were significantly changed. An increase occurred in arterial hematocrit as may occur with administration of sympathicomimetic amines. This was accompanied by a slight rise in the hemoglobin and in the oxygen content of arterial, mixed venous and coronary sinus blood. These increases in oxygen content were variable, however, and statistical significance was reached only by the increase in coronary sinus oxygen content.

It is desirable for an agent used primarily as a local vasoconstrictor in the mucous membranes of the nasopharynx and the tracheobronchial tree to have minimal cardiovascular effects. According to the present studies pseudoephedrine hydrochloride would seem to fulfill this portion of the desirable criteria.

Conclusions. 1. Pseudoephedrine hydrochloride has been administered to a group of anesthetized mongrel dogs in a dose of 1 or 2 mg/kg/minute. Systemic and coronary hemodynamics have been determined before and during its administration. 2. A minor but consistent increase occurred in systemic arterial pressure accompanied by a decrease in cardiac rate. 3. There was a slight but significant increase in hematocrit and in coronary sinus oxygen content. 4. No significant change occurred in cardiac output, coronary blood flow or other measured parameters, consequently the hemodynamic effects may be considered to be minimal.

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Heterozygosity and Homozygosity in von Willebrand's Disease.* (29938)

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Biosynthesis of the antihemophilic factor (AHF, F. VIII) in humans is clearly under the control of at least 2 genetic loci. Mutation at a locus on the X-chromosome produces classical hemophilia A, while mutation at an autosomal locus results in von Willebrand's disease (v.W.d.). In both disorders the plasma AHF activity is reduced(1). The genetic situation is complex, however, because a type of *non-reciprocal* "complementation" is observed when the mutant phenotypes are cross-transfused. Briefly, a transfusion of hemophilia A plasma apparently stimulates persons with v.W.d. to synthesize AHF, while a transfusion of v.W.d. plasma has no effect on plasma AHF activity of persons with hemophilia A(2).

The descriptions of families with v.W.d. seem to define only a single abnormal phenotype(1). The affected members appear to form a continuum, although they vary somewhat in severity of symptoms and AHF levels. Recently, we have encountered a family with v.W.d. which contains 2 mutant phenotypes responding quite differently to transfusion of hemophilia A plasma, the test now regarded as the hallmark of v.W.d.(1). The genetic relations within this family suggest that the mild bleeders are heterozygotes and the severe ones homozygotes. The differences between the abnormal phenotypes are of considerable clinical and genetic interest, but they are important chiefly because of the light they shed on the alternative hypotheses of AHF biosynthesis. In short, the differences appear to exclude the "regulatory" hypothesis but not the "combining subunit"

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hypothesis, as enunciated most recently(3).

Materials and methods. Blood was drawn for diagnostic and assay purposes by venipuncture and mixed with 3.2% sodium citrate. The 2-syringe technique and 20-gauge needles were used. *Blood for transfusion* was obtained by a plasmapheresis technique. Venipuncture was performed with 16-gauge needles and the blood drawn into plastic bags containing ACD solution. Sufficient blood was obtained from each donor, normal or hemophilic, to provide approximately 500 ml of plasma. This plasma was given to a volunteer v.W.d. recipient of the same blood type, the erythrocytes being returned to the donor. The following precautions were taken to minimize the possibility of hepatitis in recipients: 1) the prospective donors were carefully screened to exclude those with a history suggestive of past or present hepatitis, 2) the recipients were given gamma globulin (0.1 ml/kg) intramuscularly, before or immediately after the transfusion, and 3) each recipient received plasma from only one donor. After transfusion, the plasma AHF level of each recipient was determined at intervals of 8 hours until the peak value had been achieved. The peak was observed at 32 or 40 hours in most instances.

AHF assays were performed by the method of Langdell *et al*(4) using human hemophilia A plasma as substrate. The normal range is 68 to 165% of the mean with a standard deviation of ± 28%. The substrate plasma was "activated" before use with 5 mg kaolin per ml plasma for 10 minutes at 28°C, then stored at 0°C throughout the assay period. Partial thromboplastin times, bleeding times, consumption of prothrombin, and other hematologic procedures were performed as described earlier(5).

"New synthesis" of AHF was estimated as follows in persons with v.W.d. The plasma volume was determined from the I^{131} albumin space(6) at 2 points in time: before transfusion and at the peak of the plasma AHF rise after transfusion. The plasma AHF levels at these points were multiplied by the respective plasma volumes to provide a measure of "total AHF," expressed as *ml% AHF*. The difference between "total AHF" before transfusion plus "total AHF" infused and "total

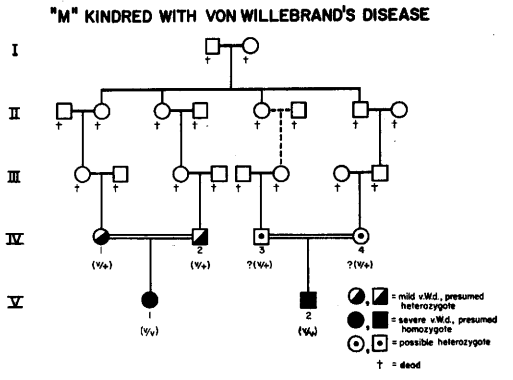

FIG. 1. The "M" kindred with v.W.d. showing genetic relationships. The presumed genotypes are indicated beneath symbols. Broken line indicates an illegal union and illegitimate issue.

AHF" at the post-transfusion peak is the measure of "new synthesis." We have described elsewhere how the extent of "new synthesis" in v.W.d. is independent of age and sex of the recipient, the pre-transfusion AHF level, and whether the donor is normal or has hemophilia A(7). During the present experiments, it was observed that reasonably accurate plasma volumes at the two critical points, (*before* and always more than 24 hours *after* transfusion) could be obtained by a simple formula based on body weight and hematocrit:

(Body weight in g) × .06 × (1- hematocrit) = plasma volume in ml.

In later experiments this formula replaced the I^{131} method because the repeated venipunctures required by the latter procedure produced more damage in bleeder subjects than was thought warranted by its greater precision.

Results. The kindred in Fig. 1 was ascertained through (V-2) a male with a severe bleeding disorder. His plasma level of AHF was too low to be titered and his bleeding time was > 30 minutes. Platelets were present in normal numbers and clot retraction was not impaired. His parents gave no history of excessive bleeding and appeared normal by laboratory test. However, 3 of his 13 siblings had exsanguinated. He was the product of a second cousin marriage. A female third cousin (V-1), equally severely affected, was also the product of a second cousin marriage; her parents were also related to both

parents of V-2 as second cousins. The parents of V-1 considered themselves to be nonbleeders, but laboratory tests showed them to be abnormal.

The crucial coagulation data are seen in Table I. The existence of v.W.d. is confirmed and the differences between parental and filial phenotypes are shown. Both parents of the female (V-1) had slightly abnormal bleeding times, borderline prothrombin consumption abnormalities, normal partial thromboplastin times, and plasma AHF at lower limit of normal. The daughter showed a high degree of abnormality by all 4 tests. The parents also gave strikingly different results than the daughter when transfused with hemophilia A plasma. During the subsequent 40 hours, the mother and father synthesized the large quantities of AHF characteristic of v.W.d. In contrast, the daughter produced very little AHF. Studies of the severely affected male (V-2) produced results remarkably similar to those of his female cousin. Tests of his parents, as mentioned above, did not produce abnormal results.

Table II summarizes our experience with "new synthesis" of AHF in the 2 presumed homozygotes of this kindred (V-1 tested twice) and compares this experience with similar results on 5 heterozygotes from other families. After adjusting the final values for the volume of plasma administered and body weight of the recipient, it appears that "new synthesis" is approximately 8 times as great in heterozygotes as in homozygotes.

Discussion. Earlier we suggested that the autosomal von Willebrand's locus might be regulatory, influencing the quantity of AHF without affecting the structure of the molecule(8). Studies of the AHF "newly synthesized" by v.W.d. patients after transfusion showed AHF which appeared normal with

TABLE I. Diagnostic Coagulation Tests and Results of Transfusion on Members of "M" Kindred Segregating for von Willebrand's disease.

	Ivy bleeding time, min	% residual prothrombin at 60 min	Partial thpln. time, sec	Plasma AHF, % control		Newly synthesized AHF, ml %
				Before transfusion	Peak after transfusion	
Normal control	1-3'	10%	60-90"	100%*	100%*	0
Mother (IV-1)	11	6	77	61	240	266,738
Father (IV-2)	8	10	71	63	120	125,113
Daughter (V-1)	>15	62	123	<5	13	13,086
Father (IV-3)	5	8	58	180	—†	—†
Mother (IV-4)	8½	7	65	104	—†	—†
Son (V-2)	>30	54	148	<5	11	11,875

* A single experiment.
† Experiments technically unsatisfactory. Subjects refuse further study.

TABLE II. Effect of Plasma Transfusions in 5 Persons Heterozygous and 3 Homozygous for v.W.d. The type of plasma infused is indicated within parentheses in second column (H = hemophilic; N = normal).

v.W.d. genotype	AHF transfused, ml %	Plasma AHF, % of control		"New" synthesis, ml %	ml % AHF synthesized/ml admin plasma/ kg body wt
		Before transfusion	Peak after transfusion		
Homozygote	2,300 (H)	<5	13	13,086	.39
"	36,210 (N)	<5	24	10,853	.32
"	3,250 (H)	<5	11	11,875	.24
Heterozygote	2,995 (H)	35	72	116,345	2.16
"	2,955 (H)	32	70	87,257	2.30
"	169,785 (N)	33	140	139,552	3.64
"	2,500 (H)	32	70	91,916	3.04
"	3,425 (H)	8	47	94,815	1.96

	Mean	Std dev	Range
5 heterozygotes	= 2.62	± .70	1.96 – 3.64
3 homozygotes	= .32	± .07	.24 – .39

respect to heat and pH stability and thromboplastin generating qualities(9). This result is consistent with a regulatory hypothesis, but closer analysis(7), showed that it is also consistent with a "combining subunit" hypothesis of AHF structure (2 structural loci) because of the physiological nature of our assays.

It occurred to us that a comparison of the extent of "new synthesis" of AHF in heterozygotes and homozygotes for v.W.d. might provide a crucial test of the regulatory hypothesis. If the only defect in v.W.d. is diminished AHF production because of reduced "effector" substance, both abnormal genotypes should respond strongly and essentially equally when given a large transfusion of hemophilia A plasma deficient in AHF but rich in the hypothetical "effector." The data presented here clearly show that persons who can be presumed to be *homozygous* for v.W.d. synthesize only 1/8 as much AHF as presumed *heterozygotes* under these conditions. This appears to mean that it is not possible to explain the defect in v.W.d. or the control of AHF biosynthesis with the "regulatory" model proposed earlier(3) and suggests that this idea can now be discarded.

Summary. Two persons believed to be homozygous for v.W.d. and their heterozygous parents were transfused with hemophilia A plasma. The parents showed extensive "new synthesis" of AHF while the children showed only 1/8 as much "new synthesis" as an unrelated group of v.W.d. heterozygotes. This result is taken to mean that the v.W.d. locus is not occupied by a regulatory gene. The result is consistent, however, with the idea that both the autosomal and the X-locus which affect AHF biosynthesis are occupied by structural genes coding sub-units of the molecule. There is still no direct evidence that the latter hypothesis is correct, however.

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Inhibition of Influenza Virus Cytotoxicity by Ammonium Ion in Commercial Medium #199. (29939)

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During studies on the cytotoxicity of influenza virus in tissue culture, variations were encountered in the susceptibility of a rabbit heart cell line to a standard, massive inoculum of influenza B (Lee) virus. The variations in viral susceptibility of the cells were traced to the presence of inhibitory concentrations of ammonium ion in 2 commercial lots of medium #199(1) from different manufacturers. The inhibitory effect of ammonium ion on influenza virus has been previously shown by several investigators(2,3,4,5).

Materials and methods. A. *Cell line.* 1. A line of cells isolated from normal rabbit myo-