Although on stained transformation smears and radiographic prints, a rare, labeled erythroid series cell was found, it is not thought that this event contributed significantly to the total cpm found in the PWM stimulated dolphin tubes. If a consistent finding, the differential PHA, PWM response deserves further investigation in greater numbers of sea mammals.


D. M. MUMFORD, G. D. STOCKMAN, P. B. BARSALSE, T. WHITMAN and J. R. WILDUR

Department of Obstetrics and Gynecology, Baylor College of Medicine, Texas Medical Center, Houston (Texas 70025, USA), and M. D. Anderson Hospital and Tumor Institute, Houston (Texas, USA), 28 October 1974.

Defective Ristocetin and Bovine Factor VIII-Induced Platelet Aggregation in Normal Rats

Recent work has indicated that high molecular weight factor VIII-related material (HMW-F-VIII) of different animal origin induces aggregation of human platelets. The identity of HMW-F-VIII with von Willebrand factor has been suggested. Rat platelets are indeed unresponsive to purified bovine HMW-F-VIII; in addition, they are not aggregated by bovine fibrinogen preparations.

Platelets from rats, in contrast to platelets from guinea-pigs, were reported to not be aggregated by a bovine fibrinogen preparation (Kabi) lately shown to contain HMW-F-VIII as the actual aggregating stimulus. The present study was undertaken to evaluate whether rat platelets are indeed unresponsive to purified bovine HMW-F-VIII and whether, in analogy with platelets from Bernard-Soulier patients, they are also refractory to ristocetin.

Blood was obtained by venipuncture from normal human volunteers and by intracardiac puncture from guinea-pigs and rats. One-tenth (v/v) 3.8% trisodium citrate was used as anticoagulant. 3 different strains of rats were used: Sprague Dawley (Charles River), Wistar (Morini) and Long Evans (Servier). The animals were separated from plasma either by gel filtration or by centrifugation-resuspension technique, repeated 4 times. In each test, the platelet number was adjusted to about 300,000/µl.

Ristocetin A (lot 3) (kindly supplied by Lundbeck & Co., Copenhagen, Denmark), containing less than 8% ristocetin B, was dissolved in isotonic saline; adenosine-5'-diphosphate (Sigma; St. Louis, Missouri, USA) and Thrombofax (batch No. 8L 118, Ortho Diagnostic; Raritan, N.J., USA) were used as previously described.

Human, rat and guinea-pig platelets were similarly aggregated by ADP (up to 10^-7 M) and Thrombofax (1/5 dilution); in contrast, 5 to 10 times more concentrated collagen suspensions were required to obtain similar changes in light transmission for rat as compared to both human and guinea-pig platelets, a finding already reported by Constantine; neither ristocetin (up to 3 mg/ml) nor bovine, porcine or human neuraminidase-treated HMW-F-VIII preparations induced rat platelet aggregation (Figure 1); no consistent differences were observed among the 3 strains of rats used.

These results indicate that the previously reported absence of rat platelet aggregation by bovine fibrinogen was, in fact, due to the lack of response of rat platelets to bovine HMW-F-VIII; this is reinforced by the observation that other preparations of HMW-F-VIII, capable of inducing human and guinea-pig platelet aggregation, failed to clump rat platelets.

Although ristocetin in high concentration is known to precipitate human fibrinogen, we were unable to detect any precipitate after addition of ristocetin (3 mg/ml) to rat PRP; however, at concentrations above 3 mg/ml, the addition of ristocetin to rat PRP provoked an immediate decrease of light transmission beyond the 0% value (PRP was calibrated at 15% transmission, taking the corresponding PPP as 100%); some granular material, resembling PPP as 100%), some granular material, resembling PPP as 100%), resembling PPP as 100%), resembling PPP as 100%), resembling PPP as 100%).

Addition of either human or guinea-pig PPP to rat PRP before ristocetin did not provoke platelet aggregation. Both gel-filtered and extensively washed rat platelets (resuspended either in rat phosphate buffer pH 7.4 or in rat serum or in human PPP) were insensitive to all HMW-F-VIII preparations (at all concentrations tested) and to ristocetin (up to 3 mg/ml); the same platelet preparations, however, were aggregated by ristocetin at a concentration of 5 mg/ml; extensively washed human platelets, resuspended in rat PPP, were normally aggregated by ristocetin. Both observations clearly indicate that a plasmatic defect can be excluded in rat and that the refractoriness to ristocetin is due to rat platelets themselves.

Recent work has shown that platelets obtained from bovine, porcine, canine and urinie species were not aggregated, in their own plasma, by ristocetin; a species specificity for this substance, at least in vitro, has been suggested; that the defective ristocetin-induced platelet aggregation would be due to some in vitro artifact, can be excluded by in vivo experiments showing that both bovine HMW-F-VIII and ristocetin induced a marked fall in platelet count in guinea-pigs, but were without effect in rats (Figure 2). Similar results have been obtained by F. Declerck (personal communication) after infusion in guinea-pigs and rats of an HMW-F-VIII-rich bovine fibrinogen preparation.

The lack of response of rat platelets to heterologous HMW-F-VIII and to ristocetin seems not to be of importance for the haemostatic mechanism of this animal species, as none of the strains studied suffered from haemorrhagic diathesis; although the question whether rats need von Willebrand factor for normal haemostasis remains unanswered, our observations add further support to the hypothesis put forward by Constantine that rat platelets are less sensitive than are platelets from other animal species, to stimuli which may be related to the in vivo formation of platelet thrombi in rat vessels. In addition, our results could be relevant for a better understanding of some human platelet disorders.

Indeed, absence of platelet aggregation by both ristocetin and bovine fibrinogen preparations (the latter being rich in HMW-F-VIII) has recently been reported in some patients affected with Bernard-Soulier syndrome; it has been suggested that Bernard-Soulier platelets, possibly due to a reduced amount of sialic acid on their membrane, are lacking a receptor for ristocetin of for a ristocetin-HMW-F-VIII (von Willebrand factor) complex. Since patients affected with Bernard-Soulier syndrome are relatively rare, rat platelets could be used as a model for future investigations on the glycoprotein composition of the platelet membrane possibly involved in bovine HMW-F-VIII- and ristocetin-induced aggregation of human platelets.

![Fig. 1](image1.png)

**Fig. 1.** In vitro platelet aggregation induced in guinea-pig PRP by bovine HMW-F-VIII (2 μg/ml) (curve A) and by ristocetin (1.5 mg/ml) (curve B). Absence of rat platelet aggregation by the above-mentioned substances (curve C). No shape change was observed in any system. The tests were performed in a Born-Michal IV Aggregation and Shape Change Monitor (Pharmacological Research, England) connected to a two-channel chart recorder (Servoscribe 2, type RF 520, Smith’s Industries Ltd, England).

![Fig. 2](image2.png)

**Fig. 2.** In vivo platelet aggregation (expressed as percent of initial platelet count) induced in guinea-pig by i.v. injection of ristocetin (20 mg/kg body weight, curve C) or of bovine HMW-F-VIII (60 μg/kg body weight, curve D). Absence of rat platelet aggregation by the above-mentioned substances (curves A and B respectively). Each curve is the mean of 2 different experiments. The method of MacKenzie et al. was followed for studies in guinea-pigs and the method of Kobayashi and Dennisheim for studies in rats.