A Different Cleavage Site for High Molecular Weight Kininogen in Vivo following Intravenous Injection of Dextran Sulfate in the Rabbit

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SUMMARY. Purified radiolabeled rabbit Hageman factor, prekallikrein, and high molecular weight kininogen were used to examine Hageman factor system molecular dynamics after the intravenous injection of heparin-like dextran sulfate polymer in the rabbit. Hageman factor system proteins rapidly disappeared from the circulation following dextran sulfate injection, as measured by radial immunodiffusion, by kaolin-releasable kinin formation, and by measuring circulating levels of radiolabeled Hageman factor, prekallikrein, and high molecular weight kininogen. 125I-Hageman factor was distributed mainly to lung, liver, and spleen following dextran sulfate injection. Proteolysis of circulating 125I-Hageman factor occurred at a site within a disulfide loop into fragments of 50,000 and 30,000 molecular weight. Proteolysis of 125I-prekallikrein also occurred with visualization of a 50,000 molecular weight fragment. Although extensive proteolysis of 125I-high molecular weight kininogen was observed, the cleavage fragments were not the same as those generated during contact activation in vitro. The major fragment of high molecular weight kininogen observed in vivo was at 80,000 molecular weight, in contrast to the 65,000 molecular weight fragment generated by kallikrein in vitro. These results indicate that high molecular weight kininogen can undergo proteolysis in vivo into fragments not known to be associated with kinin release. (Circ Res 58: 595–604, 1986)

INTRAVENOUS injection of ellagic acid into dogs produces hypotension and a "hypercoagulable state" which has been attributed to Hageman factor system activation (Botti and Ratnoff, 1964). In the present study, heparin-like sulfated dextran was injected intravenously into rabbits in an attempt to measure the effect of activating the Hageman factor system in vivo. The Hageman factor system is known to be activated by dextran sulfate in vitro (Kluft, 1978). Hageman factor system activation would be expected to generate bradykinin in the vascular compartment and, therefore, to be associated with hypotension (Cochrane and Griffin, 1982; Kaplan, 1983). The heparin-like effect of dextran sulfate would be expected to inhibit procoagulant events (Walton, 1953, 1954). After dextran sulfate injection into rabbits, hypotension did indeed occur (30–45% fall in mean arterial pressure), together with bradycardia, wheezing, bowel peristalsis, micturition, and defecation (Wiggins et al., 1985). However, the hypotension observed was shown to be due to platelet release of serotonin and to be independent of the Hageman factor system. This latter fact was demonstrated by the persistence of dextran sulfate-induced hypotension in rabbits depleted of circulating Hageman factor by infusion of goat antirabbit Hageman factor immunoglobulin G (IgG), and, also, by the failure of the angiotensin-converting enzyme, SQ20881, to potentiate the hypotension observed (Wiggins et al., 1985). Because of these unexpected results, a detailed analysis of Hageman factor system protein turnover and proteolysis in the rabbit following intravenous injection of dextran sulfate was undertaken.

Methods

Hageman Factor Purification

The Hageman Factor (HF) used for these studies was purified from rabbit plasma by a previously described method (Wiggins, 1983). Briefly, citrated rabbit plasma was passed over a Sepharose 4B immunoaffinity column to which goat anti-rabbit HF IgG had been bound by the cyanogen bromide method. After extensive washing, the HF was eluted with 4 M guanidine. The guanidine-containing fractions were pooled, dialyzed, and passed over a DEAE-Sephadex column at pH 8.1. The HF was eluted from this column by a linear salt gradient. The fractions containing HF were again pooled, dialyzed, and passed over an SP-Sephadex column at pH 5.3. HF was eluted by a linear salt gradient. The peak of HF activity was pooled. The HF so purified was homogeneous as judged by analysis using sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis and a Laemmli buffer system (Laemmli, 1970) giving a single band at approximately 80,000 molecular weight under both reducing and non-reducing conditions. The HF was also homogeneous when analyzed by alkaline disc gels, according to the method of Davis (1964). The specific clotting activity in human HF-deficient plasma was 86 clotting units per mg protein.
where one clotting unit is the amount of activity present in 1 ml of normal citrated rabbit plasma (different from 1 unit of human HF). All HF assays were performed using human HF-deficient plasma.

**Prekallikrein Purification**

Prekallikrein was purified from citrated rabbit plasma as previously described (Wiggins et al., 1981). Briefly, a goat antirabbit prekallikrein Sepharose 4B immunoaffinity column was used to bind the prekallikrein in rabbit plasma. After extensive washing, the prekallikrein was eluted with 5 mM guanidine. The guanidine-containing fractions were dialyzed and passed over an SP-Sephadex column at pH 5.3. The prekallikrein was eluted with a salt gradient. The prekallikrein-containing fractions were pooled, dialyzed against 0.1 M phosphate buffer, pH 7.5, containing 1 mM NaCl, and passed over a concanavalin-A-Sepharose column. The prekallikrein was eluted with α-D-methyl glucoside. The prekallikrein so purified was a homogeneous single protein band at 83,000 molecular weight as judged by SDS-polyacrylamide gel electrophoresis under reducing and nonreducing conditions.

**High Molecular Weight Kininogen Purification**

High molecular weight kininogen was purified from citrated rabbit plasma as previously described (Wiggins et al., 1981). Briefly, a goat antirabbit kininogen Sepharose 4B column was used to bind the protein in citrated rabbit plasma. After the column was washed extensively, the high molecular weight kininogen was eluted with 4 mM guanidine. The guanidine-containing fractions were pooled, dialyzed against 0.4 mM Tris buffer, pH 8.2, and passed over a DEAE-Sephadex A50 column. A salt gradient was used to elute the kininogens from the column so that low and high molecular weight kininogens were separately eluted in the proximal and distal parts of the major protein peak, respectively. The high molecular weight kininogen-containing fractions were pooled, dialyzed against 0.1 mM acetate buffer, pH 5.3, and passed over an SP-Sephadex column. The high molecular weight kininogen was eluted with a salt gradient. The material so eluted was predominantly (about 85%) a single protein band with an apparent molecular weight of 105,000 under reducing and nonreducing conditions. Under reducing conditions, minor containment proteins or fragments appeared at 90,000 and 65,000 molecular weight.

**Kallikrein Generation**

Kallikrein was generated by incubating prekallikrein with two-chain 80,000 molecular weight-activated Hageman factor (α-HF), which had been purified as previously described (Wiggins, 1983). Prekallikrein (220 μg, 1 ml) was incubated with α-HF (50 μl, 10 μg) in the presence of 10 μl 2 mM Tris, pH 8.0, for 60 minutes at 37°C. The enzymatic activity was measured using the tripeptide substrate N-benzoyl-l-pro-l-Phe-l-arginine-pNA as a standard. The assay was sensitive to 1 ng bradykinin.

**Radial Immunodiffusion Assays**

These assays were performed according to the method of Mancini (1965). The antiserum used were raised against the purified protein in goats, and gave single precipitin lines against normal citrated rabbit plasma when tested by immunodiffusion analysis.

**Trypsin and Kaolin-releasable Kinin Measurements by Bioassay**

This was performed by a modification of the method reported by Uchida and Katori (1979). To measure total kininogen, citrated rabbit plasma (200 μl) was acidified to pH 2 by the addition of 1.8 ml 0.03 M HCl. After incubation at 37°C, the mixture was neutralized by the addition of 0.05 ml of 1 M NaOH. Trypsin (200 μg) in 200 μl of 40 mM Tris buffer, pH 7.8, was then added and incubated for 30 minutes at 37°C. Ethanol (7 ml) was then added to the mixture. After centrifugation, the supernatant was evaporated to dryness and, after reconstitution in buffer, was assayed as described below.

Kinin release from high molecular weight kininogen was measured using kaolin to activate the HF system. Citrated plasma (200 μl) was diluted with 1.8 ml 60 mM Tris buffer, pH 7.4, and mixed with O-phenanthroline at a final concentration of 2 mg/ml plasma. To this mixture was added kaolin (10 mg/ml plasma). The mixture then was incubated for 30 minutes at 37°C. Incubation was stopped by boiling the mixture for 4 minutes. The mixture was centrifuged and then was centrifuged (10,000 g) for 5 minutes, and the supernatant was stored at −70°C for assay.

The bioassay system used was an estrogen-sensitized rat uterus, as previously described (Wiggins et al., 1980). Briefly, female Brown Norway rats were injected with diethylstilbestrol (100 μg in mineral oil) 18 hours prior to assay. The uterus was removed, and a single horn was used in a constant temperature Schultz-Dale apparatus for kinin assay. Synthetic bradykinin (Sandoz Inc.) was used as a standard. The assay was sensitive to 1 ng bradykinin. The results were quantified by measuring the time from stimulus to contraction. A linear relationship was found between the log of the time between stimulus and contraction and the dose of bradykinin. Results were expressed as bradykinin equivalents in nanograms. Recoveries of bradykinin added back to O-phenanthroline-treated plasma were 66–80%.

**Radiolabeling**

Purified proteins were radiolabeled by the chloramine-T method (McConahey and Dixon, 1966). The specific activity of all radiolabeled proteins was about 1 μCi/μg. All radiolabeled proteins were assayed by adding the label to citrated rabbit plasma and then incubating the plasma with kaolin (final concentration, 5 mg kaolin/ml plasma) for 20 minutes at 37°C. The radioactive profiles of the protein were then analyzed by SDS-polyacrylamide gel electrophoresis under reducing and nonreducing conditions. In all cases prior to incubation with kaolin, most of the radioactivity (more than 90% for 125I-HF and 131I-prekallikrein and more than 74% in the case of 131I-high molecular weight kininogen) was in a single major peak corresponding to the molecular weight of the native protein, as can be seen in the gel profiles in Figures 4 and 7). After incubation with kaolin and analysis under reducing conditions, the radiolabeled proteins underwent proteolysis into fragments associated with activation. In the case of 125I-Hageman factor, both the 50,000 and 30,000 molecular weight fragments were labeled, although most of the radioactivity was in the light chain. In the case of 125I-prekallikrein, only the 50,000 molecular weight heavy chain was consistently labeled. In the case of 131I-high...
molecular weight kininogen, only the 65,000 molecular weight heavy chain was labeled.

**Animal Studies**

All studies were performed on 2- to 2.5-kg rabbits anesthetized with sodium pentobarbitone (10-15 mg/kg). The anesthetized rabbits were placed supine on boards, and femoral arterial and venous catheters (Intramedic PE 50 tubing) were introduced under local anesthetic. Urinary catheters were also placed so that radioactive urine could be collected safely. Systemic arterial pressure was measured with a Statham pressure transducer (P37B) and a Statham SP1400 blood pressure monitor. Radiolabeled proteins, either as the pure protein or as citrated plasma (3-8 ml) from the rabbits used to screen the label, as described below, was injected into each rabbit via the marginal ear vein. Labeled proteins were allowed to equilibrate for 60-180 minutes. Venous blood samples (1.8 ml) were collected at various time intervals (as described in the Results) into 200 μl of 3.8% sodium citrate in a plastic tube. After mixing, the tubes were immediately centrifuged at 10,000 g for 5 minutes. The plasma was removed with a plastic pipette and aliquotted into plastic tubes for storage at -70°C for subsequent polyacrylamide gel electrophoresis SDS-PAGE analysis. One hundred microliters of glycerol (3%) and bromophenol blue were added to each sample before layering on the top of the stacking gel. Gels were run at 2 mA/gel overnight.

To maximize the amount of radioactivity available for analysis, large (1.5 cm × 10 cm) 10% polyacrylamide gels were used. These gels give good resolution with as much as 5 mg of protein loaded. A Laemmli buffer system was used (Laemmli, 1970). The gels were run in a Bio-Rad electrophoretic system (Bio-Rad Laboratories). One hundred microliters of glyceroaldehyde (3%) and bromphenol blue were added to each sample before layering on the top of the stacking gel. Gels were run at 2 mA/gel overnight. The completed gel, including stacking gel, was removed from the glass tube, rolled up in a piece of parafilm, and frozen at -30°C. The frozen gel was allowed to thaw slightly, and then the stacking gel was sliced off (slice 1). The rest of the gel was sliced into 2-mm slices which were each put in a glass tube. All slices were counted for radioactivity for 5 min/slice, in an automatic γ-counter. Each slice was then corrected for 125I-cross-over, and the gel profile was plotted on graph paper.

**SDS-Polyacrylamide Gel Analysis of Labeled Protein in Plasma and Tissue**

To study the degradation of 123I-prekallikrein in tissues, each rabbit received 40 μCi of 123I-prekallikrein and 8 μCi (approximately 8 μg) of 125I-prekallikrein and 8 μCi (approximately 8 μg) of 125I-high molecular weight kininogen. The labeled proteins were allowed to circulate for 3 hours before injection of dextran sulfate.
then centrifuged to pellet the nonsolubilized material. In four experiments, 87, 83, 89, and 92% of the 125I-HF and 100, 92, 93, and 100% of the 131I-albumin was in the nonpelleted material. The amount of radioactivity from tissues loaded onto each gel varied between 5,000 and 22,000 counts/min. Radioactivity profiles could be obtained with as little as 5,000 counts/min loaded onto each gel.

Plasma samples (50 μl) were boiled with 50 μl of 10% SDS containing 8 M urea and 5% β-mercaptoethanol. As positive controls to act as internal standards for activation fragments, plasma samples obtained prior to injection of dextran sulfate were preincubated with kaolin (5 mg/ml) or dextran sulfate (500 μg/ml) for 20 minutes at 37°C and 4°C, respectively, prior to analysis. The radioactivity in the pellet was counted in a 7-counter. For the turnover experiments, the data for each animal were calculated as the percent change from a first plasma sample.

### Statistics

The data in Table 1 were compared using the t-test and were expressed as the mean plus or minus the standard error of the mean. For all other diagrams, data are expressed as the mean ± the standard error of the mean.

### Results

#### Levels of Circulating Proteins

Radial immunodiffusion analysis was used to measure the amounts of HF, prekallikrein, Factor XL, C3, plasminogen, fibrinogen, and IgG in plasma after intravenous injection of dextran sulfate. The results are shown in Figure 1. Immediately after injection of dextran sulfate, a decrease in antigen levels of Hageman factor system proteins (HF, prekallikrein, and Factor XI) was observed, while not much change occurred in C3, plasminogen, fibrinogen, or IgG.

### Measurement of Trypsin and Kaolin-releasable Kinin

An estrogen-sensitized rat uterus bioassay was used to measure kinin release from plasma obtained at various times after injection of dextran sulfate. Total releasable kinin was measured by incubating trypsin with plasma (see Methods). Hageman factor system-dependent kinin release was measured by preincubating plasma samples with kaolin (see Methods). The results are shown in Figure 2. Immediately after injection of dextran sulfate, kaolin-releasable bradykinin became undetectable. Trypsin-releasable kinin fell by about 15%, although this change was not statistically significant. Since the kaolin assay requires an intact Hageman factor system for kinin release, the failure to measure kinin following incubation of plasma with kaolin could be due to decrease or inactivated Hageman factor, prekallikrein, or high molecular weight kininogen, or a combination of the above.

### Turnover and Distribution of 125I-HF after Dextran Sulfate Injection

Circulating 125I-HF was measured in two groups of three rabbits. One group received dextran sulfate (20 mg/kg), intravenously. As shown in Figure 3, there was an immediate fall in circulating 125I-HF level by 28% 3 minutes after dextran sulfate injection. Analysis of the circulating 125I-HF by SDS-polyacrylamide gel electrophoresis is shown in Figure 4. Before injection of dextran sulfate, a single peak of radioactivity at 80,000 molecular weight was present under both reducing and nonreducing conditions.

### Table 1

Distribution of 125I-HF in Tissues at Various Times after Intravenous Injection of Dextran Sulfate

<table>
<thead>
<tr>
<th>Tissue</th>
<th>5 Min</th>
<th>20 Min</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DxSO4</td>
<td>Control</td>
</tr>
<tr>
<td>Lung</td>
<td>7.8 ± 5.7</td>
<td>0.3 ± 0.3</td>
</tr>
<tr>
<td>Liver</td>
<td>2.4 ± 1.0</td>
<td>0.4 ± 0.3</td>
</tr>
<tr>
<td>Spleen</td>
<td>3.2 ± 1.3</td>
<td>0.6 ± 0.1</td>
</tr>
<tr>
<td>Kidney</td>
<td>1.6 ± 0.3</td>
<td>0.7 ± 0.1</td>
</tr>
<tr>
<td>Heart</td>
<td>1.9 ± 1.4</td>
<td>0.6 ± 0.1</td>
</tr>
<tr>
<td>Skin</td>
<td>0.8 ± 0.4</td>
<td>0.1 ± 0.1</td>
</tr>
<tr>
<td>Muscle</td>
<td>0.2 ± 0.3</td>
<td>0.3 ± 0.1</td>
</tr>
<tr>
<td>Fat</td>
<td>0.2 ± 0.1</td>
<td>0.1 ± 0.1</td>
</tr>
<tr>
<td>Stomach</td>
<td>0.2 ± 0.3</td>
<td>0.2 ± 0.3</td>
</tr>
<tr>
<td>Small bowel</td>
<td>0.3 ± 0.1</td>
<td>0.6 ± 0.5</td>
</tr>
<tr>
<td>Large bowel</td>
<td>0.4 ± 0.4</td>
<td>0.2 ± 0.4</td>
</tr>
</tbody>
</table>

Data expressed in μg HF/g tissue calculated assuming that each rabbit had a circulating HF level of 12 μg/ml before injection of dextran sulfate, as described in Methods. Data are expressed as mean ± SEM. ND = not done. NS = P > 0.05.
conditions (Fig. 4). After dextran sulfate injection, peaks of radioactivity at approximately 30,000 and 50,000 molecular weight were present under reducing conditions similar to those seen when the control plasma (obtained prior to injection of dextran sulfate) was incubated with dextran sulfate in vitro (Fig. 4). A comparison of the profile obtained under reducing and nonreducing conditions shows that, 5 minutes after dextran sulfate injection, more proteolysis was seen in the presence of reducing agent. This result is similar to that of the positive control (dextran sulfate incubated with control plasma in vitro), and indicates that proteolysis of 125I-HF occurred at a site within a disulfide loop. This cleavage site is the one associated with activation of the HF molecule (Cochrane and Griffin, 1982; Dunn and Kaplan, 1982). The additional high molecular weight peak of radioactivity seen when dextran sulfate was incubated with plasma in vitro probably represents an enzyme-inhibitor complex, which may be rapidly removed from the circulation in vivo.

### 125I-Hageman Factor Tissue Distribution after Dextran Sulfate Injection

Two distribution studies were performed to determine where the 125I-HF had gone, following dextran sulfate injection. The tissue distribution of 125I-HF was studied 5 and 20 minutes after dextran sulfate injection. For these studies, 131I-rabbit albumin was injected 3 minutes before sacrifice to act as a blood marker. Two groups of three rabbits were used for each study. One group received dextran sulfate, while the other acted as a control. The results are shown in Table 1. The values given are calculated in μg HF per g tissue for different organs, assuming that the circulating plasma HF level prior to dextran sulfate injection was 12 μg/ml (personal observation...
Turnover and Proteolysis of $^{125}$-Prekallikrein

When dextran sulfate was injected into rabbits with circulating $^{125}$-prekallikrein, there was a rapid disappearance of about 22% of $^{125}$-prekallikrein from the circulation (Fig. 6, top panel). When plasma obtained at various times after injection of dextran sulfate was analyzed by SDS-polyacrylamide gel electrophoresis in the presence of reducing agent, proteolysis of the $^{125}$-prekallikrein into a major peak at about 50,000 molecular weight was seen (Fig. 7). The smaller 30,000 molecular weight fragment was not seen, because no label was bound to this part of the molecule (see Methods). The extent of proteolysis in relation to time after injection of dextran sulfate is shown in Figure 6, bottom panel. The proportion of circulating $^{125}$-prekallikrein molecules which had undergone proteolysis reached a maximum of about 30% after 1 hour and remained at this level for a further hour. These results are compatible with the concept that activation of the HF
system occurred after intravenous injection of dextran sulfate.

**Turnover and Proteolysis of \(^{131}I\)-High Molecular Weight Kininogen**

Turnover and proteolysis of circulating \(^{131}I\)-high molecular weight kininogen was examined in the same animals as those described above containing \(^{125}I\)-prekallikrein. After intravenous injection of dextran sulfate (20 mg/kg), there was a rapid fall in the level of \(^{131}I\)-high molecular weight kininogen similar to that seen for \(^{125}I\)-prekallikrein (Fig. 6, to panel). When proteolysis was examined and expressed as proportion of molecules remaining at 105,000 molecular weight, as much as 53% of molecules had undergone proteolysis by 5 minutes. However, as shown in Figure 7, the cleavage products were different from those seen when contact activation occurs. The major fragment seen after activation by kallikrein was at approximately 65,000 molecular weight. This is illustrated in vitro on the right-hand side of Figure 7, where the \(^{131}I\)-high molecular weight kininogen (and \(^{125}I\)-prekallikrein) was incubated either with kallikrein alone or when kallikrein was added to the plasma prepared from blood removed immediately prior to dextran sulfate injection (and which therefore contained both \(^{131}I\)-high molecular weight kininogen and \(^{125}I\)-prekallikrein). In both cases, the major fragment of \(^{131}I\)-high molecular weight kininogen seen was at about 65,000 molecular weight (between native prekallikrein at 80,000 dalton and the 50,000 molecular weight cleavage fragment of prekallikrein). In contrast, the major cleavage fragment of \(^{131}I\)-high molecular weight seen in vivo coincided with the native prekallikrein peak at about 80,000 dalton. Only a small peak of radioactivity was seen at 65,000 molecular weight. This result was reproducible in the six rabbits studied. Nonreduced gels were not analyzed in this experiment. Since only the heavy chain portion of the high molecular weight kininogen molecule was labeled (see cleavage patterns on the right side of Fig. 7), and assuming that the label was distributed at several sites on the heavy chain, we can conclude that cleavage of most of the \(^{131}I\)-high molecular weight kininogen molecules probably occurred within the light chain region of the molecule. Furthermore, since kinin release is associated with proteolysis into the 65,000 and 40,000 molecular weight fragments of high molecular weight kininogen, it seems likely that kinin release was not associated with the cleavage resulting in production of the 80,000 molecular weight fragment.

**Discussion**

The surprising finding in this study was that the \(^{131}I\)-high molecular weight kininogen molecules had undergone proteolysis to form an 80,000 molecular weight fragment. There is no doubt that this did in fact occur, because the \(^{125}I\)-prekallikrein, which was circulating in the same animals, provided an internal marker in the SDS-polyacrylamide gels. The question for discussion is therefore how one interprets this result.

Plasma kallikrein normally cleaves high molecular weight kininogen into fragments of 65,000 and 45,000 dalton (Thompson et al., 1978; Kerbiriou and Griffin, 1979). Cleavage at this site is associated with bradykinin release. The 80,000 molecular weight fragmentation is not consistent with bradykinin release. We can therefore conclude that if all the kininogen molecules were cleaved at this site, then bradykinin-mediated events, such as hypotension, would not be expected. In fact acute hypotension was observed following dextran sulfate injection, but the hypotension was shown to be independent of Hageman factor, and was not potentiated by the angiotensin-converting enzyme inhibitor, SQ20881. The dextran sulfate-mediated acute hypotension was shown to be mediated by platelet serotonin release via an autonomic reflex (Wiggins et al.,1985). Therefore, the finding that the kininogen molecules were cleaved at a non-kallikrein site fits in with the observation that the hypotension was not kinin-related.

Proteolysis of the kininogen molecule at a non-kallikrein cleavage site presumably occurred as a result of enzymatic activity generated either in
FIGURE 7. SDS-polyacrylamide gels analyzed under reducing conditions showing proteolytic cleavage patterns of $^{125}$I-prekallikrein (---) and $^{131}$I-high molecular weight kininogen (-----) from rabbits injected with dextran sulfate in vivo (left side) or plasma incubated with dextran sulfate in vitro (right side). In control plasma, both $^{125}$I-prekallikrein and $^{131}$I-high molecular weight kininogen are present as single peaks of radioactivity corresponding to 80,000 and 105,000 molecular weight, respectively (top panels of both left and right sides). The in vitro addition of purified kallikrein (50 ng) to $^{125}$I-prekallikrein and $^{131}$I-high molecular weight kininogen in 0.1 M Tris buffer, pH 7.4, containing bovine serum albumin (1 mg/ml) and incubation for 10 minutes at 37°C resulted in cleavage of the $^{131}$I-high molecular weight kininogen molecule and formation of a major peak at 65,000 molecular weight without change in the $^{125}$I-prekallikrein peak (middle frame, right side). If kallikrein was added to plasma containing $^{125}$I-prekallikrein and $^{131}$I-high molecular weight kininogen, then proteolysis of both $^{125}$I-prekallikrein and $^{131}$I-high molecular weight kininogen occurred, with the major cleavage fragments being at 50,000 and 65,000 molecular weight for prekallikrein and high molecular weight kininogen, respectively (lower frame, right side). In parallel gels, the same peaks of radioactivity were seen when kaolin was incubated with a similar plasma sample (data not shown). In contrast, after injection of dextran sulfate into rabbits in vivo, the major fragment of $^{125}$I-high molecular weight kininogen comigrated with the native $^{125}$I-prekallikrein peak at 80,000 molecular weight at both 5 and 20 minutes after dextran sulfate injection (left side, lower two frames). The major fragment of $^{125}$I-prekallikrein was at 50,000 molecular weight similar to that seen in vitro (left side, lower two frames). Comparable results were present in six rabbits examined.
the Hageman factor system (Kluft, 1978). Furthermore, following injection of dextran sulfate, 125I-
Hageman factor accumulated in lungs, presumably as a complex with the dextran sulfate which could
be seen trapped in lung capillaries in association with degranulating platelets, basophils, neutrophils,
and eosinophils within 2 minutes after injection (Wiggins et al., 1985). In addition, the levels of
antigenic Hageman factor system proteins in plasma rapidly fell following injection of dextran sulfate.
Kaolin-releasable kinin became undetectable, possibly as a result of (1) inactivation by proteolysis of
either Hageman factor or prekallikrein, or (2) the cleavage of kininogen molecules at the 80,000 mo-
lecular weight site, or (3) binding of circulating Hageman factor, prekallikrein, or high molecular
weight kininogen to circulating dextran sulfate so that these molecules cannot be activated by kaolin,
or (4) inactivation of kinins by peptidase(s) which are released into blood following dextran sulfate
injection but which are not inhibited by o-phenanthroline, or (5) a combination of these factors. The 15%
fall in trypsin-releasable kinin was not statistically significant, but could be accounted for in part
by a 25% fall in high molecular weight kininogen concentration in plasma, together with cleavage of
a small proportion of the kininogen molecules at the kallikrein site to form kinin-free kininogen, or
the presence of a peptidase not inhibited by o-phenanthroline.

Interpretation of the results in this way requires that the radiolabeled molecules behave in a fashion
which is representative of the normal population of molecules. One cannot be absolutely certain that
this is the case with respect to cleavage of 125I-high molecular weight kininogen by an unknown en-
zyme. However, all the labeled molecules used (Hageman factor, prekallikrein, and high molecular
weight kininogen) underwent proteolysis in kaolin-activated plasma in vitro into the fragments that are
well established to be the activation fragments of these molecules (Mandle and Kaplan, 1977; Ulevitch
et al., 1980; Cochrane and Griffin, 1982). Furthermore, to remove any molecules that had been
damaged during purification or radiolabelling so that they might be more likely to be rapidly cleared from
the circulation (and possibly cleaved by unknown enzymes), each batch of labeled molecules was al-
lowed to circulate in one rabbit for 3 hours before being injected into the experimental animal. There-
fore, the results cannot be explained in terms of changed molecules rapidly cleaved from this circu-
lation. It therefore seems likely that the proteolytic fragments observed were indeed representative of
the normal population of molecules.

Thus it appears that while Hageman factor and prekallikrein were probably activated, the high mo-
lecular weight kininogen had been inactivated by proteolysis. The most likely explanation for this
result is that the kininogen-cleaving enzyme was rapidly liberated following dextran sulfate injection
and cleaved kininogen molecules before the Hage-
man factor system was activated. It is quite probable that some Hageman factor and prekallikrein mole-
cules were cleaved after blood had been drawn from the rabbit, since dextran sulfate activates the Hage-
man factor system better in cold plasma than at 37°C (Kluft, 1978). This seems to be the most likely
explanation of the apparent paradox of finding pre-
kallikrein cleaved, and yet the kininogen molecule,
which circulates as a complex with prekallikrein
(Mandle et al., 1976) and is rapidly cleaved by
kallikrein during contact activation (Alving et al.,
1978; Kerbiriou and Griffin, 1979), cleaved at a non-
kallikrein site.

The results of this study emphasize the caution
required in interpreting cause and effect in complex biological systems. Further studies are required
to identify what enzymes are responsible for cleaving the high molecular weight kininogen molecule at a
site which apparently made it inaccessible to kalli-
krein cleavage.

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INDEX TERMS: Hageman factor • Prekallikrein/kallikrein • High molecular weight kininogen • Dextran sulfate • Kinin/bradykinin
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