs the functions of neurotransmitter and tissue hormone in the body, is involved in the regulation of biochemical and physiological processes under both normal and pathological conditions [4]. The effect of 5-HT on cell proliferation and, in particular, on hematopoiesis is particularly interesting. There is conclusive evidence that exogenous 5-HT has a stimulating effect on erythropoiesis [8, 10] and immunogenesis [1]. High concentrations of endogenous 5-HT have been found in hematopoietic and immunocompetent tissues [2], as well as marked changes in the 5-HT level in these tissues in response to stimulation of erythropoiesis [3]. It has been shown that the amine stimulates cell proliferation in a culture of fibroblasts [5, 7]. It will be noted that the effects of 5-HT on hematopoiesis have been studied as a rule in systems for evaluating the functional activity of mature cells. Taking account of the fact that hematopoiesis is based on the hematopoietic stem cell (HSC), it was decided to study the effect of 5-HT on this particular population of hematopoietic stem cells.

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