In Vivo Behavior of Human Radioiodinated Antithrombin III: Distribution Among Three Physiologic Pools

By Timothy H. Carlson, Toby L. Simon, and A.C. Atencio

It has recently been shown that antithrombin III (AT) distributes between plasma, a noncirculating vascular-associated pool and an extravascular pool in rabbit. Study of the in vivo behavior of autologous human 131I-AT demonstrates that in humans AT also distributes among three pools that are analogous to those found in rabbit. From the in vivo kinetic behavior of the 131I-labeled AT, the fractions of total-body AT in the plasma, noncirculating vascular-associated, and extravascular pools were calculated to be 0.393 ± 0.015, 0.109 ± 0.016, and 0.496 ± 0.014, respectively. From three-exponential plasma radioactivity disappearance curves, an average plasma fractional catabolic rate, $j_1$, of 0.576 ± 0.034 day$^{-1}$ was obtained for five healthy young men. This is almost identical to the result obtained if plasma 131I-AT disappearance is assumed to fit a two-exponential curve (0.546 ± 0.038), where the constant $C_1$ from $A(t) = C_1 e^{-j_1 t} + C_2 e^{-j_2 t}$ is assumed to be equal to 1 - $C_r$. The fraction of the total vascular AT catabolized daily, $j_2$, was calculated to be 0.457 ± 0.034, and the fractional catabolic rate of total-body AT, $j_r$, averaged 0.227 ± 0.0176. The results give further support to a model of in vivo behavior in which the vascular AT distributes between plasma and an endothelial receptor. Thus, the latter may serve to mediate activation of AT for its reaction with coagulation proteases and to mediate its entrance into the endothelial cell, where it is either transported to the extravascular fluids or is catabolized.

The COAGULATION process is regulated in vivo in part by antithrombin III (AT), a protease inhibitor whose deficiency leads to a high risk of venous thrombosis. In vitro AT inactivates thrombin and other coagulation proteases by forming stable 1:1 complexes. This reaction occurs as the result of AT initially acting like a serine-protease substrate, but results, at a later stage in the reaction, in the formation of a covalent complex, probably the stabilized acyl-enzyme intermediate.

Heparin greatly enhances the rate of AT-coagulation protease reactions in vitro. For thrombin, this effect probably occurs as the result of high-affinity binding of both the inhibitor and protease to a single heparin molecule. Heparin binding produces a conformational change in AT, which probably contributes to enhancement of the reaction rate. The pharmacologic activity of heparin as an anticoagulant is generally considered to be caused by its effect on AT-coagulation protease reactions.

Using the rabbit as a model, we have recently demonstrated from the kinetic behavior that intravenously (IV) injected 131I-AT very rapidly equilibrates with a non-plasma pool. Because of the rapid rate of this equilibration, and because blood cells were not involved, it was concluded that this pool must result from AT interaction with the endothelium. The nature of the AT-endothelial interaction was suggested earlier by the findings of Lollar and Owen, who showed that the in vivo rate of AT-thrombin reaction is much faster than the in vitro rates of AT-coagulation protease reactions. Thus, it has been proposed that heparin-like material present on the vascular endothelium serves to bind and activate AT in vivo. Support for the heparin-like binding of AT to the endothelium comes from studies of two isoforms of rabbit AT differing in affinity for heparin. Thus, as would be predicted from the heparin affinities, it was found that a larger fraction of the higher-affinity form is associated with the noncirculating vascular-associated pool. The hypothesis is also supported by the finding that in the presence of endothelial cells or intact vascular beds, the AT-thrombin reaction is significantly enhanced. In addition, material from vascular tissue with heparin-like activity has been isolated, and this enhances in vitro AT activity by a mechanism that is apparently identical to that seen with heparin itself.

The studies reported here were undertaken to determine if an AT pool could be demonstrated in humans similar to the endothelial-associated rabbit one. Using methods to analyze the disappearance of 131I-AT used in rabbit-AT model studies, but previously developed to study the in vivo kinetic behavior of other plasma proteins, we show that a substantial portion of human vascular AT is in the noncirculating vascular pool. Furthermore, kinetic analysis indicates that this pool serves to mediate AT transport from the plasma to the extravascular fluids.

MATERIALS AND METHODS

Purification of antithrombin III. AT was prepared by previously described methods to modify produce sterile, pyrogen-free human protein. It was assayed as heparin-cofactor using a chromogenic substrate (Chromozym TH, Boehringer-Mannheim, Indianapolis).

The purification involved a two-step procedure using new glassware that was autoclaved and subjected to 170℃ dry heat for 16 hours to destroy pyrogens, and with buffers prepared using pyrogen-free water and autoclaved before use. First, heparin–agarose was prepared using sterile buffers and sterilized during its preparation. For each subject, a fresh 1.5 × 6.0 cm heparin–agarose column was washed with 100 vol sterile pyrogen-free 0.02 mol/L Tris, 0.01 mol/L sodium citrate, pH 7.2 (TCB) containing 3.0 mol/L NaCl, and was then equilibrated with TCB containing 0.225 mol/L NaCl.

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Each subject fasted overnight; then 0.5 units of his blood was drawn in one-ninth vol sterile pyrogen-free 3.8% sodium citrate. Plasma, obtained after centrifuging twice at 2,000 g, was diluted with TCB containing 0.3 mol/L NaCl to 0.25 mol/L NaCl by assuming a 0.15 mol/L plasma NaCl concentration. After loading, the column was washed with 100 mL of 0.25 mol/L NaCl containing TCB and was then eluted with a 100-mL linear gradient from 0.25 mol/L to 1.8 to 2.0 mol/L NaCl. The expected location of the AT among the eluted fractions was determined from another identical heparin--agarose column that had been loaded with human plasma and eluted as described above. The absorbency of the eluted fractions was measured on each side of the expected AT peak until an A180 nm of -0.1 mol/L was obtained. Those fractions that apparently had A180 nm values of >0.1 were pooled.

In the second step, the conductivity of the fractions with absorbances of -0.1 on each side of the pool was used to determine the NaCl concentration of the AT pool. The pool was then slowly diluted with TCB to a calculated NaCl concentration of 0.30 mol/L NaCl and was loaded on the same column described in step 1 after it had been stripped with TCB containing 3.0 mol/L NaCl and reequilibrated at 0.30 mol/L NaCl. After being washed with TCB containing 0.30 mol/L NaCl, the column was eluted with buffer containing 3.0 mol/L NaCl at 1.0 mL/8 min per fraction. From each fraction, 0.080 mL was removed and diluted with TCB to 0.80 mL. The A180 nm and an extinction coefficient of 7.04 were used to determine AT concentration, which ranged from 1.5 to 3.5 mg/mL.

For radiolabeling, 0.25 mL of the AT was mixed with an equal molar amount of sodium iodide, ±100 μCi Na131I (ICN, Radiochemical Division, Irvine, Calif), 0.5 mL 0.2 mol/L phosphate buffer (pH 7.2), one-fifth vial (100 μL) Enzymobead reagent (Bio-Rad Laboratories, Richmond, Calif), and 250 μL 1% d-glucose, which had been allowed to mutarotate overnight. The reaction was allowed to proceed at 4°C for two hours. After centrifugation to remove Enzymobead, the mixture was diluted with TCB and loaded on the same column initially used to purify the AT. The column had been washed with TCB containing 3.0 mol/L NaCl and reequilibrated with 0.3 mol/L NaCl. After 131I-AT loading, it was washed extensively with the latter buffer to remove unbound 131I, and eluted with a linear 100-mL NaCl gradient between 0.30 mol/L and 2.0 mol/L NaCl. Fractions with the highest radioactivity were pooled. Each pool was subjected to filtration through 0.2-μm sterile filters, which had been precoated with an aliquot of the subject’s plasma. The resulting 131I-AT was shown to be free of endotoxins by the limulus--amann test. Two 1-mL aliquots of each collection were counted for 20 to 40 minutes each for radioactivity, along with two 1-mL aliquots of the supernatant of trichloroacetic acid precipitated sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE).

Before this procedure was applied to human subjects, it was tested in rabbits, using 131I-AT prepared from plasma collected by cardiac puncture from anesthetized rabbits under aseptic conditions. The resulting 131I-AT was checked by the standard US Public Health Service rabbit pyrogen test28 and was found to be pyrogen free. Furthermore, the kinetic behavior of this rabbit 131I-AT was identical to that obtained previously for the rabbit.

Subjects and turnover studies. These studies were approved by the human research review committee of the University of New Mexico School of Medicine and conducted in the General Clinical Research Center. Five men, laboratory workers and students between 25 and 38 years of age, from whom informed consent was obtained, were studied. Beginning three or four days before 131I-AT injection and continuing throughout the duration of the study, each subject was given doses of saturated potassium iodide to block concentration of 131I in the thyroid and other organs. The first two subjects were given ten drops of the KI solution three times on the first day and ten drops once per day once each of the subsequent days until the study was completed.39 The 131I-AT was injected on the fourth day of this regimen. The third subject developed swelling of the cutaneous tissue around the eyes after the first day of K1 administration; thereafter, the dosage was reduced to two drops, without further complication.

The subjects were admitted to the General Clinical Research Center and were hospitalized for the first 24 hours of the studies. Each subject was injected with ±10 μCi of autologous 131I-iAT on day 1 of the study and 15 to 17 blood samples (anticoagulated in 3.8% sodium citrate) were obtained over the subsequent eight to nine days. Duplicate microhematocrits of the whole blood and anticoagulated blood were obtained at the time of each blood sample. Aliquots of plasma (125 μL) were frozen at the time of each sampling for AT rocket immunoassay. Two 1-mL aliquots of plasma were counted for 20 to 40 minutes each for radioactivity, along with two 1-mL aliquots of the supernatant of trichloroacetic acid precipitated plasma. No association of 131I-AT with blood-formed elements was detected. Corrections for background, decay, dilution by anticoagulant, and unbound iodide were made as described previously.1

Relative whole-body radioactivity was determined by a Picker Dyna 4/11 γ-camera in the Nuclear Medicine Department of the University of New Mexico Hospital. The camera was used without a collimator and with a 20% window. Total counts from one or two ten-minute passes were recorded beginning within an hour of 131I-AT injection and once per day thereafter for the duration of the study. A blood sample was taken immediately before or after whole-body counting. Whole-body radioactivity was corrected for background and radioactive decay.

Data analysis. Plasma 131I-AT disappearances were fitted by curve peeling to two-exponential or three-exponential equations of the form

\[ A_p(t) = C_1 e^{-a_1 t} + C_2 e^{-a_2 t} \]

or

\[ A_p(t) = C_1 e^{-a_1 t} + C_2 e^{-a_2 t} + C_3 e^{-a_3 t}. \]

The fractions of total-body, vascular, and plasma AT catabolized daily were determined as described previously.1 Thus, the fraction of total-body AT catabolized daily, \( j_T \), was determined from the steady state 131I-AT disappearance using the equation

\[ j_T = 1 - e^{-a}. \]

The fraction of vascular AT, plasma plus noncirculating vascular, which we have called \( j_{V.o} \), was determined from

\[ j_{V.o} = (C_1 + C_2)(C_3/\alpha_1 + C_2/\alpha_2). \]

The fractional plasma catabolic rate, \( j_s \), was determined from

\[ j_s = (C_4 + C_5)(C_6/\alpha_1 + C_5/\alpha_2 + C_5/\alpha_2). \]

From these first order rates, the relative size of the plasma \( (A_p) \), noncirculating vascular-associated \( (A_r) \), and extravascular \( (A_o) \) AT compartments were determined.1 Thus,

\[ A_o = j_r/j_s. \]

\[ A_r = (j_f/j_s) - A_p. \]
and

\[ A_r = 1.00 - A_y - A_c \]  \hspace{1cm} [8]

\( A_r \) was also calculated by mass balance\(^{28}\) from plasma, estimated total-body unbound \( ^{3}I \)(\(^{19}\)) and whole-body radioactivity, and \( A_c \) was determined by an alternative method from \( A_r \) and \( C_1 + C_2 \).\(^{1}\)

RESULTS

The \( ^{3}I \)-labeled AT had essentially the same heparin-cofactor activity as the unlabeled material, and it migrated as a single band on SDS-PAGE. Figure 1 shows the results obtained when a trace of one \( ^{3}I \)-AT preparation was mixed as a single band on SDS-PAGE. Figure 1 shows the results determined by an alternative method from \( A_r \) and \( C_1 \).\(^{28}\) to remove lipoproteins and frozen before chromatography. It can be seen that the labeled-AT eluted slightly ahead of the unlabeled-AT, a finding also observed previously for iodinated rabbit-AT\(^{1}\).

Figure 2 shows the results of a typical autologous \( ^{3}I \)-AT turnover study. The plasma \( ^{3}I \)-AT radioactivity is fitted to a three-exponential equation. A comparison of the two-exponential and three exponential fits for plasma \( ^{3}I \)-AT for each subject is given in Table 1. The better fit of the three-term exponential equation was shown by a comparison of \( \Sigma C_i \). Thus, for the two-term fit, \( C_1 + C_2 \) averaged 0.930 \( \pm \) 0.017, while \( C_1 + C_2 + C_3 \) for the three-term equations averaged 1.022 \( \pm \) 0.015. Furthermore, when the latter were summed for \( t = \) ten to 20 minutes, the time of the first blood sample, a value of 0.998 \( \pm \) 0.011 was obtained. Because the \( \Sigma C_i \) is theoretically equal to one at the time of the initial sampling,\(^{3}1 \) the data indicated that the three-exponential equation gave the best fit.

Figure 2 also shows the whole-body radioactivity fitted to a single-exponential equation, for \( t \geq \) 2 days. Table 2 gives data that describe these equations for each subject and gives the AT half-lives for each subject, as determined from the slope of the whole-body and plasma \( ^{3}I \)-AT radioactivity curves. The latter were significantly longer (\( P \leq 0.01 \)) by the paired Student's \( t \) test). However, for the single subject, from whom quantitative urine collections were obtained during the whole course of the study (T.C.), the half-life of the total remaining \( ^{3}I \) was slightly longer than that determined from the plasma data (\( t_{1/2} = 3.38 \), \( a_{\infty} = .205 \)), probably due to extrarenal iodine excretion.\(^{19}\)

Also demonstrated in Fig 2 is the delay in excretion of radioactivity that was seen in each of these studies. Thus, as demonstrated in dog and man,\(^{32,33}\) both whole-body and urine (injected minus urinary excreted) (1 - \( u \)) radioactivities were greater than would be expected if catabolism began.

Table 1. Comparison of Constants Obtained by Curve Peeling, Describing Two-Exponential and Three-Exponential Fits of Plasma \( ^{3}I \)-AT Disappearance Data

| Subject | \( C_1 \) | \( a_1 \) | \( C_2 \) | \( a_2 \) | \( \Sigma C \) | \( \Sigma C \)  
<table>
<thead>
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<tr>
<td>L.M.</td>
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<td>.256</td>
<td>.584</td>
<td>1.547</td>
<td>.953</td>
<td>.941</td>
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<td>I.S.</td>
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<td>.280</td>
<td>.558</td>
<td>1.753</td>
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<td>.913</td>
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<tr>
<td>T.C.</td>
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<td>.228</td>
<td>.555</td>
<td>1.191</td>
<td>.906</td>
<td>.899</td>
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<td>D.H.</td>
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<td>1.374</td>
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<td>.939</td>
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<tr>
<td>D.D.</td>
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<td>.286</td>
<td>.556</td>
<td>1.238</td>
<td>.921</td>
<td>.916</td>
</tr>
<tr>
<td>Average</td>
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<td>.258</td>
<td>.577</td>
<td>1.421</td>
<td>.930</td>
<td>.922</td>
</tr>
<tr>
<td>±SD</td>
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<td>.023</td>
<td>.025</td>
<td>.207</td>
<td>.017</td>
<td>.016</td>
</tr>
</tbody>
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Three-Term Constants

| Subject | \( C_1 \) | \( a_1 \) | \( C_2 \) | \( a_2 \) | \( C_3 \) | \( a_3 \) | \( \Sigma C \) | \( \Sigma C \)  
<table>
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<td>.201</td>
<td>5.197</td>
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<td>.941</td>
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<td>I.S.</td>
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<td>1.466</td>
<td>.246</td>
<td>4.121</td>
<td>.928</td>
<td>.913</td>
</tr>
<tr>
<td>T.C.</td>
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<td>.228</td>
<td>.409</td>
<td>1.038</td>
<td>.273</td>
<td>6.543</td>
<td>.906</td>
<td>.899</td>
</tr>
<tr>
<td>D.D.</td>
<td>.356</td>
<td>.286</td>
<td>.556</td>
<td>1.238</td>
<td>.921</td>
<td>.916</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average</td>
<td>.354</td>
<td>.258</td>
<td>.426</td>
<td>1.240</td>
<td>.247</td>
<td>4.790</td>
<td>.930</td>
<td>.922</td>
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<tr>
<td>±SD</td>
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<td>.023</td>
<td>.025</td>
<td>.160</td>
<td>.031</td>
<td>.1016</td>
<td>.015</td>
<td>.011</td>
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*Value of \( \Sigma C \) at ten to 20 minutes after \( ^{3}I \)-AT injection.
immediately after $^{131}$I-AT injection. That this is not caused by an artifact of the whole-body counting procedure is demonstrated by the agreement of whole-body and urine data. Thus, fractions of remaining whole-body radioactivity demonstrated by the agreement of whole-body and urine by an artifact of the whole-body counting procedure is immediately after $^{131}$I-AT injection. That this is not caused daily,j,j3.5, course of each of the studies ranged from 2.3 to 4.3%. The average coefficients of variation for the AT concentrations during the rate constants describing $^{131}$I-AT catabolism. The constants were used to calculate the relative sizes of the noncirculating vascular-associated (A3) and extravascular (A4) compartments, and these are listed in Table 4. Also given are A3 calculated by the mass balance approach. For these latter calculations, whole-body radioactivity was corrected by assuming the same slope from the plasma curve. The A3 values calculated by this approach were slightly, but significantly (P < .05) by the paired Student's t test), lower than those obtained from the J3 and J4. When the latter A3 value and C1 and C2 from the three-exponential plasma equations were used to calculate A3, no difference was found from the value determined from equation 7.

Using J4, the plasma volume and AT concentration, absolute catabolic rates were determined to be 4.93 ± 0.57 mg/kg/d for the five subjects in this study. From the relative sizes of $A_3$, $A_4$, and $A_n$, and the calculated total plasma AT, the amounts of AT in $A_3$ and $A_4$ were calculated. These averaged 8.57 ± 0.92, 2.39 ± 0.42, and 10.85 ± 1.42 mg/kg, respectively.

**DISCUSSION**

In this study, we show that human AT, like its rabbit counterpart, distributes among three physiological pools or compartments. This is demonstrated by the better fit to three-exponential than two-exponential plasma $^{131}$I-AT disappearance curves. These three-exponential curves do not result from the presence of denatured material in our labeled-AT preparations as shown by: (1) no loss of heparin cofactor activity as the result of labeling; (2) comigration of $^{131}$I-AT and unlabeled AT in SDS-PAGE; (3) virtual coelution of $^{131}$I-AT and unlabeled-AT from heparin agarose (Fig 1); and (4) a delayed, rather than increased, rate of label excretion during the initial time after $^{131}$I-AT injection. Furthermore, the methods of AT purification and iodination used in these studies were essentially identical to those used in previous experiments in rabbits. In those studies, the $^{131}$I rabbit AT was studied as both biologically screened and unscreened preparations with no difference in in vivo behavior.

The fractional catabolic constant, J4, reported here is nearly identical to that reported by others when our data was analyzed by two compartment methods and assuming C2 = 1 - C1. When this method is applied to the data, an average J4 of 0.546 ± 0.038 is obtained as opposed to 0.577 ± 0.033 by three-compartment analysis (Table 3). Thus, the absolute catabolic rates reported here are not significantly different than previously reported. The three AT pools in humans and rabbit must include a vascular-endothelial-associated one, in addition to the plasma and extravascular ones. This is demonstrated by the lack of $^{131}$I-AT associated with blood cells and the relatively high rate of plasma-second pool equilibration. The latter is somewhat slower in humans as compared with the rabbit, as shown by a smaller $a_3$ from the third term of the equation describing plasma $^{131}$I-AT disappearance. This is probably because of a longer circulation time than in rabbit.

Two models of distribution have been suggested to account for the presence of three AT pools. The first, model A, is similar to the general model originally proposed by Mat-
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Thus, for this model AT is seen as distributing between the plasma pool ($A_p$) and vascular-associated ($A_v$) and extravascular ($A_e$) compartments independently. In the second model, model B (Fig 3), plasma AT is in direct equilibrium with only $A_v$, from which AT passes in one direction to $A_e$. In this model, AT returns to the plasma from $A_e$ in a unidirectional manner, independent of $A_v$. If model A is tested by computerized fitting of the plasma, $^{131}$I-AT disappearance by compartmental analysis as described previously, extremely variable first-order rate constants are obtained and, in several cases, the best solutions were obtained when some of these are negative. Thus, model A does not seem to describe AT distribution. When model B is tested with the assumption that AT catabolism occurs from the plasma compartment, more reasonable solutions for the rate constants are obtained. When these are used in the integration of the differential equations describing model B as described previously, the predicted compartment sizes are $0.356 \pm 0.079$, $0.208 \pm 0.062$, and $0.434 \pm 0.114$ for $A_p$, $A_v$, and $A_e$, respectively. These values are in fairly good agreement with those obtained from the kinetic constant and mass balance methods (Table 4). However, the size of $A_v$ is significantly overpredicted, probably because AT is not catabolized from the plasma (see below).

A difference in the rabbit and human studies is in the slopes of the $^{131}$I-AT plasma and whole-body radioactivities. In rabbit, these were identical, while a more rapid whole-body slope was seen in the present human studies. The reason for this is not clear, but it could be an artifact resulting from a progressive redistribution of the $^{131}$I-AT, which in turn causes a change in the whole-body counting efficiency. In support of this supposition is the finding in one study, in which quantitative urine collection was maintained throughout the course of blood sampling, that radioactivity $1 - u$ at some slower slope than that seen for the whole-body disappearance of radioactivity. It is unlikely that these results were caused by $^{131}$I loss through sweat, because this study was conducted during the coldest time of year (December), and the subject limited his physical activity. However, the $1 - u$ slope was even slower than that obtained from plasma $^{131}$I-AT disappearance, suggesting that some catabolic products were excreted by nonurinary mechanisms.

A similarity between human and rabbit $^{131}$I-AT behavior is in the delay in metabolism, as demonstrated by the initial delay in the decline of whole-body radioactivity. This result has been previously demonstrated in dog and humans, and the early agreement between the whole-body and urinary excretion data in the present study demonstrates that the effect is not an artifact of the whole-body counting procedure. The cause of this behavior has been proposed to be the result of AT catabolism following its association with cell surfaces within the extravascular AT pool. Compartmental kinetic analysis of rabbit $^{131}$I-AT data suggested that AT catabolism does not occur exclusively from the plasma compartment. However, this analysis assumes instantaneous mixing of tracer within compartments. Therefore, if breakdown occurs from the extravascular compartment, where mixing is slow, this type of analysis may be misleading. The site of AT catabolism is strongly suggested by our recent study of the comparison of the distribution and catabolism of the two isoforms of rabbit AT, differing in heparin affinity. Thus, a larger fraction of the isoform with higher heparin affinity was apparently associated with the endothelium, and this difference was virtually identical to the difference in the rates of catabolism of the isoforms. The most reasonable explanation for this is that AT metabolism occurs following binding to cell surface heparin-like material, as was originally proposed, but virtually exclusively within the vascular endothelial cell. Because the endothelial cell cytoplasm is actually part of the extravascular compartment, AT catabolism is pictured in the model of AT in vivo behavior shown in Fig 3 as occurring in $A_v$, but near the vascular endothelial pool ($A_e$).

If the AT compartment lining some or all of the vascular endothelium is responsible for AT activation in vivo, it is implied that a significant fraction of AT is always activated. Thus, in vessels with normal endothelium containing the AT binding-site, traces of coagulation proteases present should be rapidly inhibited by contact with activated inhibitor. This supposition is supported by the animal studies of Lollar and Owen, who found that within 30 seconds of injection of subcoagulant levels of $^{125}$I-thrombin, 80% of the plasma radioactivity was present in a complex with properties identical to those of the AT-thrombin complex. In contrast to this finding, when thrombin and then AT were perfused through intact vascular beds, only a slight or no enhancement of the rate of the AT-thrombin reaction was observed. The reason for the difference between these and the in vivo data is not entirely clear, but one explanation is that there are two possible fates for circulating active thrombin. First, it may react with AT activated by association with an endothelial cofactor (or more slowly with circulating AT). In vessels in which there is a large amount of AT associated with the endothelium, this is the preferred route when low levels of the protease are present. The second fate of circulating active thrombin is to become reversibly associated with its high-affinity endothelial binding site. When thrombin is present in vessels depleted of surface-bound AT, this is the fate of thrombin. This probably also results when thrombin is perfused in vitro through vascular

![Fig 3. A model of the in vivo behavior of AT. Within the vascular system AT is seen as equilibrating between plasma ($A_p$) and endothelial receptor pools ($A_v$). The latter, resulting from a heparin-like binding site, is proposed to activate AT in vivo, and to mediate its transport to the nonluminal side of the endothelium for release into the extravascular fluids ($A_e$). AT catabolism ($A_e$) occurs as an alternative to transport, in the intracellular endothelium. The dashed arrow represents the possible transport of AT by non-receptor-mediated mechanisms.](image-url)
because very small amounts of AT remain associated with the endothelium, resulting from either preperfusion with buffer, or to an initial undetected rapid AT-thrombin reaction. Endothelial-bound thrombin may be protected from rapid inhibition during AT perfusion of AT-depleted vessels, because the time available for the AT to interact with its cell surface cofactor during passage through the vascular bed may not be long enough to lead to significant association. Thus, the rate-limiting step of the endothelial enhanced AT-thrombin reaction may be the association of AT with endothelial cofactor.

The above paradigm implies that when large amounts of thrombin are present in vivo, such as occurs near the site of vessel injury, or when other changes in the endothelium take place that would reduce the amount of endothelial-activated AT present, clot formation and endothelial thrombin binding are enhanced. It further implies that the status of AT function in vivo cannot be assessed by determination of plasma AT levels alone, because the amount, affinity, and distribution of endothelial binding must also play a role. The amount of endothelial AT cofactor could, for instance, decrease with age, thus contributing to the high incidence of venous thrombosis in elderly patients undergoing hip joint surgery, or to the generally postadolescent onset of thrombosis in individuals with hereditary AT deficiency. Future studies to determine the site(s) and nature of AT-endothelial interaction are needed to further elucidate its possible physiological role.

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REFERENCES


DISTRIBUTION OF ANTITHROMBIN


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