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Blood coagulation studies in guineapigs (*Cavia porcellus*)

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Summary

Blood coagulation studies were performed on 45 healthy, adult guineapigs. Additionally thrombelastograms of 30 animals were recorded. Guineapigs revealed short partial thromboplastin times and euglobulin lysis times, but long prothrombin times and thrombin times. Fibrinogen values were within the range of human normal values. Biphasic ADP-induced aggregation of platelets, as occurs in man, was found in 29% of the animals. Short *r* (reaction time until the beginning of clot formation) and *k* times (time from the beginning of clot formation until an amplitude of 20 mm) of their thrombelastograms indicate, that whole blood clotting is enhanced in guineapigs. Higher maximum amplitudes in this species suggest a stronger clot stability than in man.

Keywords: Guineapig; Plasmatic coagulation; Platelet aggregation; Thrombelastography

Blood coagulation of healthy and ill human beings has been described in countless reports, but there is only limited information available concerning blood clotting in laboratory animals. Systematic investigations on plasma coagulation, platelet aggregation and fibrinolysis, carried out on the same group of test animals with contemporary methods of coagulation research are rare. The reports about coagulation of guineapigs deal only with simple aspects of haemostasis (Archambault & Tremblay, 1965*a*, 1965*b*; Constantine, 1966; Daver *et al.*, 1968; Mayer *et al.*, 1965, 1966; Sinaskos & Caen, 1967; Hwang & Wosilait, 1970; Macmillan & Sim, 1970) or were performed with procedures which are considered as obsolete today (Flute & Howard, 1959; Poplawski & Poplawska, 1970). For this reason we decided to carry out a study on blood clotting of guineapigs that was not restricted to plasma coagulation but also included fibrinolysis, platelet count, platelet aggregation and whole blood clotting parameters measured by thrombelastography, in order to obtain a coagulation screening profile of this species.

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Materials and methods

Forty-five conventional, clinically healthy, adult guineapigs (26 female and 19 male, mean weight 650 g) were used to perform the coagulation tests and platelet aggregation and a further group of 30 guineapigs (13 female and 17 male, mean weight 720 g) for thrombelastographic investigations. All were supplied from a closed bred colony of the same commercial breeder (Tannreuther, Hannover). They were kept in wire-mesh cages in one animal room, with 21 ± 1°C room temperature, relative humidity of 50 ± 5% and illumination from 0700 to 1900 h. Blood was obtained by percutaneous heart puncture (1 ml for thrombelastography and 5 ml for the other procedures) from animals anaesthetized by ether. It was directly withdrawn into syringes containing sodium citrate, resulting in a final blood/sodium citrate ratio of 10 : 1 after gently mixing of the samples. Plasma with signs of haemolysis was rejected in all cases and is not included in the evaluation. Platelet rich

plasma (PRP) was obtained by centrifugation with 75 *g* for 20 min and platelet poor plasma (PPP) by further centrifugation with 1000 *g* for 10 min. Platelet rich plasma (PRP) was used immediately for the measurement of ADP-induced aggregation. Plasma for the euglobulin lysis time was stored up in melting ice at 4°C for up to 2 h. Plasma for coagulation tests was frozen at −20°C for up to 5 days before use.

Plasma coagulation analysis was carried out using a coagulometer according to Schnittger and Gross for the following coagulation tests. Prothrombin time according to the method of Quick (Calcium-Thromboplastin, lyophilized thromboplastin extract from human placentas; Behringwerke AG, Marburg), partial thromboplastin time (Pathrombin, lyophilized lipid extract from human placentas and 5% kaolin suspension; Behringwerke AG), thrombin time (Test-Thrombin, lyophilized bovine thrombin 30 IU/ml; Behringwerke AG), fibrinogen according to Clauss (Multifibren, lyophilized α-thrombin from bovine plasma; Behringwerke AG) factor II- and factor V (human factor II- or factor V-deficient plasma; Behringwerke AG). All tests were carried out according to the instructions of the producer (Behringwerke AG, 1979). The principle of the coagulometer according to Schnittger and Gross is as follows: two platinum electrodes of the coagulometer plunge in a set time interval into a polystyrol test tube containing the tested plasma and the coagulation activating substance. At the point of time when a clot is formed between the electrodes a conductive contact occurs between them and the stop-watch of the coagulometer (started manually at the beginning of the test) stops automatically. The time represents the clotting activity of the plasma directly (partial thromboplastin time, thrombin time) or is converted into the percentage of clotting activity by means of a calibration curve (prothrombin time according to Quick, clotting factors). Calibration curves for prothrombin time (Quick), factor II and factor V were determined using a plasma pool of 8 healthy guineapigs. Plasma was diluted with physiological saline solution and the clotting time of a 100%, 50%, 25% and 12·5% plasma solution was determined. All coagulation values are based on double tests. Commercial available human control plasma and human fibrinogen control plasma (Behringwerke AG) served as controls.

Because precipitation did not occur in undiluted plasma, the euglobulin lysis time was modified in the following way: guineapig citrated plasma was diluted 1 : 20 and euglobulin was precipitated by means of diluted acetic acid at a pH of 5·9. The precipitate was resolved in diethyl-barbiturate-acetat buffer and its coagulation finally activated by 3 IU thrombin. All procedures until the coagulation of the euglobulin precipitate by thrombin were carried out in melting ice at +4°C, because of the known labile nature of activators of fibrinolysis (Lechner, 1982).

Platelets in PRP and whole blood were counted manually in a Neugebauer counting chamber by phase-contrast microscopy. Blood was haemolysed in 3% novocaine solution and the counting chamber was stored up in a humid chamber for 15 min. The number of platelets found in 25 squares of the counting chamber was multiplied by the dilution factor. Platelet aggregation of constantly stirred PRP was induced by 2 μl of an ADP solution (4·71 mg/50 ml; reagent from Merck, Darmstadt; final concentration: 200 μM)) in an aggregometer according to Born (Braun-Melsungen AG, Melsungen) at 37°C and the percentage of primary and secondary aggregated thrombocytes was calculated on the base of the difference of light transmission of PRP before and after aggregation. Changes in light transmission were recorded automatically. A rise in light transmission indicated aggregation while the reverse indicated disaggregation. The amount of ADP used was determined in a previous series of tests. Lower amounts of ADP produced only a weak primary aggregation, while higher amounts resulted in a superimposition of the first and second phase of aggregation in most of the guineapigs examined. The number of platelets in platelet rich plasma was not standardized by

dilution with platelet poor plasma, because the platelet poor plasma was needed for the coagulation tests. Thrombelastographic measurements of citrated whole blood were realized by means of the thrombelastograph according to Hartet after recalcification. *r* Time (reaction time until the beginning of clot formation), *k* time (time from the beginning of clot formation until an amplitude of 20 mm) and maximum amplitude of the diagram recorded by the thrombelastograph were obtained by measurement of the length of the distances indicated in Fig. 2 and calculating the coagulation times (*r* time, *k* time) based on a paper speed of the thrombelastograph of 2 mm/min.

Results

Plasmatic coagulation and fibrinolysis data are given in Table 1 as mean values, standard deviation and range of values. The prothrombin time (representing the extrinsic pathway of plasmatic coagulation) is longer in guineapigs than the partial thromboplastin time (measuring the intrinsic part of the coagulation cascade). In man the speed of the extrinsic pathway exceeds that of the intrinsic pathway (Barthels & Poliwoda, 1980). The thrombin time of the animals was considerably longer than the known human normal values (17–24 s; Behringwerke AG, 1979), but the results of fibrinogen analyses were within the range of human values (2·0–4·5 g/l; Behringwerke AG, 1979). The euglobulin lysis time ($\bar{x}$ 33·25 min) of guineapigs was short.

Table 1. Mean values, standard deviation and range of coagulation and fibrinolysis findings in guineapigs

Methods	*x*	*DS*	*range*	*n*
Prothrombin time (according to Quick)	85%[a]	17·7	50–100	45
Partial thromboplastin time	16·8 s	2·3	13·0–22·9	45
Thrombin time	35·2 s	5·3	27·5–48·0	43
Fibrinogen	2·39 g/l	0·5	1·78–4·22	45
Factor II	100%	15·3	85–150	39
Factor V	100%	19·4	68–140	39
Platelets	447·0 g/l	85	194–713	43
Euglobulin lysis time	33·25 min	19·7	15–100	40

[a]100% = 20·7 s

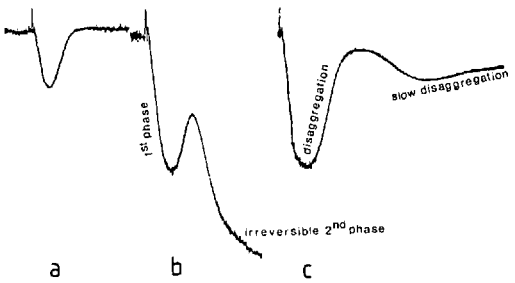

Fig. 1. ADP-induced aggregation in guineapigs: (a) primary aggregation, (b) primary aggregation followed by an irreversible second phase of aggregation, (c) biphasic aggregation with slow disaggregation after the second phase

In Fig. 1 the three observed patterns of thrombocyte aggregation in guineapigs are presented. Seventy-one per cent (27/38) of the animals showed only a single phase of aggregation (a) but 29% (11/38) revealed secondary aggregation (b). Although secondary aggregation is considered as irreversible (Dodds, 1974) 2 of 11 showed an incomplete second phase with slow disaggregation of the platelets (c). Primary aggregation resulted in clumping of 32% of the thrombocytes on the average, whereas animals with two phases of aggregation had a stronger primary aggregation of 43% and also a stronger final aggregation of 62% after the second phase (Table 2).

Table 2. Percentage of aggregated platelets after ADP-induced primary and secondary aggregation of 38 guineapigs

	x	*SD*	*n (%)*
Animals with primary aggregation	32%	14	27 (71)
Animals with primary and secondary aggregation			11 (29)
primary aggregation	43%	14	
secondary aggregation	62%	21	

If comparing the two simultaneously recorded thrombelastograms of human blood (Fig. 2, top) and guineapig blood (Fig. 2, bottom) differences in the speed of whole blood clotting become obvious. The *r* and *k* times of guineapigs (Table 3) are shorter due to a faster whole blood coagulation. Furthermore, the maximum amplitude

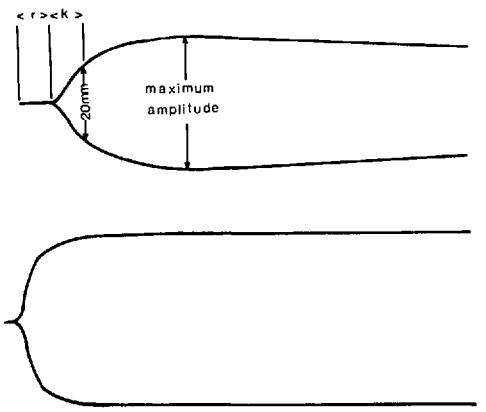

Fig. 2. Thrombelastograms of man (top) and guineapig (bottom)

is higher. In spite of an extension of thrombelastographic measurements up to 6 h in several animals, a relaxation of the clot, recognizable in man (Fig. 2) as an approach of the both thrombelastogram lines, was not visible in guineapigs.

Table 3. *r* Time, *k* time and maximum amplitude of 30 guineapigs measured by thrombelastography of recalcified citrated whole blood

	x	*SD*	*n*
r time	4·3 min	0·99	30
k time	1·8 min	0·46	30
Maximum amplitude	6·72 cm	0·59	30

Discussion

It was the aim of this study to give a survey of the clotting behaviour of guineapigs. Therefore the investigations included not only plasmatic coagulation and fibrinolysis tests but also ADP-induced platelet aggregation and whole blood clotting.

The speed of coagulation in the intrinsic system (partial thromboplastin time) is higher in guineapigs than the speed of coagulation in the extrinsic system (prothrombin time according to Quick). The extrinsic system is retarded in guineapigs probably due to a nearly complete lack of factor VII (Chenkin & Weiner, 1965; Archambault & Tremblay, 1965b; Mayer *et al.*, 1966; Daver *et al.*, 1968; Hwang & Wosilait, 1970). The high speed contact activation observed, is in accordance with findings that higher concentrations of factors of the intrinsic pathway like HMW-kininogen, prekallikrein (Saito *et al.*, 1974; Saito *et al.*, 1976), factor XI, factor V, factor VIII (Archambault & Tremblay, 1965a, 1965b; Mayer *et al.*, 1966) and factor XII (Hawkey, 1975) in guineapigs were discovered. Whether the extremely prolonged thrombin time of guineapigs has its cause in physicochemical differences of the fibrinogen molecule (Fantl & Ebbels, 1953) or in the existence of a heparin-like inhibitor of thrombin-fibrinogen interaction in the plasma of guineapigs (Beaulac *et al.*, 1968) is still unknown. Fibrinolysis (measured by the euglobulin lysis time) is a very rapid process in guineapigs as demonstrated in this study and previously (Clifton & Mootse, 1967; Donner & Houskova, 1970; Baillie & Sim, 1971). Although the normal values of the euglobulin lysis time of man vary to a great extent in the literature (Hallmann, 1980: 4–6 h; Lechner, 1982: 2–12 h; Vinazzer, 1972: 4–8 h; Sirridge & Shannon, 1983: > 90 min) depending on the method of acidification (acetic, CO_2) and the amount of thrombin used, the euglobulin lysis time of guineapigs is considerably shorter. In human blood an euglobulin lysis time, shortened to such an extent, would be interpreted as a state of accelerated fibrinolysis.

Platelets of guineapigs show an ADP-induced aggregation behaviour comparable to human thrombocytes. In both species biphasic aggregation occurs. Biphasic aggregation has not been found in other small laboratory animals (rats, rabbits), but occurs in cats and dogs (Macmillan & Sim, 1970). Therefore, guineapigs are able to serve as a suitable laboratory animal model for secondary aggregation. The degree of primary and secondary aggregation varied to a great extent between different animals. It is known, that the response of platelets to an aggregation inducing substance is influenced by the concentration of thrombocytes. In PRP with high concentrations of platelets often a stronger response is found (Born & Cross, 1963). Since the number of platelets was not standardized in

our study, we can not completely exclude an influence of platelet concentration, but the observation that strong responses (even secondary aggregations) occurred also in animals with low numbers of platelets in PRP, just as weak primary aggregations occurred in animals with high numbers of thrombocytes, suggests that the degree of aggregation in our study was mainly influenced by differences in the sensitivity of the platelets to ADP. Because the amount of ADP used was constant all the time, the differences found must be considered as the consequence of a different responsiveness to ADP in different individuals. Strain differences also seems to exist in ADP-induced platelet aggregation, because Macmillan & Sim (1970) were able to induce biphasic aggregation by ADP in rosett and piebald guineapigs, but not in an albino strain. Calkins *et al.* (1974) also failed to induce aggregation by ADP in English smooth hair guineapigs. Further investigations involving standardization and concentrating entirely on platelet function studies will be necessary to solve that problem.

Thrombelastographic measurements of whole blood revealed a faster clotting in guineapigs than in man. In contrast to the usual withdrawal of blood from a peripheral vein in humans, blood from guineapigs was obtained by heart puncture in our study. However we can assume that there is no difference between the coagulation speed of both procedures, because Kraft (1973) found equal clotting times in a thrombelastographic study on cats. The lack of clot relaxation in guineapigs may be due to a higher concentration of factor XIII (Lopaciuk *et al.*, 1978), resulting in the formation of a stronger clot and a longer duration of clot stability. To our knowledge, this is the first report on thrombelastographic measurements of recalcified guineapig citrated whole blood. Thrombelastrophic values of guineapig are dependent on the assay method used. *r* Time values measured in recalcified whole blood in our study were nearly identical with the reaction time values Fiedler & Taube (1971) found in recalcified PRP of guineapigs. The study of Chenkin *et al.* (1959) on citrated plasma revealed longer *r* times in consequence of the absence of thrombocytes in the clotting process, while the investigations of de Nicola *et al.* (1957) on native whole blood yielded shorter times for clot development because of the omission of the time consuming recalcification process in the coagulation reaction. In our opinion thrombelastograms of recalcified citrated whole blood are the most convenient method of measuring whole blood clotting by thrombelastography in small laboratory animals. Both parts of the coagulation system (thrombocytic and plasmatic) can be investigated and the blood can even be stored for a while because of the prevention of clotting by sodium citrate. The latter seems to be especially important with regard to the fast clotting of small laboratory animals native whole blood.

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