Synthesis of Human Plasminogen by the Liver

Abstract. *Genetic types of plasminogen were determined from a donor and a recipient before and after hepatic homotransplantation. Examination of the plasminogen types demonstrated that the liver is the principal site of synthesis of human plasminogen.*

The glycoprotein plasminogen is the zymogen form of the proteolytic enzyme plasmin (E.C. 3.4.21.7). Plasmin functions in degradation of fibrinogen and the fibrin blood clot. The site of synthesis of the plasma protein plasminogen has been the subject of controversy. Genetic typing of proteins in a liver donor and a recipient before hepatic homotransplantation and from the recipient after transplantation has established that the liver is the primary site of synthesis of haptoglobin, Gc-globulin, orosomucoid, transferrin, α_1-antitrypsin, and the complement proteins, factor B, C3, C6, and C8 (*1*).

Initially, plasminogen was localized in the eosinophilic series of granulocytes in the bone marrow by immunofluorescence (*2*). In that experiment, no plasminogen was detected in surgical biopsy specimens of liver, spleen, lymph nodes, or lung. This was interpreted to mean that plasminogen is synthesized within developing and maturing eosinophils of the bone marrow. Other investigators observed no production of plasminogen by the isolated perfused rat liver (*3*). In addition, plasminogen was detected in human granulocytes and monocytes, depending upon the maturation stage (*4*). The zymogen appeared in promyelocytes, reached highest activity in metamyelocytes and in band cells, and then gradually decreased during maturation. No differences in plasminogen content among mature neutrophils, eosinophils, and basophils were detected. Plasminogen has also been detected in red blood cells (*5*), although at a low level.

In contrast, a study of patients with abnormal liver functions revealed that 75 percent had low levels of plasminogen (*6*). In a study with rats, streptokinase was injected intravenously to deplete the circulating plasminogen (*7*). After sudden depletion, concentration of plasmin in the renal vein was twice that in the renal artery, while no arteriovenous difference was detected for the liver. These studies did not resolve whether plasminogen was synthesized de novo in the kidney or activated there. Siefring and Castellino described de novo biosynthesis of plasminogen in the anephric rat (*8*). None of these investigators examined the production of plasminogen in vivo after homotransplantation, although experiments of this type would establish the principal site of synthesis of plasma proteins.

We have delineated a common genetic polymorphism in human plasminogen by treating serum or plasma samples with neuraminidase. Genetic differences in asialo plasminogens were detected by using isoelectric focusing in polyacrylamide gel and either immunofixation or caseinolytic overlay after urokinase activation (*9*). Briefly, serum or plasma samples (7 μl) were treated with 10 μl of *Clostridium perfringens* neuraminidase (10 U/ml, *p*H 6.8) (Sigma, type VI) containing 0.005*M* EDTA. The mixture was subjected to constant dialysis against a phosphate buffer for 5 hours at room temperature. Ampholytes (LKB Instruments) ranging in *p*H from 3.5 to 10 at a final concentration of 2 percent were incorporated into 5 percent acrylamide gels with 0.2*M* taurine. Gels were polymerized with riboflavin and light. Samples of the mixture (approximately 15 μl) were applied by moistening small rectangles of Whatman No. 1 filter paper and placing them on the gel surface near the anode. Isoelectric focusing was performed for 16 to 18 hours at 450 V. After isoelectric focusing, the gels were exposed to 1 ml of urokinase at 100 U/ml (Calbiochem) and overlaid with a casein-agarose gel. The overlaid plates were then incubated for 2 to 4 hours at 37°C in a moist chamber. Plates were fixed in 10 percent trichloroacetic acid for 30 to 60 seconds and washed overnight in distilled water.

Plasminogen patterns consisting of clear proteolyzed areas in a milky casein background were immediately discernible. Two common plasminogen alleles, *PLGN*A* and *PLGN*B* (*10*), have been observed in each major race. Several rare alleles with isoelectric points more acidic than PLGN A or more basic than PLGN B have also been observed. After a plasma sample is treated with neuraminidase, each allele product resolves into two major bands. Mixtures of plasma from a person with PLGN A and from one with PLGN B mimic the pattern produced by PLGN AB plasma. A similar polymorphism has been described for plasminogen in untreated plasma (*11*). However, there is ambiguity in typing plasminogen in untreated plasma.

Plasminogen types were determined in plasma samples from a recipient of kidney homotransplantation and from his mother, the donor, before transplantation and in two samples from the

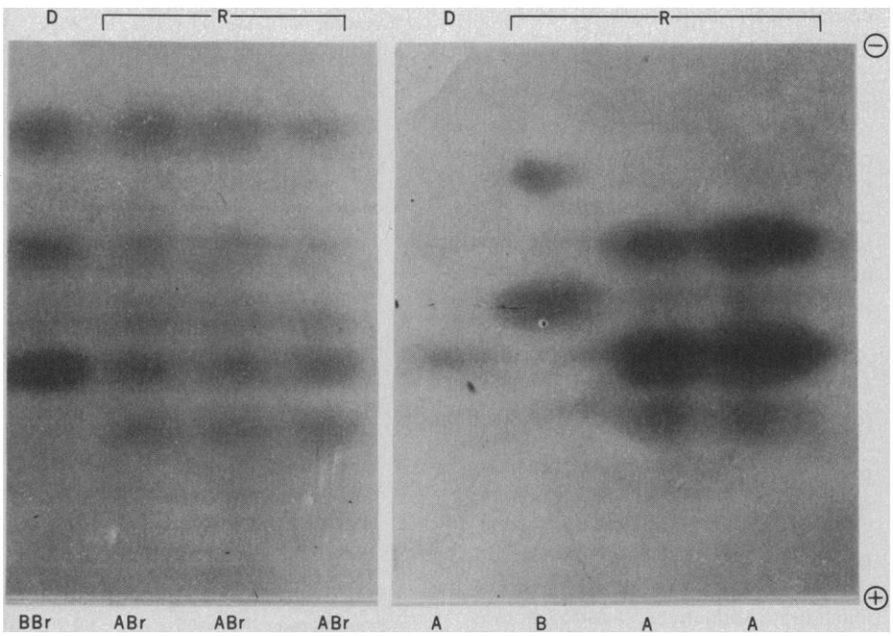

Fig. 1 (left). Plasminogen types in a donor (*D*) and recipient (*R*) before and after renal homotransplantation, demonstrated by caseinolytic overlay. From left to right: donor, recipient before transplantation, recipient 1 week after transplantation, and recipient 2 weeks after transplantation. Fig. 2 (right). Plasminogen types in a donor and recipient before and after liver homotransplantation, demonstrated by caseinolytic overlay. From left to right: donor, recipient before transplantation, recipient 1 month after transplantation, and recipient 6 months after transplantation. The specimen from the donor was obtained immediately prior to death, and it appeared to show reduced plasminogen activity at that time.

0036-8075/80/0530-1036$00.50/0

recipient after transplantation. Figure 1 shows the plasminogen type of the donor, PLGN BBr (rare), and of the recipient, PLGN ABr, before renal transplantation. The Br (B rare) pattern consists of three major bands, two of which are shared with the B variant. The recipient did not lose the two major A bands in the 2 weeks after the transplantation. Figure 2 shows the plasminogen type of a donor, PLGN A, and of the recipient, PLGN B, before hepatic transplantation. In plasma samples obtained from the recipient after the liver transplant, the plasminogen type shows a change to that of the donor.

We conclude that most, if not all, plasminogen in human plasma is synthesized in the liver. These experiments confirm that virtually all plasma proteins except for the immunoglobulins are synthesized by the liver in vivo (*12*).

DONALD RAUM
DEBORAH MARCUS, CHESTER A. ALPER
Center for Blood Research, Boston, Massachusetts 02115
RAPHAEL LEVEY
Department of Surgery, Children's Hospital Medical Center, Boston, Massachusetts 02115
PAUL D. TAYLOR
THOMAS E. STARZL
Department of Surgery, University of Colorado Medical Center, Denver 80262

References and Notes

1. T. E. Starzl, T. L. Marchioro, D. T. Rowlands, Jr., C. H. Kirkpatrick, W. E. C. Wilson, D. Rifkind, W. R. Waddell, *Ann. Surg.* **160**, 411 (1964); N. Kashiwagi, C. G. Groth, T. E. Starzl, *Proc. Soc. Exp. Biol. Med.* **128**, 247 (1968); C. A. Alper, A. M. Johnson, A. G. Birtch, F. D. Moore, *Science* **163**, 286 (1969); M. J. Hobart, P. J. Lachmann, R. Y. Calne, *J. Exp. Med.* **146**, 629 (1977); C. A. Alper, D. Raum, Z. L. Awdeh, B. H. Petersen, P. D. Taylor, T. E. Starzl, *Clin. Immunol. Immunpathol.*, in press.
2. M. I. Barnhart and J. M. Riddle, *Blood* **21**, 306 (1963).
3. R. Mattii, J. L. Ambrus, J. E. Sokal, I. Mink, *Proc. Soc. Exp. Biol. Med.* **116**, 69 (1964).
4. J. Prokopowicz, *Biochim. Biophys. Acta* **154**, 91 (1968); _____ and H. Stormorken, *Scand. J. Haematol.* **5**, 129 (1968).
5. N Sakuragawa, *Acta Haematol. Jpn.* **29**, 910 (1966).
6. R. D. Davis and R. C. Picoff, *Am. J. Clin. Pathol.* **52**, 661 (1969).
7. R. F. Highsmith and D. L. Kline, *Am. J. Physiol.* **225**, 1032 (1973); D. L. Kline and R. F. Highsmith, *Ser. Haematol.* **7**, 513 (1973).
8. G. E. Siefring, Jr., and F. J. Castellino, *J. Appl. Physiol.* **38**, 114 (1975).
9. D. Raum, D. Marcus, C. A. Alper, *Clin. Res.* **27**, 458A (1979); *Am. J. Hum. Genet.*, in press.
10. The nomenclature for genetic polymorphism of human plasminogen outlined here is designed to conform to the recently formulated international system for human gene nomenclature (B. Shows *et al.*, *Cytogenet. Cell Genet.*, in press).
11. M. J. Hobart, *Ann. Hum. Genet.* **42**, 419 (1979).
12. R. P. Witherspoon, M. S. Schanfield, R. Storb, E. D. Thomas, E. R. Giblett, *Transplantation* **26**, 407 (1978); L. L. Miller and W. F. Bale, *J. Exp. Med.* **99**, 125 (1954); L. L. Miller, C. G. Bly, W. F. Bale, *ibid.*, p. 133.
13. This work was supported by grants AI 14157, AI 13626, AM 16392, and AI 15033 from the National Institutes of Health, grant 6-183 from the National Foundation-March of Dimes, and the Charles E. Merrill Trust.

3 December 1979; revised 21 January 1980

Progesterone Administration in vivo Stimulates Release of Luteinizing Hormone–Releasing Hormone in vitro

Abstract. *The release of luteinizing hormone-releasing hormone (LHRH) from tissue from the mediobasal hypothalamic-anterior hypothalamic-preoptic area of prepuberal female rats was measured in a perifusion system. Measurements were also made of the concentrations of LHRH in these tissue fragments and of luteinizing hormone in serum obtained when the rats were killed. Four groups of immature rats were studied: intact, ovariectomized, ovariectomized and implanted with estradiol-containing capsules, and ovariectomized rats primed with estradiol and injected with progesterone. The release of LHRH from the tissue of ovariectomized animals was significantly less than that of intact females and was not modified when the ovariectomized rats received estradiol. However, there was a four- to fivefold increase in LHRH release from tissue of ovariectomized rats primed with estradiol when they were killed 6 hours after they received an injection of progesterone. The concentrations of LHRH in tissue and of luteinizing hormone in serum varied among groups and with the time of day that the animals were killed. The interactions among luteinizing hormone, gonadal steroids, and the photoperiod seem to set the appropriate conditions for neural processes triggering a complete and normal release of luteinizing hormone.*

Thirty years ago data were presented indicating a neural basis for the spontaneous release of luteinizing hormone (LH) in adult female rats (*1*). Subsequent evidence demonstrated that an estrogen-induced LH surge in female rats is synchronized with the photoperiod (*2*) and that this photoperiod-dependent LH surge occurs in immature female rats treated with steroids or gonadotropins (*3*). Furthermore, estrogen can induce LH release in intact but not in ovariectomized immature rats (*4*), whereas progesterone can facilitate the LH-releasing action of estrogen in both immature and adult rats (*5*). It has been assumed that the administration of estrogen or progesterone to immature rats trigger the release of LH-releasing hormone (LHRH) from the hypothalamus to the portal vessels and thus stimulate the release of LH from the anterior pituitary gland. This hypothesis is supported by the demonstration of increased concentrations of LHRH in the portal vessels of proestrous (*6*) and ovariectomized, steroid-treated rats (*7*). These observations prompted us to examine the release of LHRH from portions of tissue from the mediobasal hypothalamic-anterior hypothalamic-preoptic area (MBH-AHA-POA) (*8*) using a perifusion technique (*9*) and a specific radioimmunoassay for LHRH (*10*). Tissue samples were obtained from four groups of prepuberal rats killed either in the morning or afternoon of the photoperiod. We used four groups of rats: 1, intact; 2, ovariectomized; 3, ovariectomized and implanted with capsules containing 17β-estradiol; and 4, ovariectomized, implanted with 17β-estradiol, and injected with a single dose of progesterone (*11*). The effects of these hormonal manipulations in vivo on the release of LHRH from the MBH-AHA-POA tissue in vitro, on LHRH tissue concentrations, and on the concentrations of LH in serum were examined.

The release of LHRH from the tissue of ovariectomized animals was significantly less (*12*) than that of intact females and was not modified when ovariectomized rats received estradiol implants. Only the combination of estradiol implants and progesterone reinstated LHRH release to levels observed in intact females (see Fig. 1, a and b). None of the groups showed significant photoperiodic changes in LHRH release in vitro. High LHRH concentrations were measured in tissue from the rats in groups 1 and 4, with the highest LHRH values being obtained for the rats in these groups killed in the morning (see Table 1). The concentrations of LHRH from tissue of rats in groups 2 and 4 killed in the afternoon did not differ from the concentrations for rats in these groups killed in morning, but the LHRH concentrations for groups 1 and 3 were significantly reduced in rats killed in the afternoon (*13*). As expected, serum LH concentrations in the serum samples varied significantly as a function of time of death in all groups except group 2 (Table 1), with lower values being obtained from the rats killed in the morning.

These results show that hormonal manipulations in vivo in immature female rats influence (i) the concentrations of LHRH in tissue and (ii) the manner by which hypothalamic fragments perifused in vitro release this decapeptide. When compared to tissue from immature intact females, tissue from ovariectomized females showed a decrease in LHRH release in vitro, in spite of the fact that ovariectomy was followed by an in-

0036-8075/80/0530-1037$00.50/0