

## The Action of Calcium and Thrombin on Isolated 5-Hydroxytryptamine Organelles of Blood Platelets

In isolated blood platelets, thrombin, especially in the presence of calcium, causes a marked liberation of endogenous 5-hydroxytryptamine (5HT)<sup>1-7</sup>. By combined electronmicroscopic and biochemical studies, it has recently been demonstrated that the 5HT of the platelets is mainly localized in specific subcellular organelles which also contain large amounts of adenosine-triphosphate (ATP). These organelles, which are surrounded by a membrane and have a highly osmiophilic content (probably due to 5HT), can be isolated in virtually pure form by density gradient centrifugation<sup>8-10</sup>. Therefore, it was of interest to compare the action of thrombin and calcium on isolated organelles versus intact platelets.

**Experimental.** Rabbits of 2-3 kg, fasted for 16 h, were bled in ether anaesthesia through a polyethylene cannula in the carotid artery. Disodium-ethylene-diamine-tetracetate (1/10 vol., 5%) was added to the blood in order to prevent coagulation. Platelets were isolated as previously described<sup>11</sup>, homogenized by ultrasonication, and submitted to density gradient centrifugation in order to isolate the 5HT organelles<sup>12</sup>.

In the control experiments, isolated intact platelets, washed once with incubation medium (see below), or 5HT organelles were incubated at 37°C for 30 min in modified tyrode solution (containing 1% bovine albumin (Fluka) but no calcium). In similar experiments, the tyrode buffer was supplemented with various amounts of CaCl<sub>2</sub> or 1 IU/ml thrombin (Roche). At the end of the incubation period, the platelets as well as the organelles were separated by centrifugation at 2°C (platelets 2710 g for 5 min, organelles 44,000 g for 10 min), and their 5HT and ATP content was measured by previously described methods<sup>13,14</sup>. Furthermore, isolated 5HT organelles, which had been exposed to calcium, were examined by electron microscopy<sup>10</sup>.

**Results.** (1) As described earlier, in the control experiments, the platelets lose practically no 5HT and ATP during the incubation period, but in the isolated 5HT organelles these constituents are diminished (in the present experiments by about 2/5 and 1/5, respectively)<sup>12,15</sup>. Thrombin causes a marked decrease of 5HT and ATP in the platelets, some of which agglutinate whereas others show a normal appearance on light microscopy. In the isolated organelles, however, the spontaneous liberation of neither 5HT nor ATP is significantly enhanced by thrombin ( $p > 0.05$ ) (Table).

(2) Calcium alone does not markedly affect the 5HT and ATP content of the intact platelets, e.g. concentrations of 2.2 mM calcium (corresponding to subnormal plasma levels) decrease the 5HT and ATP by not more than about 10%. In contrast, isolated 5HT organelles are very sensitive to calcium since concentrations as low as 0.7 mM calcium diminish both 5HT and ATP by about 50% whereas 2.2 mM calcium reduce these constituents by some 75% (Figure 1).

(3) Electronmicroscopical pictures of the isolated organelles exposed to calcium for 30 min show mainly ghost-like structures. Most of the organelles are collapsed, have no osmiophilic content and their membrane exhibits an irregular, ill-defined structure. This contrasts with the organelles not exposed to calcium most of which contain osmiophilic material and which, even if empty, appear spherical and show a well-defined membrane (Figure 2).

**Discussion.** According to the present results, isolated 5HT organelles react differently from intact platelets to thrombin and calcium. The 5HT and ATP of the organelles are highly sensitive to calcium, but little influenced

by thrombin. In contrast, in the intact platelets, thrombin has a more marked effect than calcium.

Since the 5HT organelles in isolated form seem to be strongly altered by calcium (Figure 2), they are probably

Effect of 1 IU/ml thrombin on the 5-hydroxytryptamine (5HT) and adenosine-triphosphate (ATP) content of isolated platelets and organelles incubated for 30 min

	Platelets	Organelles
5HT	15 ± 2	92 ± 5
ATP	24 ± 2	97 ± 8

The values are indicated in % of those of controls incubated for 30 min without thrombin. Each figure represents an average with S.E. of 4 experiments.

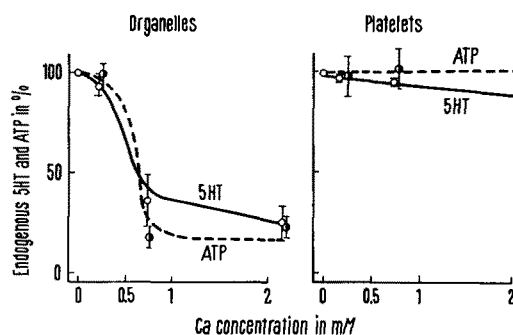


Fig. 1. Effect of various concentrations of CaCl<sub>2</sub> on the 5-hydroxytryptamine (5HT) and adenosine-triphosphate (ATP) content of isolated 5HT organelles and intact blood platelets of rabbits. Incubation time 30 min. Organelles and platelets incubated for 30 min without CaCl<sub>2</sub> served as controls (= 100). Each point represents an average with S.E. of 5 experiments.

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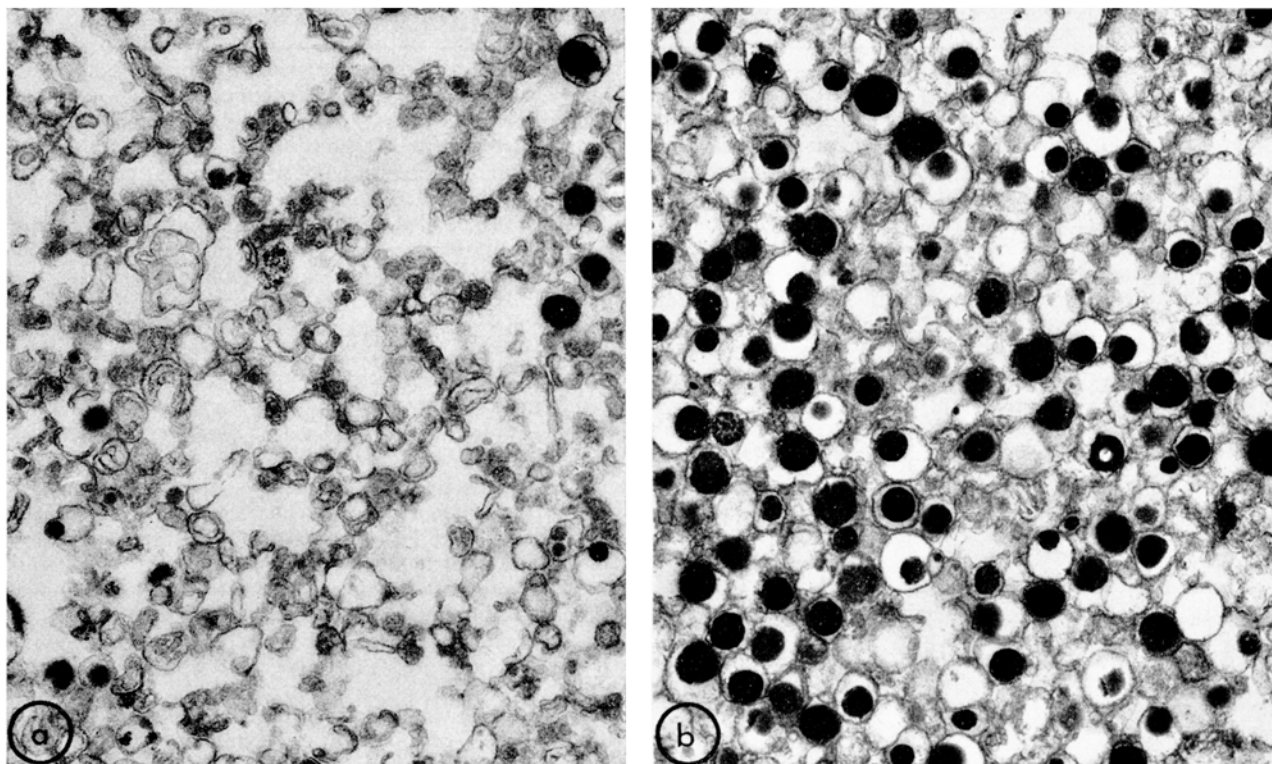


Fig. 2. Isolated 5-hydroxytryptamine organelles of rabbit platelets incubated for 30 min in modified tyrode solution. (a) with 2.2 mM  $\text{CaCl}_2$ , (b) without  $\text{CaCl}_2$ .  $\times 28,000$ .

protected from the action of this cation within the cell. It is also possible that calcium added to the incubation medium cannot penetrate in relevant amounts to the organelles of the intact platelets. Thrombin does not seem to decrease the 5HT and ATP content of the intact platelets by a direct action on the 5HT organelles. It might, however, act through structural changes of the platelet membrane which would permit a contact between the organelles and free calcium. Thus, if platelets agglutinate as especially in the presence of thrombin plus calcium<sup>6</sup>, the organelles are possibly disconnected or expelled in toto from the cell, but subsequent contact with calcium (e.g. of the plasma or the platelets) might lead or contribute to their alteration and to the liberation of 5HT and ATP. The presence of rare extracellular 5HT organelles has indeed been demonstrated in fresh experimental platelet clots in vivo<sup>16</sup>.

**Zusammenfassung.** In isolierten 5-Hydroxytryptamin-(5HT)-Organellen aus Blutplättchen von Kaninchen, im

Gegensatz zu intakten Plättchen, bewirkt Thrombin keine wesentliche Freisetzung von 5HT und Adenosin-Triphosphat (ATP). Andererseits erzeugen geringe Konzentrationen von Kalzium starke Verminderung von 5HT und ATP in den Organellen, hingegen nicht in intakten Plättchen. Nach elektronenmikroskopischen Befunden verursacht Kalzium hochgradige morphologische Veränderungen der isolierten 5HT-Organellen.

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### Determination of Plasma Volume in the Mouse with Screened Iodine-Labelled Proteins

A blood sample collected a few minutes after an i.v. injection of a labelled protein is utilized to calculate the plasma volume (PV) from the isotopic dilution. Evidently, this determination depends upon 2 factors. One is the type of protein used, e.g. albumin, transferrin,  $\gamma$ -globulins, etc. with their various distribution between vascular and extravascular spaces. The second is the time between injection and collection of the blood which differs from

30 sec to 15 min for the rat<sup>1-3</sup>. In this manner, diffusion from the blood vessels will occur more or less rapidly, depending upon the molecular weight and the time elapsed.

A third factor often not taken into account, may however play an important role, i.e. the degree of denaturation of the protein. As pointed out by REEVE<sup>4</sup>, REEVE and FRANCK<sup>5</sup>, DEWEY<sup>6</sup> and others, denatured proteins