Antihemophilic Factor Release by Perfused Liver and Spleen: Relationship to Hemophilia

Abstract. *As judged by perfusion of isolated organs an antihemophilic factor is produced in the liver and the spleen. Perfusates (from hemophiliacs) that are deficient in the human antihemophilic factor stimulate production of the factor during perfusion. Apparently there is an antihemophilic-factor–stimulating substance in the plasma of hemophiliacs. The data suggest that splenic homotransplantation might alleviate the symptoms of hemophilia.*

There are approximately 10,000 hemophiliacs in America today. These individuals bleed because they lack factor VIII (antihemophilic factor, AHF). Factor VIII has received considerable attention in regard to exogenous replacement and endogenous half-life (*1*). Its sites of origin, however, have been less well studied (*2*). After splenectomy patients and animals do not develop hemophilia, and it is likely that multiple compensatory sites of origin (bone marrow, spleen, liver, lymph nodes) exist. If an available, transplantable site of enduring AHF synthesis could be identified, temporary or permanent replacement of the deficiency in hemophiliacs might be possible. We have observed an increase in AHF activity in outflow samples, compared to inflow samples, of the pig liver during clinical trials of heterologous liver perfusion for overcoming hepatic failure (*3*). Several other lines of converging evidence implicate both the liver and the spleen. These organs contain abundant reticuloendothelial elements that were suggested as possible sites of AHF activity (*4*). Short-term cross-circulation between normal dogs and hemophiliac dogs temporarily maintains normal concentrations of AHF in the hemophiliac animals; splenectomy in the normal animals before cross-circulation results in decreases in AHF (*5*). In normal humans splenectomy prevents rises in AHF activity after injection of adrenalin (*6*). Extraction of various animal organs for AHF activity shows that the spleen is the most potent source (*7*). Perfusions of normal dog spleen with dog hemophilic blood results in increased amounts of AHF in the perfusate, whereas perfusion of the hemophiliac dog spleen does not (*8*) cause this increase.

Table 1. Generation of factor VIII activity by isolated perfused pig liver. Figures are averages in percent of normal human plasma standard. The numbers in parentheses are the numbers of perfusions. ROH, residual open heart.

Perfusion time (hr)	Control* (3)	Fresh homologous (2)	Heterologous	
			Fresh human (2)	ROH (7)
0	30	502	130	36
1	23	831	49	45
2	19	624	49	50
3	19	627	41	49
4	18	609	35	44

*No organ in the circuit.

Table 2. Generation of factor VIII activity in isolated perfused spleen. The results are averages in percentage of normal human plasma standard. The numbers in parentheses indicate the number of perfusions. The numbers in square brackets indicate the range.

Perfusion time (hr)	Control (4)	Autologous (2)	Homologous (2)	Heterologous	
				ROH (8)	Human hemophiliac (4)
0	22.1	206	176	8.6	5.2 [2.5–10.5]
¼*	20.9			4.9	8.6 [6–10.5]
½†	17.0			4.5	10.5 [7–12.5]
1	17.0		176	4.2	12.0 [8–15]
2	19.4	174	95	3.0	14.8 [9.5–22.5]
3	18.0		86	2.2	18 [11–28.5]
4	17.0			0.5	18 [12–30]

* Fifteen minutes; † 30 minutes.

These previous and parallel studies prompted us to undertake a series of isolated organ-perfusion experiments with various perfusates. The details of pig-liver perfusion have been reported (*9*). Pig-spleen perfusions were undertaken with somewhat similar techniques. The spleens were excised under sterile conditions from female Yorkshire or Chester white shoats (about 30 kg; 2½ months old). The animals had been treated with heparin to prevent clotting of the organ and with 1000 ml of Hartmann's solution, containing 5 percent dextrose and buffered with 10 ml of 0.6*M* tris, to improve the microcirculation of the spleen (hemodilution). After *ex vivo* cannulation of the splenic artery beneath the surface of saline (4 liters) to avoid air emboli, the specimens were rendered asanguinous with 2 to 4 liters of oxygenated, heparinized (2500 unit/liter), and buffered (3.3 ml of 0.6*M* tris per liter) Hartmann's solution containing dextrose, the pressure being maintained at 100 to 120 mm-Hg. Between excision and perfusion, there was a lapse of 15 to 20 minutes.

The asanguinous spleens were perfused in a sterile, siliconized system with fixed-phase, pulsatile, disc-oxygenated splenic arterial inflow. Open splenic venous return was collected by gravity drainage and propelled to the oxygenator by a nearly occlusive roller pump. Perfusate temperature, *p*H, pO_2, and pCO_2 were monitored and maintained within physiological limits.

Hemodiluted (the hematocrit was 20 to 30) priming volumes for liver perfusions (2000 ml) and the priming volumes for spleen perfusions (1000 ml) consisted of autologous or homologous (pig) blood or heterologous (human) fresh blood—the residual blood from open-heart surgical procedures (ROH), or AHF-deficient blood.

Samples of perfusates, taken during perfusion, were assayed for AHF activity by a modified thrombo-plastin-generation test (*10*) with standardized reagents, the standard error of assay being of ± 5.46 percent. Normal values are seen in isolated congenital deficiencies of all blood coagulation factors except AHF deficiency. Heparin in concentrations up to 5 unit/ml does not affect the result. The possibility that activity resembling that of factor VIII during

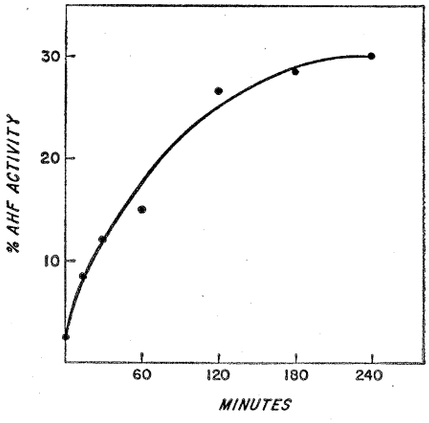

Fig. 1. Generation of AHF activity by isloated pig spleen perfused with heterologous AHF-deficient (human hemophiliac) blood.

perfusions originated from activation of plasma factors or from contamination by "tissue thromboplastin" has been explored. The assay method for factor VIII was insensitive to the addition of active factor I (activated Christmas factor-PTC′) and active factor XI (activation product); it was sensitive to "tissue thromboplastin" only in concentrations greater than 1 : 10, concentrations greatly in excess of those that might occur physiologically. The activity of factor VIII perfusate when generated with human hemophilic plasma priming volumes was destroyed by heating to 56°C for 10 minutes. These observations supported the validity of this two-stage assay as representing factor VIII in the perfusates rather than "tissue thromboplastin" or the products of early-stage coagulation reactions. The standard reference plasma, assigned an arbitrary level of 100 percent, was commercial lyophilized plasma (*11*) prepared from 50 or more donors.

The results of 35 studies are summarized in Tables 1 and 2. Before each perfusion, no AHF activity could be detected in the effluent after the specimens were flushed. In control perfusions of both liver and spleen, without specimens in the circuits, AHF activity progressively decreased. Pig livers perfused with homologous blood produced overall increases in AHF activity of the perfusate. Livers perfused with residual blood from open-heart surgical procedures and with fresh heterologous (human) priming volumes produced increases and decreases in AHF activity, respectively.

Perfusion of spleens with autologous, homologous, and heterologous (human) blood resulted in decreases in AHF activity of the perfusate. However, pig spleens perfused with heterologous (human) AHF-deficient priming volumes resulted in progressive increases in AHF activity of the perfusate, 0.7 to 12 times baseline determinations. A representative experiment with pig spleen and AHF-deficient perfusate is shown in Fig. 1. Thus, the liver and spleen are sites of release of AHF activity by synthesis or storage mechanisms, and the targets of an AHF-stimulating substance in the plasma of hemophiliacs.

Splenic homotransplantation is feasible. Five human allografts have been undertaken. After operation in one, a child with hypogammaglobulinema, evidence of transplanted splenic function was present, as indicated by a postoperative increase in serum γ-globulin (*12*). The application of whole-organ transplantation of a permanent AHF source in hemophilia is suggested. The spleen, as indicated by the data and the technical ease of procurement and transplantation, would seem suitable for this purpose.

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Murine Lymphoma: Augmented Growth in Mice with Pertussis Vaccine–Induced Lymphocytosis

Abstract. *Injection of* Bordetella pertussis *vaccine caused an excessive lymphocytosis, associated with an augmented growth of a lymphoma cell homotransplant in mice. A markedly impaired reactive cell proliferation was revealed in the spleens of the vaccine-pretreated mice after stimulation with phytohemagglutinin or Freund's complete adjuvant.*

Lymphocytes play a central role in immune response, especially in homograft rejection (*1*). Lymphopenia can be induced in animals by various procedures, namely, x-irradiation (*2*), neonatal thymectomy (*3*), cortisone treatment (*4*), thoracic duct drainage (*5*), and others. Animals rendered lymphopenic by these methods become immunologically deficient. This deficiency manifests itself not only by delayed homograft rejection (*5*, *6*) but also by enhanced carcinogenesis, viral (*7*) or chemical (*8*), and also, possibly, by enhanced growth of transplanted tumors.

A study was performed to investigate the effect of lymphocytosis on the growth of a tumor homograft. The tumor used in this experiment is an undifferentiated lymphoma induced in a female Swiss mouse inoculated with a long-term tissue culture line of the Rauscher leukemia virus (*9*). All mice used derive from a random-bred Swiss line of the Texas Inbred Mouse Company, Houston.

An extreme lymphocytosis ranging from 50,000 to 118,000 per cubic millimeter was induced in 3- to 4-week-old mice by injecting 0.3 ml of commercially available *Bordetella pertussis* vaccine (BPV) intravenously. Control mice received 0.3 ml of saline intravenously. Subcutaneous tumor inoculations were made by injecting 5000 viable lymphoma cells in the scapular area. Periodic observation was then made of the occurrence of palpable tumors and their growth; tumor size was recorded as an average of the longest diameter and the one crossing it at right angles.

It appears that the tumor is antigenic in these mice and the "take" rate of the inoculum consisting of 5000 viable cells is 50 percent in mice of the age group used. At least one antigenic marker could be attributed to leukemia virus antigens (*10*).

In the first set of experiments, lymphoma cells were inoculated at the time of maximal lymphocytosis, that is, 4 days after BPV injection. Figure 1 (left) shows the results of a typical experiment. An augmented tumor growth was manifested by earlier appearance of measurable tumors and a larger tumor size on each measurement, as compared to the control group. In a total of four such experiments, using 32 mice in the pretreated and 28 mice in the control groups, it