luteinizing hormone or follicle-stimulating hormone release, and in other work also these have not proved necessary for steroid-induced receptivity (*7*). In work in our laboratory preliminary to the experiments reported here, the LRF effect was most easily detected when the amount of estrogen given was just below that necessary for increased receptivity with estrogen alone. It should also be noted that LRF-facilitated lordosis in our hypophysectomized rats was not accompanied by all the signs of maximum behavioral receptivity; our females did not hop and dart, they sometimes kicked the male even during tests where other mounts resulted in lordosis, and during prolonged tests the incidence of lordosis tended to decrease.

Luteinizing hormone–releasing factor both triggers the ovulatory discharge of luteinizing hormone and facilitates lordosis. This combination of facts may help to explain the close temporal relations between endocrine and behavioral manifestations of estrus during the normal female rat cycle. In turn, behavioral events associated with copulation are known to influence subsequent endocrine processes in the female associated with impregnation (*8*). The present findings also raise the possibility that some LRF-producing neurons project, either directly or via axon collaterals, to other neurons concerned with lordosis, as well as to the median eminence. In this regard, electrophysiological effects of LRF would be of considerable interest. Finally, these results contribute to the search for biochemical effects, linked to binding of steroid sex hormones by neurons (*9*) and to the facilitation of lordosis by steroid hormones (*10*). Steroid hormone effects on the production or release of LRF may be of relevance for mating behavior mechanisms as well as for neuroendocrine events leading to ovulation.

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Von Willebrand Factor: Dissociation from Antihemophilic Factor Procoagulant Activity

Abstract. *Factor VIII corrects both the clotting defect in hemophilia A and an abnormality of platelet aggregation in von Willebrand's disease. These two activities of factor VIII (antihemophilic factor and von Willebrand factor) are both detected in the void volume when human plasma or cryoprecipitate is chromatographed on Bio-Gel 5M under conditions of isotonic salt concentration. In contrast, antihemophilic factor procoagulant activity is detected with proteins of lower molecular weight when the chromatography is performed with a buffer containing 0.8M NaCl. In this way, the two activities of factor VIII can be dissociated. It remains to be determined whether these components are separate molecules associated as a complex of high molecular weight in plasma or whether they are subunits of a complex macromolecule.*

Factor VIII is a glycoprotein (*1, 2*) that is eluted in the void volume when normal plasma or cryoprecipitate is chromatographed on agarose gels with exclusion limits greater than 2×10^6 to 5×10^6 daltons (*1, 3, 4*).Two biological properties have been ascribed to this protein. It corrects the clotting defect in the sex-linked bleeding disorder known as hemophilia A, a property termed the antihemophilic factor (AHF). Factor VIII also corrects abnormalities of platelet function which are recognized in the autosomally inherited disorder known as von Willebrand's disease; these abnormalities are the reduced retention of platelets in glass bead filters (*5, 6*) and the defective aggregation of platelets by the antibiotic ristocetin (*7*). This property of factor VIII may be called the von Willebrand factor (VWF), and a deficiency of this factor may account for the prolonged bleeding time observed in patients with von Willebrand's disease. The finding that VWF activity is normal in hemophilia (*7*) suggests that the procoagulant (AHF) and platelet (VWF) factor activities of factor VIII are located on different subunits of the molecule, or possibly on different molecules that are associated as a complex of high molecular weight in plasma.

We examined the relation between these factors by determining the elution pattern of the VWF activity when plasma is chromatographed on Bio-Gel 5M with buffers with high salt concentration. In previous studies, we and others have shown that, under these conditions, AHF procoagulant activity is detected with proteins with a much lower molecular weight than plasma

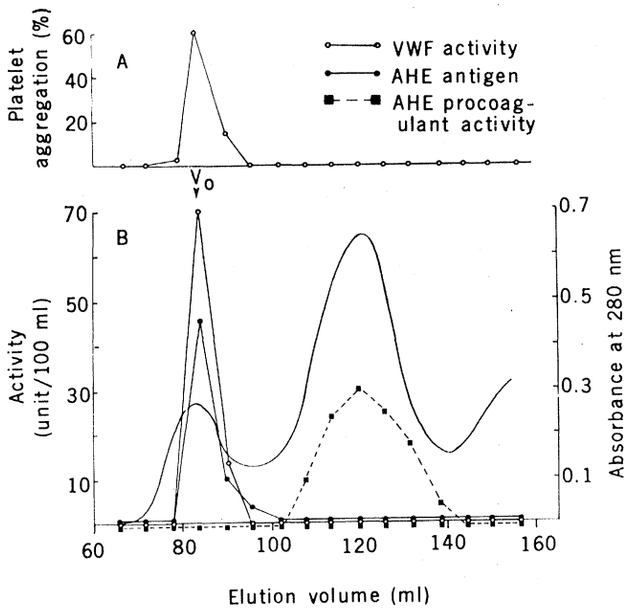

Fig. 1. Chromatography of normal human cryoprecipitate on Bio-Gel 5M with a buffer with high salt concentration. Cryoprecipitate from 30 ml of pooled human plasma was used. (A) The presence of VWF activity was detected by the capacity of the fractions to correct the abnormality of ristocetin-induced aggregation of platelets from a patient with von Willebrand's disease. (B) The VWF activity was assayed by a method (*13*) based on the observation that aggregation of washed normal platelets by ristocetin is proportional to the amount of normal plasma added. Both VWF activity and AHF antigen (*13*) were eluted at the void volume (V_0), whereas AHF procoagulant activity (*4*) was found with proteins of lower molecular weight. Under conditions of isotonic salt concentration, these three properties of factor VIII are all detected in void volume fractions (*1–7, 10, 13*).

AHF (*4, 8, 9*). This experiment was performed to determine whether VWF undergoes a similar decrease in molecular size under conditions of high salt concentration.

Glass columns (2.5 by 60 cm) were packed with Bio-Gel 5M, 200 mesh (Bio-Rad Laboratories, Richmond, California) to a gel height of 55 cm and equilibrated with a buffer containing one part of 0.253*M* imidazole, *p*H 7.3, at 4°C, and four parts of 1*M* NaCl. The void volume was determined with *Salmonella typhosa* lipopolysaccharide, molecular weight 1.5×10^8. Cryoprecipitate from 30 ml of pooled, normal human plasma (*4*), diluted to 2.0 ml with supernatant plasma, was applied to the top of the column, and the column was eluted with buffer at 18 ml/hour. All procedures were done at 4°C. Fractions were analyzed as follows: (i) Absorbance at 280 nm was measured. (ii) AHF procoagulant activity was determined by one-stage assay (*4*). Before assay, the fractions were diluted with five parts of distilled H_2O to reduce the ionic strength to that appropriate for assay of AHF. (iii) AHF antigen was measured by radioimmunoassay (*10*). (iv) The presence of VWF activity was determined in two ways after the salt concentration of the fractions was reduced to isotonicity by dialysis against isotonic saline buffered with tris(hydroxymethyl)aminomethane, *p*H 7.3. In the first method, 0.5-ml portions were added to 1.3 ml of platelet-rich plasma of a patient with von Willebrand's disease. We then added 0.1 ml of a solution of the antibiotic ristocetin (Abbott Laboratories, North Chicago, Illinois) to the mixture (final concentration, 1.5 mg/ml) and measured platelet aggregation in an aggregometer (*11*) (Fig. 1A). [In patients with von Willebrand's disease, ristocetin-induced platelet aggregation is reduced or absent (*7, 12*), and this defect is specifically corrected by factor VIII (*7*).] We also measured VWF activity in the fractions by means of a recently described quantitative assay that is based on the observation that ristocetin aggregates washed normal platelets in proportion to the amount of normal plasma added (*7, 13*).

Results are shown in Fig. 1. As previously reported, AHF activity was not detected in the void volume fractions under conditions of high salt concentration, but was present in later fractions that contain proteins of lower molecular weight (*4, 9*). By contrast, VWF activity and AHF antigen were both detected in void volume fractions. The same results were obtained in two similar experiments. The retention of AHF antigen in void volume fractions was similar to that in a previous study in which the salt concentration was increased by the use of 0.25*M* $CaCl_2$ rather than NaCl (*14*).

The effect of thrombin on the VWF activity and AHF antigen content of plasma was also strikingly different than its effect on AHF procoagulant activity. As reported previously, thrombin produced a marked increase in the AHF activity of normal plasma (*8, 15*). No such increase in VWF activity or AHF antigen occurred under these conditions (Fig. 2).

Two common hereditary bleeding disorders are associated with reduced AHF procoagulant activities. In hemophilia A, AHF procoagulant activity is

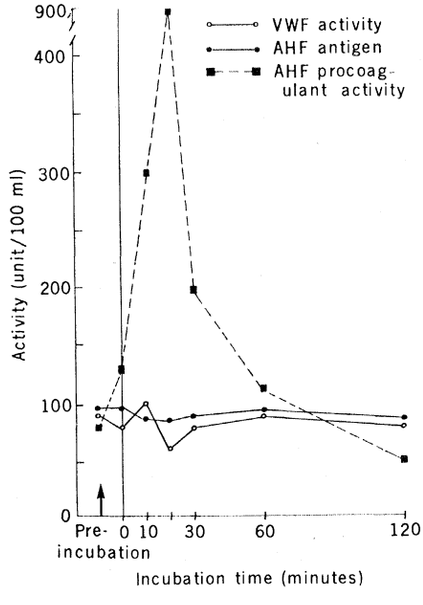

Fig. 2. Incubation of plasma with thrombin. Citrated, platelet-poor human plasma was incubated at 37°C with 1/20 volume of lipid (Platelin, Warner-Chilcott) plus 1/20 volume of bovine thrombin (Parke-Davis) (final concentration, 0.05 unit/ml). Immediately after addition of thrombin and lipid and at intervals thereafter, portions were removed for measurement of AHF procoagulant activity (*8*), VWF activity (*13*), and AHF antigen content (*10*). Preincubation indicates samples removed before thrombin and lipid were added.

reduced or absent, but there is normal or increased concentration of a protein with antigenic determinants that react with a rabbit antibody to AHF (*10, 16, 17*). In von Willebrand's disease, AHF procoagulant activity and AHF antigen are proportionally decreased (*10, 16*). In studies of AHF procoagulant activity, VWF activity, and AHF antigen, we observed a high degree of correlation ($r > .80$) between pairs of these variables in normal plasmas and in plasmas from patients with von Willebrand's disease (*13*). This correlation is further evidence that these three factors are either located on the same molecule in plasma or on separate but closely associated molecules.

The dissociation of AHF procoagulant activity from VWF activity and AHF antigen by chromatographic separations, as well as the selective effect of thrombin on AHF procoagulant activity, indicates that two separate components—one associated with AHF activity and the other with VWF activity—are present in the plasma complex that has both activities. It remains to be determined whether these components are separate molecules or whether they are subunits of a complex macromolecule. Immunologic studies demonstrated that there are common antigenic determinants on the factor VIII components of high molecular weight (VWF and AHF antigen) and low molecular weight (AHF activity) (*13*). This suggests that the intact complex is a polymer that includes repeating subunits. The VWF activity and AHF antigen (detected by immunoprecipitation by rabbit antibodies) appear to be found only in the polymeric form, whereas AHF procoagulant activity is retained by the dissociated smaller fragment. Additional studies are necessary to achieve a fuller characterization of this macromolecule.

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Xenotropic Viruses: Murine Leukemia Viruses Associated with NIH Swiss, NZB, and Other Mouse Strains

Abstract. *Murine leukemia virus activity is present in tissues from NIH Swiss and other mouse strains after cocultivation with nonvirus-yielding rat cells transformed by Harvey sarcoma virus. The resulting pseudotype sarcoma virus has the same type-specific coat as the virus previously isolated from New Zealand black (NZB) mice, and, like the NZB virus, it is unable to infect mouse cells. The results show that this NZB type virus is endogenous in other strains of mice and is xenotropic; that is, it grows only in cells foreign to the host. This is the first clear demonstration that NIH Swiss mice also carry indigenous infectious murine leukemia virus.*

A C-type virus which differs from standard murine leukemia viruses (MLV) has been isolated from New Zealand black (NZB) mice (*1*). This virus can be recovered from NZB embryo fibroblasts as well as from adult NZB tissues and does not propagate in mouse cells but does grow in rat and human cells. It rescues the murine sarcoma virus (MSV) genome from nonvirus-producing rat and hamster cells transformed by MSV and yields a pseudotype sarcoma virus that transforms rat and human cells (*1*). Although the NZB mouse cells contain the MLV group-specific (gs) antigen, the NZB virus added exogenously induces gs antigen only in rat and human cells; even at high titer, it does not produce MLV XC plaque formation (*2*) in these cells (*1*). The NZB virus contains reverse transcriptase but is not significantly neutralized by antiserums prepared against the standard murine C-type viruses [FMR (Friend, Moloney, Rauscher) and Gross AKR]; it is only neutralized by certain serums from NZB mice and by antiserums prepared by hyperimmunization of rabbits with concentrates of the NZB virus (*1*).

NIH Swiss are a random-bred colony of mice that have been used for studies with murine C-type viruses. One reason for their general use has been the failure to demonstrate the presence of an infectious indigenous C-type virus in these animals. Despite this fact, MLV gs antigen (*3*) and C-type particles (*4*) have been detected at times in their tissues. By using techniques which revealed the NZB virus, we have been able to demonstrate the presence of infectious MLV similar to the NZB virus in tissues from NIH Swiss as well as other mouse strains.

Spleen and thymus from individual mice were removed, minced, and plated in 60-mm petri dishes. Kidneys were separated from their capsules, minced, trypsinized, and plated. The NIH Swiss mice were obtained from three separate sources: Microbiological Associates, Bethesda; Dr. Arthur Furst; and Dr. Paul Arnstein. The C57/B16J and $(NZB \times C57/B16J)F_1$ were provided by Dr. Beatrice Mintz. The $(NZB \times NZW)F_1$ mice were supplied by Dr. Norman Talal. Cell cultures were grown to confluency in Eagle's minimum essential medium supplemented with 10 percent unheated fetal calf serum and 1 percent antibiotics and glutamine. At this time most of the cells were fibroblastic stromal cells, although the kidney cultures also contained several islands of epithelial cells. Cultures were trypsinized, and the cells were plated at 50 percent confluency. Simultaneously or 24 hours later, 10,000 normal rat kidney (NRK) Harvey cells were added. The NRK