Further studies with this model system, including transfer of cells into enzyme-treated mice, should permit a more rational analysis of the mode of action of this enzyme as it affects various parameters of the immune mechanism, including humoral and cellular responses.

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Kidney: Primary Source of Plasminogen after Acute Depletion in the Cat

Abstract. *The kidney was the primary source of plasminogen to restore normal plasma levels, after acute plasminogen depletion was produced by injection of streptokinase in cats. The concentration of plasminogen in the hepatic vein remained below that in the artery during the time when concentrations in the artery and renal vein were returning to normal.*

Plasminogen (profibrinolysin), a plasma protein, is the precursor of the proteolytic enzyme, plasmin (fibrinolysin). The primary sites of synthesis or storage (or both) of plasminogen have not been firmly established in any species. Perfusion of isolated rat livers (*1*) yielded negative results, and use of the fluorescent antibody technique by Barnhart and Riddle (*2*) showed that plasminogen was localized in the eosinophilic granules of bone marrow cells. The extremely small number of eosinophils in the circulating blood, compared to the relatively high concentration of plasminogen in plasma, led us to look elsewhere for possible sites of plasminogen production.

Adult male cats were anesthetized with 5 percent pentobarbital sodium (0.7 ml per kilogram of body weight, intraperitoneally), and a control sample of arterial blood was obtained by left cardiac puncture. All cats were then rapidly infused through the left femoral vein with 10^6 units of streptokinase (SK) (*3*) in 3 ml of sterile saline. Streptokinase is an effective activator of plasminogen in this species, as well as in humans and other primates (*4*). Arterial and venous samples were taken within a few minutes after infusion in order to verify the depletion of circulating plasminogen. Samples were taken from some animals hourly; and the animals were given supplementary pentobarbital as needed. Some animals were permitted to recover from the anesthetic and were reanesthetized for later sampling. Arterial blood was obtained by cannulation of the left femoral artery, and venous blood was obtained by venipuncture. All samples were treated and assayed as follows. The euglobulin fraction was precipitated by 20-fold dilution of the plasma with distilled water and acidification to *p*H 5.3 with 2 percent acetic acid. The resulting precipitate was collected by centrifugation, and was suspended in 0.05*M* H_2SO_4 in a volume equal to that of the original plasma sample (*5*). After extraction for 10 minutes, the suspension was centrifuged, and the supernatant was analyzed for plasminogen and plasmin by α-caseinolytic assay at *p*H 7.4 according to the specifications of the Committee on Thrombolytic Agents (CTA) (*6*). Plasminogen activity was calculated by subtracting spontaneous plasmin activity from the total activity measured after addition of SK to the assay. Completeness of activation in the assay was verified by the use of urokinase in parallel with SK in duplicate samples obtained before and after SK infusion. To insure that plasminogen was completely precipitated in the euglobulin fraction and that true changes in plasminogen content were being measured, we used an alternate method of treating samples without precipitation of plasminogen. Acidification and neutralization of whole plasma (*7*) obtained before and after SK infusion gave the same values for circulating plasminogen as those found with the euglobulin precipitation method. Results are expressed in CTA units per milliliter of plasma.

After the infusion of SK, plasminogen in arterial blood dropped within 30 minutes from 5 CTA units per milliliter of plasma to unmeasurable levels.

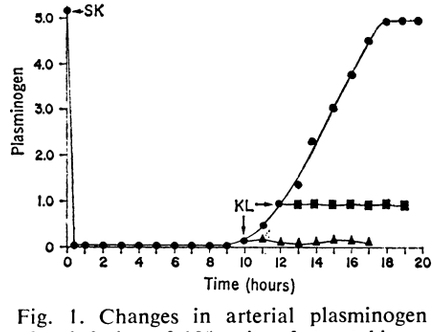

Fig. 1. Changes in arterial plasminogen after infusion of 10^6 units of streptokinase (SK). Results are expressed as CTA units per milliliter of plasma. Solid dots (●) represent the means in at least three animals. Squares (■) indicate levels in one animal after kidney vessel ligation (KL) at 12 hours. Triangles (▲) indicate levels in one animal after kidney ligation at 10 hours. Times of infusion and ligation are indicated by arrows.

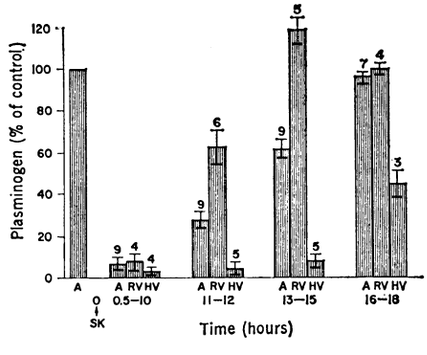

Fig. 2. Changes in arterial (A), right renal vein (RV), and hepatic vein (HV) plasminogen concentrations at different time intervals after infusion of streptokinase (SK). Results are expressed as percentage of control arterial values with mean ± standard error indicated by vertical bars. Number of animals sampled at each time period is shown above the vertical bars.

After 10 to 12 hours, plasminogen began to rise, and gradually returned to normal during the next 4 to 6 hours (Fig. 1, dots). In all animals studied, the arterial plasminogen concentrations became very low after the infusion of SK, but were completely restored within 18 hours.

Having established the time course of response to SK depletion, we compared plasminogen content, after SK infusion, of the hepatic and renal venous effluents to that in the left femoral artery. Since each cat had a different arterial concentration of plasminogen before SK infusion, each animal served as its own control (Fig. 2). Results are expressed as the percentage of arterial plasminogen before infusion. From 30 minutes to 10 hours after SK infusion, concentrations of plasminogen in the artery, hepatic vein, and renal vein all remained very low. At the beginning of restoration (11 to 12 hours after infusion), the renal vein concentration of plasminogen was double that in the artery, but that in the hepatic vein failed to rise. By 13 to 15 hours after infusion, the arterial concentration had risen to 60 percent of the control value, the renal vein concentration was 20 percent greater than the control value, but the hepatic vein concentration remained low. By 16 to 18 hours after infusion, the concentrations in the artery and renal vein had reached the control value, but the hepatic vein concentration had only reached half this value.

In another experiment, an animal was depleted of plasminogen, and both renal arteries and veins were ligated 10 hours after infusion (Fig. 1, triangles). For the next 7 hours, there was no change in the arterial concentration of plasminogen. After kidney ligation, in another animal, at 12 hours after infusion, the arterial plasminogen remained low for 7 hours (Fig. 1, squares). The arterial plasminogen level had begun to rise before ligation, a result indicating that the kidneys in this animal were indeed capable of producing plasminogen.

Spontaneous plasmin was never detectable in renal vein blood, an observation which suggests the absence of significant amounts of plasminogen activator activity in blood leaving the kidney. No difference in plasminogen content between renal artery and vein was detectable before SK infusion. After SK injection, changes in the plasminogen levels in the splenic vein and in the inferior vena cava, below the entry of the renal veins, paralleled the changes in arterial levels (data not shown).

According to our results, the kidney in the cat is the primary source of plasminogen in amounts sufficient to restore normal levels after acute depletion by SK. The 10- to 12-hour delay in plasminogen restoration has also been reported for the human (*8*), and probably does not represent plasminogen release from already existing stores. The fact that plasminogen in the hepatic vein remained low during the time when the arterial level was increasing may be an indication of the ability of the liver to store plasminogen under the conditions of our experiments.

Plasminogen levels in the human are low in a variety of hepatic disorders (*9*). These observations suggest that the liver may play a role in human plasminogen metabolism but is not necessarily involved in plasminogen synthesis. It is hazardous to extrapolate results either from acute to chronic situations or from the cat to man. We have, accordingly, measured plasminogen levels in several anephric patients. Plasminogen was detectable in the plasma of some, but not all, of these few individuals. Our data, which implicate the kidney in the restoration of plasminogen levels in the cat after acute depletion, do not exclude a possible role for other organs in the maintenance of circulating plasminogen levels in chronic situations and should not be applied to man without further substantiation.

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Cyclic Adenosine 3′,5′-Monophosphate during Glucose Repression in the Rat Liver

Abstract. *Intragastric administration of glucose inhibits the induction of serine dehydratase and tyrosine aminotransferase by glucagon in rat liver, but has no effect on the increase in hepatic adenosine 3′,5′-monophosphate resulting from administration of glucagon. Thus, glucose repression in mammalian liver, unlike catabolite repression in microorganisms, appears to operate independently of the amounts of cyclic nucleotide in the cells.*

In mammalian liver in vivo, several enzymes have been shown to be responsive to the administration of dietary protein and glucagon (*1, 2*). The administration of glucagon increases the rate of synthesis of serine dehydratase and tyrosine aminotransferase in rat liver (*1, 2*). Evidence suggests that this induction is mediated by adenosine 3′,5′-monophosphate (cyclic AMP) (*2*) and can be suppressed to a variable extent by the oral intubation of glucose (*1, 3, 4*). It has been pointed out (*5, 6*) that the "glucose effect" in mammalian liver resembles catabolite repression in bacteria. In microorganisms glucose represses the induction of β-galactosidase, while simultaneously lowering the intracellular concentration of cyclic AMP (*7*). The concentration of cyclic AMP in mammalian liver results in part from the ratio of glucagon to insulin (*8*), and it has been suggested that glucose might tend to decrease amounts of cyclic AMP in the liver by stimulating insulin secretion (*4*). In contrast, studies demonstrating that glucose prevents the cyclic AMP–independent induction of tyrosine aminotransferase and tryptophan pyrrolase by hydrocortisone (*4, 5*) indicated that cyclic nucleotides might not be involved in all mechanisms regulating levels of these enzymes. In particular the possi-