# METHOD OF ISOLATION OF RAT PLATELETS AND ASSESSMENT OF THEIR AGGREGATION

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Increased aggregating ability of platelets is known to be a risk factor in connection with the development of thromboembolic complications in various diseases such as, for example, arterial hypertension, atherosclerosis, and ischemic heart disease [3]. Because of the limited possibilities of clinical investigations, experiments on laboratory animals and, in particular, on rats are of great importance for the study of the etiology and pathogenesis of these diseases [4]. However, information on methods of isolating washed platelets and of estimating their aggregating activity, is scanty.

This paper describes methods of taking blood from adult Wistar rats weighing 250-300 g, isolating washed platelets from it, and estimating their aggregation.

## EXPERIMENTAL METHOD

When blood was taken with a catheter the animal, anesthetized with pentobarbital (40 mg/kg) was placed on an operating table, the carotid artery was isolated, and a thin catheter connected to a 10-ml plastic syringe containing 1 ml of sodium citrate (3.8%), was introduced into it. The whole operation took 10-15 min.

When blood was taken by puncture of the left ventricle, ether anesthesia was used, because this method does not require the animal to be deeply anesthetized and takes only 2-3 min. In this case the rat was fixed on an operating table, the chest opened, and the left ventricle punctured with a siliconized needle. The blood was collected into a plastic tube with 3.8% sodium citrate solution (9:1). This is evidently the best of the methods examined, for it enables a sufficient quantity of blood to be obtained relatively quickly even from very small animals (weighing 30-50 g) and, most important of all, the blood obtained is not contaminated with saliva or other impurities, as it often is when obtained by the traditional method of decapitation.

The following mother solutions were prepared: A) NaCl 160 g, KCl 4 g, $NaHCO_3$ 20 g, and $NaH_2PO_4$ 1 g in 1 liter of distilled water, pH 7.35; B) 0.1 M $MgCl_2 \cdot 6H_2O$; C) 0.1 M $CaCl_2 \cdot 6H_2O$. Solutions A, B, and C are stable and can be kept for 2-3 months at 4°C. The following buffers were prepared before each isolation: D (washing): 50 ml of A, 20 ml of B, 3.5 g albumin, and 1 g glucose in 1 liter of distilled water, pH 6.2; E (suspending): 50 ml of A, 10 ml of B, 20 ml of C, 3.5 g of albumin, and 1 g of glucose in 1 liter of distilled water, pH 7.3. All procedures were carried out at 20°C.

Platelet-rich plasma (PRP) was obtained by centrifugation of blood (146 g, 15 min), after which the platelets were sedimented at 1100g for 10 min and washed twice in buffer D. The washed platelets were finally suspended in buffer E.

Platelets were counted in a Goryaev chamber and diluted with buffer to a concentration of $3 \times 10^5$ cells/mm$^3$, which is optimal for aggregation (Fig. 1). Platelet aggregation was recorded on a two-channel aggregometer (Payton, Canada). ADP (from Sigma, USA) was used as inducer.

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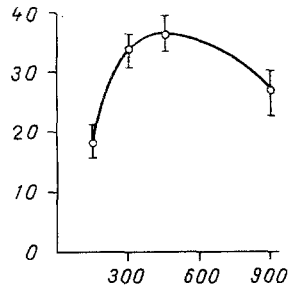


Fig. 1

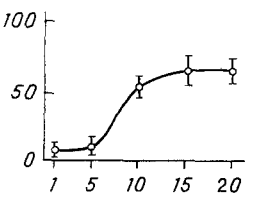


Fig. 2

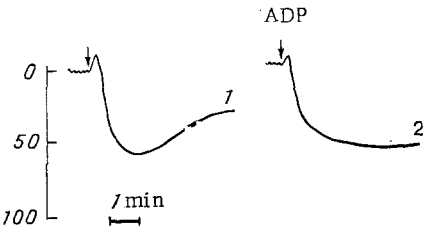


Fig. 3

Fig. 1. Dependence of amplitude of aggregation on platelet concentration in suspension. Abscissa, number of cells in 1 mm$^3$ ($\times 10^3$); ordinate, degree of aggregation (in %).

Fig. 2. Dose–response curve for platelet aggregation. Abscissa, ADP concentration (in μM); ordinate, amplitude of aggregation (in %).

Fig. 3. Typical aggregation curves of platelets in plasma (1) and in buffer (2). Abscissa, time (in min); ordinate, amplitude of aggregation (in %). Platelet concentration $3 \times 10^5$ cells/mm$^3$. ADP concentration 10 μM.

TABLE 1. Comparison of Methods of Taking Blood from Rats

| Method of sampling | Anesthesia | Volume of blood taken, ml | Contamination with foreign substances | Number of platelets in 1 mm$^3$ ($\times 10^3$) | Amplitude of aggregation, mm |
|---|---|---|---|---|---|
| Decapitation | — | 6 | + | 133 | 72±2 |
| Puncture of left ventricle | Ether | 10 | — | 300 | 76±3 |
| Catheterization of carotid artery | Pentobarbital | 12 | — | 330 | 78±3 |

## EXPERIMENTAL RESULTS

Curves showing dependence of the amplitude of platelet aggregation on ADP concentration (dose–response curves) were S-shaped; maximal aggregation was observed with ADP in a concentration of 10 μM for both PRP and washed platelets (Fig. 2).

Typical curves of ADP-induced rat platelet aggregation are illustrated in Fig. 3. Under these conditions aggregation of PRP clearly was reversible in character, unlike aggregation of washed platelets; in the latter case aggregation became reversible in lower concentrations of inducer.

When the results of platelet aggregation are interpreted, difficulties may be encountered because of the absence of generally accepted quantitative criteria for evaluation of aggregation curves [1, 2]. To estimate the velocity of the aggregation process, the writers suggest a model based on approximation of the initial regions of the curves by the exponential function $A = A_0 e^{-\alpha t}$, where $A_0$ is a constant and $\alpha$ the velocity constant (min$^{-1}$). These parameters were calculated for each curve. To do this, five values of A(t) were taken from each experimental curve, converted to logarithms: $\ln A = \ln A_0 - \alpha t$, and the value of $\alpha$ calculated by the method of least squares. By this approach it was possible to objectivize analysis of the data and to discover any particular features of platelet function, especially in arterial hypertension. For instance, the velocity constant ($\alpha$) of platelet aggregation was significantly ($P < 0.05$) higher in spontaneously hypertensive rats than in normotensive rats, in agreement with data on the increased aggregating power of the platelets in arterial hypertension [5].

Table 1 summarizes data for comparing the three different methods of taking blood from rats: decapitation, catheterization of the carotid artery, and puncture of the left ventricle.

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