Influence of Prostaglandins E_1 and E_2 on Coagulation of Rat Blood

Prostaglandin E_1 (PGE_1) inhibits in vitro both blood platelet adhesiveness (to glass) and aggregation[1], the latter process being induced by several agents, in plasma of various species[1,2]. In vivo, PGE_1 suppresses platelet thrombus formation induced by mechanical injury or electrical stimulus[2,3]. On the other hand, PGE_2 stimulates platelet aggregation in vitro, induced by ADP or thrombin[4].

We have now investigated the influence of PGE_1 and PGE_2 on the coagulation of rat blood or plasma by means of thrombelastography – to obtain information on the overall intrinsic clotting process – as well as by the 'one-stage' prothrombin time test – to gain information on the extrinsic clotting process, especially with regard to the question whether prostaglandins can interact with or replace tissue thromboplastin. Finally, the influence of PGE_1 on the calcium clotting time of plasma has been examined at various Ca-ion concentrations.

Materials and methods. Animals: adult male Wistar rats; ether-anaesthetized. Blood was collected from the jugular vein either into a siliconized test tube containing 1 vol. of 3.8% trisodium citrate. 5 aq. per 9 vol. of blood, or, for studying the effects of PGE's on thrombelastograph values in vitro, into a disposable thrombelastograph cup[5] containing 0.1 ml saline (0.85% NaCl) with various concentrations of prostaglandin. Blood was added up to a final cup content of 0.35 ml. The effect of PGE_1 in vivo was studied by injecting 0.5 ml saline containing 10 or 20 µg PGE_1 via the dorsal vein of the penis of the rats (mean body weight 237 g), 1 min before 0.35 ml blood was collected into an empty preheated thrombelastograph cup. Thrombelastography was carried out as described by HARTERT[6].

Citrated platelet-poor (cPPP) or platelet-rich plasma (cPRP) was prepared by centrifugation (30 min at 1000 *g* or 15 min at 190 *g*, respectively) of citrated blood pooled from various animals. The 'one-stage' prothrombin time was determined by the Quick procedure[7] using cPPP, which, in some cases, had to be diluted (1:1 v/v) with saline. Prostaglandin (2 µg/0.1 ml saline) was introduced instead of or in addition to thromboplastin (ex Difco). The calcium clotting time[7] of cPRP was determined using a variety of $CaCl_2$ concentrations for recalcification of the plasma.

Results and discussion. The effects of prostaglandins E on the thrombelastography values of rat blood are shown in Table I.

In vitro, neither PGE_1 nor PGE_2 interferes with the coagulation process (no differences in *r*- or *k*-values). PGE_1 however, unlike PGE_2, affects the strength of the clot formed, as the mA-values are significantly lower than the corresponding control value. A reduced mA-value suggests a deficient platelet function[8], which in addition to the results of the adhesiveness and aggregation studies[1-4] suggests a specific effect of PGE_1 on blood platelets.

Since PGE_2 stimulates platelet aggregation in vitro, it could be expected to cause an increase in the mA-value. When we consider that the controls invariably show a maximum mA-value, it is, however, understandable that our experiments do not confirm these expectations.

The failure of PGE_1 to affect the mA-value when injected i.v. may be due to a difference in the actual PGE_1 concentration in the blood between the experiments in vitro and those in vivo. PGE_1 disappears from the blood very rapidly[4,9-11], so that its concentration in the blood, sampled 1 min after i.v. injection, must have been much lower than expected. The difference in mA-value between the control in vitro and that in vivo is due to the higher degree of dilution of blood in the experiments in vitro. On the other hand, the *r*- and *k*-values are hardly influenced by the degree of dilution.

The 'one-stage' prothrombin-time test shows that PGE_1 or PGE_2, when added in addition to thromboplastin, does not affect the prothrombin time of the diluted plasma (Table II).

Table I. The effect of PGE_1 and PGE_2 added in vitro and of i.v. injected PGE_1 on the thrombelastography values of rat blood

Experiment		N	R (min)	K (min)	MA (mm)
		in vitro			
Control		43	5.9	2.1	66.9
PGE_1 (µg)	0.1	10	6.5	1.9	63.1 [a]
	0.4	11	6.2	1.8	61.0 [a]
	1.0	12	6.3	2.2	59.4 [a]
	2.0	10	6.2	2.3	57.6 [a]
PGE_2 (µg)	1.0	10	6.1	2.1	68.4
	2.0	10	5.7	2.3	64.5
		in vivo			
Control		20	5.7	1.9	70.4
PGE_1 (µg)	10	10	6.4	1.9	71.0
	20	10	5.6	1.9	69.4

N, number of animals; R, reaction time, i.e. the time needed for activation of the coagulation enzymes; K, clot-formation time; MA, maximum amplitude, which is a measure of the maximum strength of the clot. [a] Significantly different from control value ($P_2 < 0.001$).

Table II. Effect of PGE_1 and PGE_2 on 'one-stage' prothrombin time (sec), using normal and diluted cPPP of rats (n = 5)

Substance added	With thromboplastin		Thromboplastin omitted	
	Normal	Diluted	Normal	Diluted
–	13.8	15.4	–	–
Saline	–	–	60.6	81.9
2 µg PGE_1	–	16.0	59.3	85.6
2 µg PGE_2	–	15.3	64.1	80.5

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Omission of thromboplastin increases the coagulation time 4.5–5.5 times both for normal and diluted plasma. Addition of PGE_1 or PGE_2 instead of thromboplastin has no effect. We can therefore conclude that PGE_1 and PGE_2 do not interfere with thromboplastic activity and, second, that they do not possess such an activity.

The calcium clotting times of rat plasma (Table III) are not affected by PGE_1 at any of the $CaCl_2$-concentrations used for recalcification of the plasma. FERRI et al.[12], who examined the calcium clotting times of citrated rat blood dialysed against saline for 7–8 h at various $CaCl_2$-concentrations, found shorter calcium clotting times in the presence of PGE_1 (1 µg/2 ml blood) than in its absence at any of the $CaCl_2$-concentrations used. They postulate that Ca-ions can be substituted by PGE_1 in a ratio exceeding 1000:1. The discrepancy between our results and those obtained by FERRI et al.[12] can be explained by assuming that a dialysable factor – not the Ca-ions – can be substituted by PGE_1[13].

Table III. Effect of PGE_1 on calcium clotting time (sec), using cPRP of rats ($n = 6$)

Substance added	$CaCl_2$-concentration (mM)								
	25	17.5	15	13.75	12.5	11.25	10	8.75	7.5
Saline (control)	60	68	72	79	84	98	124	162	260
1 µg PGE_1	61	65	74	77	86	99	126	161	264

Zusammenfassung. Die Prostaglandine E_1 (PGE_1) und E_2 (PGE_2) haben keinen Einfluss auf die Gerinnung von Rattenblut. Im Gegensatz zu früheren Postulationen können die Ca-Ionen im Plasma nicht von PGE_1 ersetzt werden. PGE_1 beeinflusst die maximale Amplitude des Thrombelastogramms in vitro nach Zugabe von 0.3–6 µg/ml, während PGE_2 diesen Effekt nicht aufweist.

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Unsaturated Fatty Acids; Platelet-Serotonin Releasers in Tissue Extract

A factor is present in alkaline extract of mammalian tissue which releases serotonin from blood platelets[1]. Partition with organic solvents and thin-layer chromatography[2] indicated that a mixture of naturally occurring unsaturated fatty acids such as oleic and linoleic is responsible for the serotonin-releasing activity of the alkaline tissue extract and in consequence, these acids may be added to the family of serotonin releasers. In this communication, the action of partially purified tissue extract on platelet cells and isolated amine-containing granules is explored.

Methods. As reported in a previous paper[2], the active material(s) could be obtained more effectively by total lipid extraction and subsequent alkaline treatment of minced hog kidney. Purification of active principle(s) was carried out with 2 steps of thin-layer chromatography and finally the zone corresponding to mono- and di-enoic fatty acids was eluated with acetone[2]. Rabbit platelets were washed and resuspended in Tullis-Toh's solution[3]. Isolated amine-containing granules were prepared by sonication (100 W, 10 kc for 1 min) and differential centrifugation[4] and suspended in the same solution. Serotonin release from platelet cells and granules was estimated by fluorimetrical procedures[2,5].

Results and discussion. More than 90% of the platelet serotonin was released by the purified material derived from 1–2 mg original tissue per ml cell suspension fluid (ca. 10^8 cells per 4 ml) in 60 min at 37 °C. The spontaneous release of serotonin ranged from 5–10%. The action of the serotonin-releasing material of such extract was found to be markedly inhibited by addition of glucose (0.1–5.5 mM) to the incubation media and ATP (1–5 mM) had little effect.

Certain saturated fatty acids like palmitic, stearic and behenic release platelet serotonin and histamine as well and cause adhesion of the cells to the vessel wall and cell damage[6]. However, these acids require the presence of blood plasma for their platelet effect. In contrast, even when all the serotonin was released by the alkaline tissue extract no diminution in the platelet count or significant adhesion and aggregation was observed. Electron microscopy revealed morphological changes such as degranula-

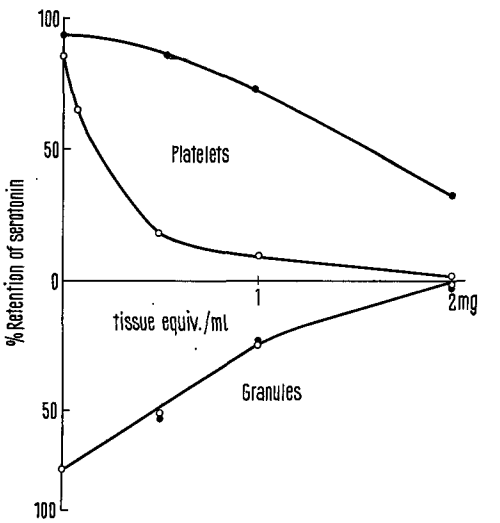

Fig. 1. Effect of the purified alkaline kidney extract on the serotonin content of platelets (top) and of granules (bottom). Incubation, with glucose (closed circle) or without glucose (open circle) in the medium, was carried out at 37 °C for 60 min and at 24 °C for 30 min, for platelets and for granules respectively.