to antisera showing the highest complement fixation titre (50). Such proofs had been programmed in order to thy to serotype our isolates by means of cross reactions, but in view of the low titre remaining in only a few antisera following absorption with *Staph. aureus* Rose, no significant results were obtained.

The results concerning the inhibition of bacterial growth in the presence of rabbit antisera and of human sera are shown in Figure A and B. Microscopical examination demonstrated that the nephelometric variations were related to different rates of multiplication and not to processes of agglutination.

The data obtained indicate that the addition of rabbit immune sera and of human sera to the cultures interferred with the bacterial multiplication. The sera of the rabbits inoculated simultaneously with bacterial suspensions and immunodepressant drugs showed the same behaviour as the sera of normal rabbits. Antisera towards *Staph. epidermidis* inhibited significantly the multiplication of the same microorganism and of *Staph. aureus* Rose: the absorption on *Staph. aureus* Rose impaired the activity towards *Staph. epidermidis*. Such data are in full agreement with the results of the immunological assays previously described. The serum of the thrombocytopenic patient failed to interfere with the multiplication of the strain of *Staph. epidermidis* present within the same patient's blood; normal human serum significantly impaired the growth of this same microorganism: such activity was significantly but not completely depressed following absorption with *Staph. aureus* Rose.

From the results of the present researches the following considerations may be drawn: human subjects carry in the circulation strains of *Staph. epidermidis* with variable properties; the low immunological reactivity sometimes observed in assays carried out with human sera and rabbit antisera appears to be almost completely related to antigenic properties common to *Staph. aureus* (see [5], also for a review of the literature); the possibility may not be excluded that, within the circulating blood, a control of the multiplication of the bacterial forms under consideration derives from a non-specific immunological situation: the fact that the blood specimens from thrombocytopenic subjects showed a very high rate of growth of *Staph. epidermidis* together with a low reactivity towards *Staph. aureus* Rose might support this hypothesis. It appears that the microorganisms here considered, once they enter the host organism, become established in a state of equilibrium. The damage that can arise from the alteration of this equilibrium will be the object of a separate study.

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Lack of Platelet Factor-3 Activation After Incubation of Platelet-rich Plasma with Kaolin in the Rat[1]

F. DE CLERCK, J. GOOSSENS and M. BEERENS

Laboratory for Thrombosis Research, Janssen Pharmaceutica, Turnhoutsebaan 30, B–2340 Beerse (Belgium),
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Summary. Stypven times, measured in rat platelet-rich plasma (P. R. P.) after incubation with kaolin, did not shorten as incubation proceeded, thus reflecting the lack of development of platelet factor-3 (PF_3) availability in this test system. Repeated freezing and thawing of P. R. P. or aggregation with collagen did result in PF-3 availability. Aggregation and PF-3 availability were inhibited by the compound VK774. These findings add another aspect to the list of species differences in platelet function.

Platelet factor-3 (PF_3) a lipoprotein fraction of platelets, contributes, by complex formation, to the activation of factor X and of prothrombin in the intrinsic pathway of coagulation.

For human cells, PF-3 can be made available by incubation of platelet-rich plasma (P. R. P.) with kaolin or collagen or by repeated freezing and thawing. PF-3 availability is then reflected by a shortening of the coagulation time of plasma mixed with Russel Viper Venom (Stypven time)[2]. The present paper reports upon aberrant behaviour of rat platelets in the determination of PF-3 availability after incubation with kaolin, as measured with the Stypven time[2].

Material and methods. 1. From rats (Wistar, male, 280–320 g), anaesthetized with pentobarbital 30 mg/kg intraperitoneally and Hypnorm® (Janssen Pharmaceutica) 1 ml/kg subcutaneously, blood was drawn by syringe from the surgically exposed inferior caval vein on citrate 3.8% (1 V/9 V of blood). The preparation of platelet-rich plasma (P. R. P), platelet-poor plasma (P. P. P.) and the counting of platelets in plasma was performed as previously described[3].

2. PF-3 availability after the activation of P. R. P. with kaolin was tested as follows[2]: 0.45 ml of plasma was incubated at 37 °C with 0.05 ml of kaolin suspension (final concentrations 20, 10, 5 mg/ml) or with buffer. Before and 20 min after the addition of kaolin or buffer, 0.1 ml of the mixture was coagulated with 0.2 ml of Stypven/$CaCl_2$. In order to check the reactivity of rat plasma in the Stypven time test, 50 µl of Thrombofax® (Ortho Pharmaceutica) was added to plasma-kaolin mixture in some experiments. Kaolin (Light, B. D. H.) was suspended in barbital-HCl buffer pH 7.4. Stypven (Burroughs, Wellcome and Co) was diluted 1/100,000 in buffer, mixed with an equal volume of $CaCl_2$ 0.05 M, and was kept on crushed ice.

3. PF-3 availability after aggregation induction with rat collagen in the presence or absence of an aggregation inhibitor was tested as follows: 0.7 ml of P. R. P. was incubated for 2 min at 37 °C with 0.1 ml of solvent or inhibitor solution and 0.1 ml of a mixture of $CaCl_2$ (final concentration 1×10^{-3} M) and $MgCl_2$ (final concentration 5×10^{-4} M) before the addition of collagen suspension or buffer. The mixture was continuously stirred in an ag-

gregometer (E.E.L. 169, Evans Electroselenium). – 10–15 min after the addition of collagen or buffer, 0.1 ml of the mixture was coagulated with 0.2 ml of Stypven/$CaCl_2$. As inhibitor of platelet aggregation VK774 (Karl Thomae)[4], dissolved in 0.15 *M* NaCl pH 7.4, was used at the final concentration of 1×10^{-3} *M*.

Results. Just as for human P.R.P., the shortening of the Stypven time after freezing and thawing 3 times, and hence the PF-3 availability, is proportional to the number of platelets in P.R.P. of rats (figure). However, in contrast to human P.R.P.[2], incubation of rat P.R.P. with kaolin, over a range of concentrations, does not result in a significant shortening of the Stypven times as compared with the values after incubation with buffer (Table 1).

Table 1. Effect of incubation at 37 °C for 20 min of rat P.R.P. (800,000–1,037,163 platelets/μl) with various concentration of kaolin on PF-3 availability

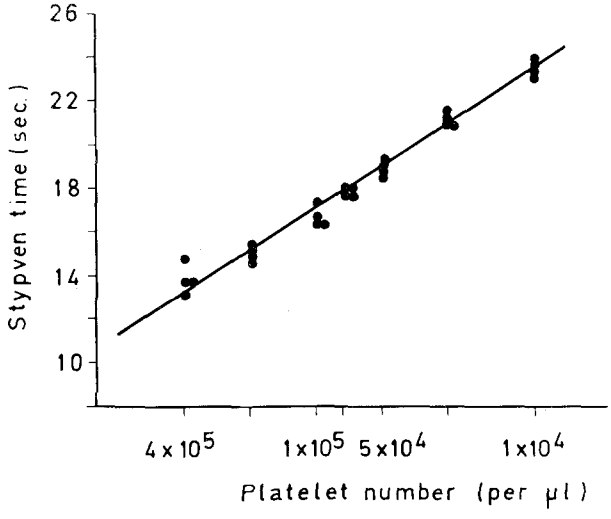
Kaolin concentration (mg/ml)[a]	Stypven time (sec)[b] before contact	after 20 min contact
20	27.3 ± 1.8	27.4 ± 2.3[c,d]
10	26.7 ± 2.9	23.8 ± 2.7[c,d]
5	26.4 ± 3.5	23.2 ± 2.4[c,d]
0 (buffer)	26.0 ± 3.6	23.7 ± 2.3[c]

[a] Final concentration of kaolin in the incubation mixture. [b] Mean ± S. D. of 4 experiments. [c] Not significant versus before contact. [d] Not significant versus buffer (Student *t*-test, two-tailed probability).

Table 2. Effect of collagen-induced aggregation of rat P.R.P. (800,000–1,037,163 platelets/μl) on the availability of PF-3.

Aggregation mixture	Stypven time (sec)[a]	Aggregation wave
A. P.R.P. + solvent without collagen	45.8 ± 7.8	Absent
B. P.R.P. + solvent + collagen	31.5 ± 5.6[b]	Strong
C. P.R.P. + VK774 1×10^{-3} *M* + collagen	44.2 ± 10.0[c]	Absent

[a] Mean ± S. D. of 3 to 5 experiments. [b] $p = 0.011$ versus A. [c] $p = 0.81$ versus A (Student *t*-test, two-tailed probability).



Relation of Stypven time to platelet number in rat P. R. P. after freezing and thawing 3 times.

On the other hand, the addition of Thrombofax (final concentration 10%) as a source of phospholipids to P.R.P.-kaolin (final concentration 10%) results in a marked, significant shortening of Stypven times at 20 min incubation (without Thrombofax 23.4 sec ± 3.15 versus 16.2 sec ± 1.2 with Thrombofax; $n = 4$; $p < 0.005$). Stypven time shortening on addition of Thrombofax is as pronounced with as without kaolin addition to P.R.P. The induction of aggregation of rat P.R.P. with collagen also results in a significant shortening of Stypven times, reflecting development of PF-3 availability during aggregation. The inhibition of aggregation by VK774 is accompanied by inhibition of PF-3 development (Table 2).

Discussion. The present study shows that rat platelets fail to develop PF-3 availability on incubation with kaolin. Lack of prolongation of Stypven times after incubation with kaolin and pronounced shortening of the Stypven time after the addition of Thrombofax, seem to exclude absorption of plasma factors or phospholipids as an explanation for the non-reactivity of rat P.R.P. in the Stypven time test. The shortening of the Stypven times after repeated freezing and thawing of P.R.P. and after aggregation with collagen indicate that, with the appropriate stimuli, PF-3 availability can develop in rat platelets.

Interesting is the finding that inhibition of collagen-induced aggregation by VK774 also results in inhibition of PF-3 availability. By this effect the drug may affect plasma coagulation, as previously suggested for its inhibitory effect on venous stasis thrombosis in the rat[5]. The former observation is not necessarily in contradiction with previous findings[2] of dissociation of PF-3 availability and platelet aggregation (mainly the release reaction phase). Indeed we showed[6] that VK774, in contrast to pure 'release inhibitors' such as suprofen (Janssen Pharmaceutica), strongly reduces the platelet retention to glass beads in rats, and also inhibits the development of PF-3 availability after kaolin activation in human P.R.P. (unpublished observation).

The fact that platelets from normal rats behave quite differently in a number of tests, as compared with human cells, has recently been stressed. DE GAETANO[7] observed that, in aggregation tests, rat platelets, in comparison with human and guinea-pig cells, are much less sensitive to collagen, to ristocetin and to some von Willebrand factor preparations. Rat platelets are also reported to adhere to tendon suspensions and to glass beads less readily than human platelets[8]. Even the primary role of the rat platelet in the very early phases of haemostasis has been questioned[9].

The present paper adds another aspect to the list of species differences in platelet function or reactivity.

[1] This study was partly supported by a grant from I. W. O. N. L.

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