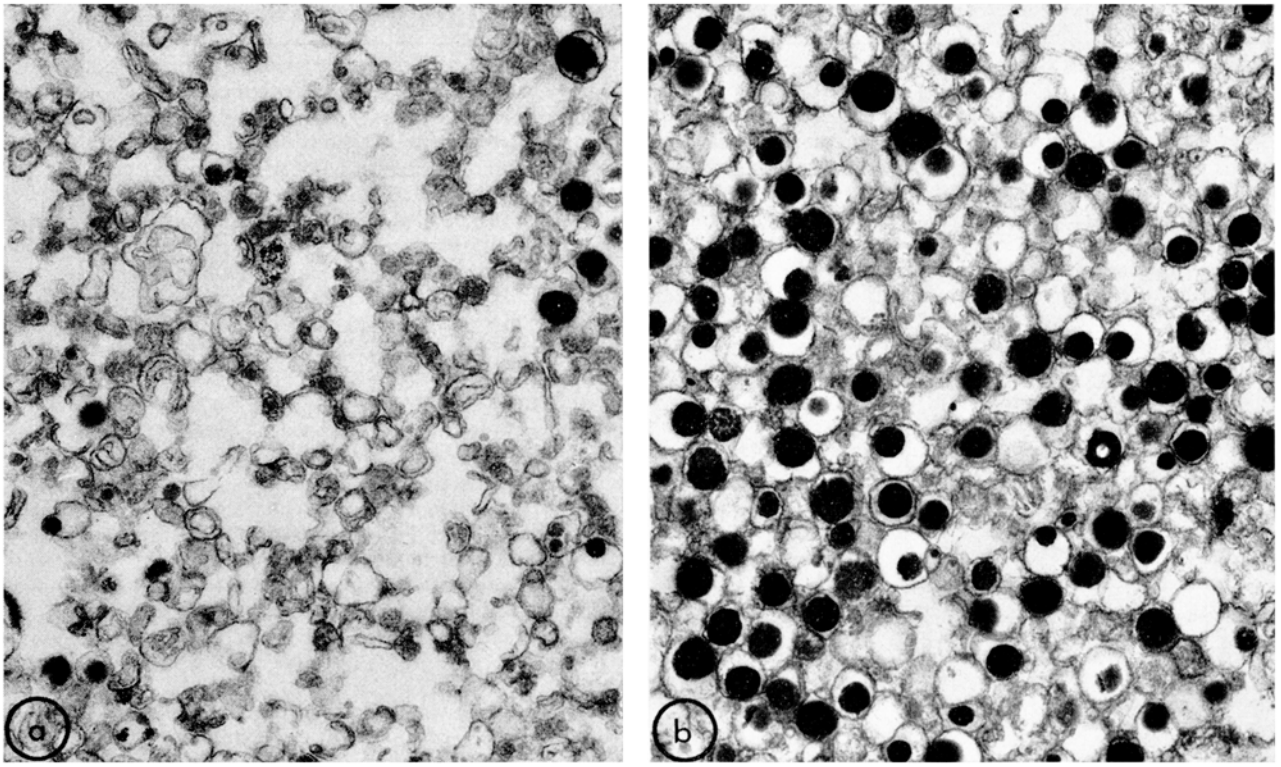

Fig. 2. Isolated 5-hydroxytryptamine organelles of rabbit platelets incubated for 30 min in modified tyrode solution. (a) with 2.2 m*M* $CaCl_2$, (b) without $CaCl_2$. × 28,000.

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[5], 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[16].

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). Anderseits 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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Forschungsabteilung der F. Hoffmann-La Roche & Co. AG, Basel (Switzerland), 3 September 1968.

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

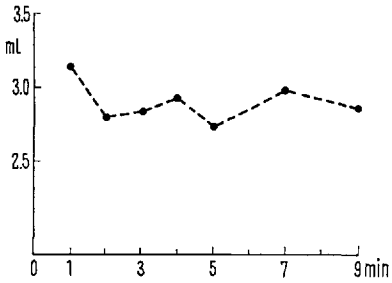
A blood sample collected a few minutes after an i.v. injection of a labelled protein is utilized to calculate the plasma volume (PV) from the isotopic dilution. Evidently, this determination depends upon 2 factors. One is the type of protein used, e.g. albumin, transferrin, γ-globulins, etc. with their 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[1–3]. 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[4], Reeve and Francks[5], Dewey[6] and others, denatured proteins

Comparison of plasma volumes obtained with different labelled proteins, screened or unscreened

Screening time (h)	Protein[a]	Iodine isotope	% free iodine	No. of mice	Time of blood collection after injection (min)	Plasma vol. ml/100 g body weight	Means and standard deviation	
0	MSA	125	–	14	2	4.04		
0	MSA	131	3.2	20	4	4.27		
0	HSA	131	2.8	4	3	3.10	4.018 (0.635)	
0	HSA	131	4.2	4	4	4.84		
0	HSA	131	0.7	9	3	3.84		
24	MSA	125	–	16	2	3.15		
29	MSA	125	3.0	24	2	3.46		3.418 (0.209)
40	MSA	131	–	30	2	3.47		
40	MSA	131	2.8	12	3	3.30	3.252 (0.272)	
40	MSA	131	–	16	4	3.71		
24	BF	131	1.5	16	2	3.18		2.976 (0.176)
40	BF	125	2.6	12	3	2.87		
40	BF	131	1.2	56	1–9	2.88		
					Significance of the difference:		$P < 0.05$	$P < 0.05$

[a] MSA, mouse seralbumine; HSA, human seralbumine; BF, bovine fibrinogen.



Plasma vol. (ml/100 g body weight) at different time intervals after injection of I^{131} labelled fibrinogen screened in vivo for 40 h. Each point represents the mean of 6–7 mice.

will be fixed in the reticuloendothelial system (RES) and this will yield excessive values for the PV.

After various attempts to determine PV in the mouse with different preparations of albumin, we have utilized bovine fibrinogen since it is commercially available and remains essentially in the blood vessels[7].

Human albumin (Cutter Laboratories), bovine fibrinogen (Sigma' type I) and mouse albumin (prepared by ethanol-trichloracetic acid fractionation and DEAE-cellulose chromatography) were labelled by the chloramine T procedure of BOCCI[8]. Free iodine was removed with Amberlite IRA-400 and the solution was injected into 4–5 mice for screening. These mice were then killed after 1–2 days. The plasma was dialysed during 1 day at 4 °C and diluted with saline to contain 0.3–1 μCi/ml of TCA precipitable activity.

BALB/c^+ mice, 2-3 months old, fed ad libitum, received an injection of 0.2 ml of the labelled protein via the tail vein. After various time intervals, the mice were either killed by decapitation or bled from the orbital plexus. Blood was mixed with heparin and the plasma counted. PV was calculated by the formula

$$PV = (\text{activity injected}/\text{activity of the plasma}) - 0.2 \text{ ml}.$$

Since there was no statistical difference between male and female PV, the results for both sexes are pooled in the Table. 2 conclusions are apparent: (1) PV determined with screened proteins differs significantly from that given by unscreened proteins; (2) PV measured with fibrinogen was consistently smaller than that obtained with albumin.

In order to check the influence of time on the determination of PV with fibrinogen, mice were killed from 1–9 min after injection of the labelled protein. The results shown in the Figure indicate that PV remains unchanged during that time[9].

Résumé. Une épuration biologique préalable («screening») des protéines marquées est indispensable pour la mesure du volume plasmatique chez la souris. La diffusion extravasculaire rapide de l'albumine gêne fortement les déterminations. Au contraire, le fibrinogène donne des résultats exacts et reproductibles.

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