Comparison of the behaviour *in vivo* of two molecular forms of antithrombin III

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The distribution and rates of catabolism were determined for rabbit antithrombin III (AT) isoforms differing in affinity for heparin-agarose. After isolation from rabbit plasma by heparin-affinity chromatography, the very-high-affinity form, AT_{vh}, was labelled with ¹³¹I, and its high-affinity congener, AT_h, with ¹²⁵I. The two forms were separated from free iodine by heparin-affinity chromatography, and then injected simultaneously into young recipient rabbits. The disappearance-of-plasma-AT-radioactivity data were fitted to three exponential equations, and the resulting constants were used to calculate fractional catabolic rates and the various pool sizes for AT_h and AT_{vh}. The fractions of plasma AT_h and AT_{vh} catabolized daily (j_3) were 0.763 ± 0.023 and 1.88 ± 0.057 day⁻¹ respectively. Average values of j_T , the daily fractional catabolic rates for the total-body AT_h and AT_{vh}, were 0.2633 ± 0.0113 and 0.3832 ± 0.0211 day⁻¹. The ratios, AT_h/AT_{vh}, of the sizes of the plasma, non-circulating vascular-associated and extra-vascular compartments were 0.593, 0.990 and 1.30. By using a previous estimate of a 9:1 AT_h/AT_{vh} ratio in rabbit plasma and the calculated compartment sizes, the calculated relative ratio of AT_h to AT_{vh} was 5.4:1 in the non-circulating vascular-associated compartment. The data fit a model of antithrombin III distribution and catabolism in which a pool of endothelial-cell heparin-like receptors is seen to mediate the transfer of the inhibitor from plasma to interstitium with catabolism serving as an alternative to this transport.

AT neutralizes thrombin (Abildgaard, 1969) and other coagulation-related proteinases (Rosenberg, 1977) by acting as a substrate-like inhibitor (Bjork & Lindahl, 1982). The rates of these reactions is greatly increased by heparin (Rosenberg, 1977), and one or more is important physiologically, as shown by the high incidence of venous thrombosis among individuals with a hereditary AT deficiency (Egeberg, 1965).

We have shown that AT exists in two forms in plasma (Carlson & Atencio, 1982). The biochemical properties of the major form have been well characterized (Rosenberg & Damus, 1973; Kurauchi *et al.*, 1976), and we have determined some of

the properties of the second form (Carlson & Atencio, 1982). Both forms of AT have heparin-cofactor and progressive-AT activities; however, the second form has an apparent 2 kDa lower molecular mass, lower electrophoretic mobility and substantially higher affinity for heparin-agarose. Because of the latter property, this form of AT is termed 'very high-affinity AT' (AT_{vh}) and the other form is termed 'high-affinity AT' (AT_h).

We have reported kinetic evidence that AT_h distributes to three physiological compartments, one of which is closely associated with, but independent of, the plasma compartment (Carlson *et al.*, 1984). This probably results from a pool of the AT endothelial binding sites that have recently been demonstrated (Busch & Owen, 1982; Marcum *et al.*, 1984). Here we compare the kinetic behaviour *in vivo* of the two forms of AT in rabbit. By using methods developed to study the behaviour of AT_h *in vivo*, we determined the behaviour of AT_{vh} and AT_h simultaneously.

Abbreviations used: AT, antithrombin III; AT_{vh}, antithrombin III with a very high affinity for heparin; AT_h, antithrombin III with a high affinity for heparin.

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Experimental

Materials

Tosylglycylprolylarginine *p*-nitroanilide acetate (Chromozym-TH) was obtained from Boehringer Mannheim Biochemicals, Indianapolis, IN, U.S.A. Crude bovine thrombin was from Parke, Davis and Co., Detroit, MI, U.S.A. Electrophoretic-grade acrylamide, *NN'*-methylenebisacrylamide, *NNN'*-tetramethylethylenediamine, ammonium persulphate, SDS, 2-mercaptoethanol, agarose and the immobilized glucose oxidase/lactoperoxidase enzyme system (Enzymobead) were products of Bio-Rad Laboratories, Richmond, CA, U.S.A. Sepharose 4B was a product of Pharmacia Fine Chemicals, Piscataway, NJ, U.S.A. Heparin (stage 14) for heparin-agarose preparation was obtained from Inolex Corp., Chicago, IL, U.S.A. Heparin-agarose was prepared as described previously, and contained 650–950 µg of heparin/ml of gel (Carlson & Atencio, 1982). Heparin for heparin-cofactor assay was 5000 units/ml from Organon, West Orange, NJ, U.S.A. Polybrene was from Aldrich Chemical Co., Milwaukee, WI, U.S.A. Na¹²⁵I and Na¹³¹I were from ICN, Chemical and Radioisotopes Division, Irvine, CA, U.S.A. Goat anti-(rabbit AT) antiserum was that reported previously (Carlson & Atencio, 1982).

Methods

Assays. Heparin-cofactor activity was assayed by the method of Abildgaard *et al.* (1977) using Chromozym-TH as the thrombin substrate. Methods for rocket immunoassay and crossed immunoelectrophoresis were described previously (Carlson & Atencio, 1982).

Preparation of AT_h and AT_{vh}. The two forms of rabbit AT were prepared by a modification of our previously described purification procedure (Carlson & Atencio, 1982), involving only heparin-agarose chromatography. A 1.5 cm × 17 cm column was used, and all steps employed a Tris/citrate buffer (0.02 M-Tris/0.01 M-sodium citrate, adjusted with HCl to pH 7.2). Pooled rabbit plasma, obtained after centrifugation of 250 ml of blood, freshly collected in $\frac{1}{8}$ vol. of 0.129 M-sodium citrate, was diluted with buffer containing 3.0 M-NaCl to give a final added NaCl concentration of 0.225 M. This material was gravity-loaded, and the column was washed overnight with 50–100 ml of buffer containing 0.225 M-NaCl. The column was developed by a 100 ml linear NaCl gradient from 0.225 M to 0.9 M, and then, after washing with an additional 30 ml of the latter buffer, by step elution with buffer containing 2.0 M or 3.0 M added NaCl.

Although samples of both forms of AT were homogeneous by sodium dodecyl sulphate/polyacrylamide-gel electrophoresis (Weber & Osborn,

1969), they were too dilute for use in labelling. The proteins were concentrated by using a 5.0 ml heparin-agarose column (Carlson *et al.*, 1984). An AT_{vh} concentration of 0.5 to 1.0 mg and an AT_h one of 2.0–3.0 mg/ml were obtained.

AT iodinations and isolation from free iodine. The solid-phase glucose oxidase/lactoperoxidase (Enzymobead) method was used to label the proteins (Carlson *et al.*, 1984). AT_{vh} concentrations of 1.5 mg/ml or greater, however, could not be achieved without the use of methods of protein concentration that could result in denaturation. Therefore AT_h was diluted with buffer to the identical concentration of the AT_{vh} before labelling to ensure that differences in ¹²⁵I-AT_h and ¹³¹I-AT_{vh} *in vivo* would not be the result of labelling a more dilute AT_{vh} preparation. The labelling efficiencies ranged from 25–50% iodine incorporation.

After labelling AT_h with ¹²⁵I and AT_{vh} with ¹³¹I, the iodination-reaction solutions were combined, and diluted to 0.3 M-NaCl with buffer. The resulting mixture was loaded on a 5.0 ml heparin-agarose column equilibrated with 0.3 M-NaCl, and after extensive washing to remove unbound iodine, eluted with a 60 ml linear 0.3–1.8 M-NaCl gradient. The resulting labelled proteins had essentially unchanged heparin-cofactor activities.

Methods *in vivo*

Healthy New Zealand White rabbits were used. Plasma radioactivity was corrected for background, decay, dilution and removal during sampling, and whole-body radioactivity for background, decay and removal by standard techniques (Carlson *et al.*, 1984).

Kinetic data analysis. Corrected plasma radioactivity data was fitted to three-exponential equations of the form

$$*A_p = C_1 e^{-a_1 t} + C_2 e^{-a_2 t} + C_3 e^{-a_3 t} \quad (1)$$

where $*A_p$ is the fraction of plasma ¹³¹I-AT_{vh} or ¹²⁵I-AT_h remaining at time in days, *t*, and the *C* and *a* terms are constants. The fitting was by least-square methods employing the PROPHET computing system (see the acknowledgements) or a programmable hand calculator.

The apparent plasma volumes, *V_p*, were determined as described previously (Carlson *et al.*, 1984). The fractions of the plasma AT catabolized each day (*j₃*) were obtained by the standard method for a three-compartment system (Matthews, 1957; Reeve & Bailey, 1962). Thus *j₃* is related to the *C* and *a* constants of eqn. (1) as follows:

$$j_3 = (C_1 + C_2 + C_3)/(C_1/a_1 + C_2/a_2 + C_3/a_3) \quad (2)$$

The fractions of the sum of plasma and non-circulating vascular AT_h and AT_{vh} catabolized

each day ($j_{3.5}$) and of total-body AT_h and AT_{vh} (j_T), were determined as described previously (Carlson *et al.*, 1984).

The fractions of total-body AT_v and AT_{vh} in the plasma, A_p , vascular-associated, A_w , and extra-vascular, A_e , compartments were determined from the rate constants j_3 , $j_{3.5}$ and j_T (Carlson *et al.*, 1984). Thus:

$$A_p = j_T/j_3 \quad (3)$$

$$A_w = (j_T/j_{3.5}) - A_p \quad (4)$$

and

$$A_e = 1.00 - A_p - A_w \quad (5)$$

These fractions for AT_{vh} were checked by the mass-balance method (Carlson *et al.*, 1984) and found to be in close agreement.

Results

A typical isolation of AT_h and AT_{vh} from rabbit plasma on heparin-agarose was as shown in Fig. 1. As reported previously (Carlson & Atencio, 1982) both absorbance peaks contained progressive-antithrombin, heparin-cofactor and AT antigenic activity. However, the second peak had a slightly lower apparent molecular mass by sodium dodecyl sulphate/polyacrylamide-gel electrophoresis, and a lower electrophoretic mobility as determined by cross-immunoelectrophoresis at pH 8.6 in the absence of heparin.

After radioiodination, the ^{125}I -AT and ^{131}I -AT were isolated by heparin-agarose as shown in Fig. 2. ^{125}I - AT_h and ^{131}I - AT_{vh} were eluted as sharp peaks slightly ahead of their respective absorbance peaks. The difference in the specific radioactivity of the fractions could have been the result of

reduced affinity for heparin of populations of I-AT with more than one atom of iodine per AT molecule (Leonard *et al.*, 1983), or could occur because of a slightly reduced affinity of singly labelled I-AT for heparin. If the first of these possibilities were the case, the distribution of iodinated AT within the total AT population would be expected to change *in vivo* over time, because it has been shown previously that plasma proteins labelled with several iodine atoms per protein molecule are more rapidly catabolized than those containing a single atom per molecule (Atencio *et al.*, 1965). To test this, the blood from three rabbits was collected by cardiac puncture 10 days after injection of ^{131}I - AT_h . The resultant plasma was subjected to heparin-agarose chromatography, and the results shown in Fig. 3 were obtained. In this case the peak of ^{131}I - AT_h was again eluted as a sharp peak, and slightly ahead of the peak at AT_h 280nm absorbance. In fact, a significantly greater separation was obtained, but this is attributable to the much more shallow gradient required to separate the larger amounts of AT_h and AT_{vh} present in this volume of plasma. We can, therefore, safely conclude that the fractions from the radioactive AT_h peak obtained immediately after labelling were not metabolically heterogeneous, and were probably not separated by heparin-agarose on the basis of iodine specific radioactivity.

Fig. 4 shows the disappearance, from a typical animal, of ^{125}I - AT_h and ^{131}I - AT_{vh} from plasma, and in addition, the non-protein-bound ^{131}I and

Fig. 1. Typical chromatogram obtained during isolation of the two forms of rabbit AT by heparin-affinity chromatography

Both the broad lower-affinity peak and the sharp higher-affinity peak contained heparin cofactor and AT antigenic activities. ●—●, A_{280} ; —, [NaCl] determined by conductivity measurements.

Fig. 2. Isolation of radioiodinated- AT_h and - AT_{vh} by heparin-affinity chromatography

After labelling 0.76 mg each of AT_h and AT_{vh} with ^{125}I and ^{131}I respectively, the mixture was diluted to 0.3 M-NaCl and chromatographed. The column was eluted with a 0.3–1.8 M-NaCl gradient. Fractional radioactivity is expressed as a percentage of that in the highest fraction. The heparin-cofactor activity of the labelled material in each peak was not changed from that of the starting unlabelled AT_h and AT_{vh} . ----, Radioactivity; —, A_{280} .

Fig. 3. Effect of $^{131}\text{I-AT}_h$ residence in vivo on its affinity for heparin-agarose

Plasma from three rabbits injected 10 days previously with $^{131}\text{I-AT}_h$ was chromatographed on heparin-agarose. The radioactivity of each fraction (●---●) was determined before measurement of its A_{280} (●—●). [NaCl] (—) was determined by conductivity measurements.

Fig. 4. A typical semilogarithmic plot of the disappearance of $^{125}\text{I-AT}_h$ and $^{131}\text{I-AT}_h$ injected simultaneously at $t = 0$. The plasma $^{125}\text{I-AT}_h$ (▲) and $^{131}\text{I-AT}_h$ (▼) radioactivities, whole-body $^{131}\text{I-AT}_h$ radioactivity (◆), and estimated whole-body non-protein-bound ^{131}I radioactivity (●) obtained from rabbit B8 are shown. The continuous lines show the best fit of plasma data to three-exponential curves, and the whole-body ^{131}I data to a single-exponential curve.

the whole-body ^{131}I radioactivities. A large difference was found in the plasma half-lives of the isoforms. This difference was found for each animal, as shown by the results reported in Table 1 for the analysis of the plasma-disappearance curves obtained from six animals simultaneously injected with $^{125}\text{I-AT}_h$ and $^{131}\text{I-AT}_h$. Table 2 gives the fractional rates of catabolism and the relative sizes of the plasma (A_p), vascular-associated (A_w) and extra-vascular (A_e) compartments for the two forms of rabbit AT. The rate of AT catabolism, j_T , is almost 1.5 times greater for AT_h than for AT_v . Furthermore, the fraction of AT_h in the plasma is about 60% of that seen for AT_v . This accounts for the large difference in the plasma fractional catabolic rate, j_3 , which is nearly 2.5 times greater for the very-high-affinity AT. The relative sizes of the non-circulating vascular-associated compartments are nearly identical for the two forms of AT.

Discussion

The distribution and rates of catabolism we report for rabbit $^{125}\text{I-AT}_h$ can be compared with our previously reported results obtained from biologically screened and unscreened $^{131}\text{I-AT}_h$ (Carlson *et al.*, 1984). Thus the sizes of the A_p , A_w and A_e compartments reported here are nearly identical with those obtained in the previous study. The AT_h catabolic rates are, however, significantly higher in the present study. For example, j_T for the six animals reported here averaged 0.2633 ± 0.0113 , as compared with 0.2346 ± 0.0175 for the 12 animals studied previously. These are significantly different by Student's t test ($P = 0.003$). This difference, which is also seen in j_3 and $j_{3.5}$, may reflect the relative ages of the animals. To test this, the j_T values for the 18 animals in this and the previous study, and five animals of less than 2.0 kg (T. H. Carlson & A. C. Atencio, unpublished work), were subjected to linear regression against rabbit weight (paralleling relative maturity). The equation describing the best fit of the data is:

$$j_T = -0.07701(\text{kg}) + 0.4367$$

for which $P < 0.0001$ and $r = 0.8563$. On the basis of these results we conclude that the rate of AT catabolism in rabbits between 1.7 and 3.0 kg is strongly dependent on weight (age), and that the average predicted j_T for the six rabbits in the present study, 0.2542 ± 0.0093 , is not significantly different from the observed average ($P = 0.196$). Therefore the smaller size of the rabbits in the present study is primarily responsible for the higher rates of AT_h catabolism seen here. On the basis of the size-dependence of AT_h catabolism and the similarities in physical and biological

Table 1. Constants describing the disappearance from plasma of $^{125}\text{I-AT}_h$ and $^{131}\text{I-AT}_{vh}$ in normal young rabbits. The curves were fitted to eqn. (1) by curve-peeling. Three different preparations of $^{131}\text{I-AT}_{vh}$ and two preparations of $^{125}\text{I-AT}_h$ were used. Half-lives are in days, and r^2 denotes the correlation coefficients for the portion of the curve from which $t_{\frac{1}{2}}$ and a_1 were determined. All rabbits except R14 were simultaneously injected with $^{131}\text{I-AT}_{vh}$ and $^{125}\text{I-AT}_h$.

Molecular form of AT	Rabbit no.	Weight (kg)	C_1	C_2	C_3	a_1	a_2	a_3	$t_{\frac{1}{2}}$	r^2 (a_1)	Plasma volume (ml/kg)
AT_{vh}	R14	2.04	0.191	0.254	0.579	0.532	2.15	9.04	1.30	0.9972	50.4
	B7	2.21	0.188	0.345	0.498	0.510	2.09	13.7	1.36	0.9999	40.1
	B8	2.23	0.206	0.456	0.368	0.516	3.18	16.3	1.34	0.9989	39.3
	B9	2.50	0.196	0.330	0.503	0.531	2.70	15.1	1.31	0.9939	37.2
	B10	2.34	0.151	0.296	0.551	0.434	2.23	11.7	1.60	0.9911	44.7
	B11	2.42	0.182	0.412	0.407	0.466	3.15	11.7	1.49	0.9943	40.3
	B12	2.52	0.162	0.252	0.616	0.447	1.82	10.9	1.55	0.9973	38.5
	Mean	2.32	0.182	0.335	0.503	0.491	2.47	12.6	1.42		41.5*
	± S.D.	± 0.16	± 0.018	± 0.071	± 0.083	± 0.038	± 0.50	± 2.3	± 0.11		± 4.2
	Mean										40.0†
	± S.D.										± 2.3
AT_h	B7	2.21	0.375	0.295	0.354	0.319	1.95	15.4	2.17	0.9994	34.1
	B8	2.23	0.364	0.376	0.285	0.312	2.68	17.4	2.22	0.9987	33.4
	B9	2.50	0.369	0.281	0.371	0.327	2.47	15.2	2.12	0.9947	31.8
	B10	2.34	0.346	0.222	0.427	0.290	2.41	8.67	2.39	0.9988	40.2
	B11	2.42	0.337	0.370	0.298	0.284	2.26	11.0	2.44	0.9974	37.1
	B12	2.52	0.357	0.311	0.354	0.301	2.10	12.6	2.30	0.9986	35.5
	Mean	2.37	0.358	0.309	0.348	0.306	2.31	13.4	2.27		35.4
	± S.D.	± 0.12	± 0.013	± 0.053	± 0.047	± 0.015	± 0.24	± 2.9	± 0.11		2.7

* Mean plasma volume for all seven animals injected with $^{131}\text{I-AT}_{vh}$.

† Mean plasma volume for the six animals injected with both $^{131}\text{I-AT}_{vh}$ and $^{125}\text{I-AT}_h$.

properties between the AT forms, a weight-dependence for j_T of AT_{vh} can also be assumed. Hence, it is best to compare $^{131}\text{I-AT}_{vh}$ and $^{125}\text{I-AT}_h$ catabolism in the same animal, as we have done here.

Previously we have shown that (1) about 10% of the AT isolated by heparin-agarose from rabbit plasma is in the AT_{vh} form (Carlson & Atencio, 1982), (2) the total rabbit plasma AT concentration is approx. 250 µg/ml, and (3) plasma volume is typically 80 ml for young rabbits (Carlson *et al.*, 1984). On the basis of these data, the plasma of a typical 2.5 kg rabbit contains 18 mg of AT_h and 2.0 mg of AT_{vh} , and hence the amounts of the total-body AT as AT_h and AT_{vh} are 52 mg and 9.7 mg respectively. Thus 16% of all the AT is AT_{vh} , and approx. 14 mg of AT_h and 3.7 mg AT_{vh} are produced daily in healthy steady-state rabbits. This means that 21% of the total AT-synthetic effort is devoted to making AT_{vh} .

Heparin-like endothelial-cell binding sites for AT have recently been demonstrated (Busch & Owen, 1982; Marcum *et al.*, 1984). We have suggested previously that the non-plasma vascular-

associated AT compartment, A_w , found in our studies *in vivo* probably consists of AT bound to a pool of these (Carlson *et al.*, 1984). If this is so, we expect the two forms of AT we have isolated on the basis of differences in heparin affinity to have different affinities for these binding sites. The heparin-like character of the binding sites is indicated by consideration of the relative amounts of AT_h and AT_{vh} in plasma and non-plasma vascular-associated compartments (A_w). From the typical plasma AT_h and AT_{vh} masses (see above) and the relative sizes of the AT_h and AT_{vh} compartments (see Table 2), the mass of the isoforms in A_w are 9.0 and 1.7 mg respectively. Hence, the ratio of AT_h to AT_{vh} goes from 9:1 in the plasma to 5.4:1 in the non-plasma vascular-associated compartment. The most reasonable explanation for this 1.7-fold difference in relative AT_{vh} mass is that the two forms of AT are competing for the same pool of heparin-like binding sites for which AT_{vh} has a higher affinity.

The greater affinity of AT_{vh} than AT_h for the putative AT-binding sites permits distinguishing between two models that fit rabbit plasma I-AT_h

kinetic data (Carlson *et al.*, 1984). One of these two models views AT distribution from a plasma compartment (A_p) to the binding-site pool (A_w) and to the extravascular compartment (A_e) as occurring independently (Model A). A second model, which also fits plasma AT radioactivity disappearance, has the passage AT from A_p to A_e mediated by compartment A_w (Model B). From Table 2 it is seen that equal fractions of AT_h are associated with the vascular system, $A_p + A_w$, and interstitium, A_e (see also Carlson *et al.*, 1984). On the other hand, A_e contains about 1.6 times more AT_{vh} than $A_p + A_w$. Assuming that the isoforms are competing for the same receptor pool, Model B predicts that if a larger fraction of AT_{vh} than AT_h is bound in A_w , then a larger fraction of it is subsequently transported to A_e . This prediction is consistent with the data. Model A, on the other hand, predicts that if a larger fraction of AT_{vh} than AT_h is associated with A_w , then more of it remains with the vascular system ($A_p + A_w$), since the distribution of AT between A_p and A_e is independent of the distribution between A_p and A_w .

As a further consequence of Model B, ratios of the fractions of AT_h and AT_{vh} in the vascular pools ($A_p + A_w$) should reflect the ratios of the sizes of the extravascular pools, A_e , assuming return of both isoforms from the interstitium via the lymphatic system is independent of A_w . The AT_{vh}/AT_h ratio of the relative A_e pool sizes was 1.30 ± 0.077 (see Table 2), whereas the ratios of the fractions of vascular AT_{vh} to AT_h in A_w alone averaged 1.36 ± 0.14 . These data suggest that the AT-binding site discovered by Busch & Owen (1982) serves both to mediate activation of the AT-proteinase reaction (Marcum *et al.*, 1984) and as a receptor for transport of AT from plasma to interstitium.

Our previous study indicated that AT is not exclusively catabolized in association with either the plasma or the extra-vascular compartments (Carlson *et al.*, 1984). The site of AT metabolism is further elucidated by consideration of the data exemplified in Fig. 5. When a linear plot of the time course of the ratio of $^{125}I\text{-}AT_h/^{131}I\text{-}AT_{vh}$ is constructed, a straight-line relationship is obtained, beginning with data collected after equilibration between plasma and non-circulating vascular compartments is complete. Before equilibration between A_p and A_w , an even more rapid relative disappearance of $^{131}I\text{-}AT_{vh}$ is obtained. Thus it appears that AT_h and AT_{vh} catabolism begins immediately after association with A_w . The expanded models of the AT behaviour *in vivo* shown in Fig. 6 includes this hypothesis, proposing that AT catabolism occurs during passage from endothelial-cell surface to the extravascular fluid space. This could occur by the following mecha-

Fig. 5. Linear plot of the ratio of plasma $^{125}I\text{-}AT_h$ to $^{131}I\text{-}AT_{vh}$ during the course of a typical turnover study. The data was obtained from rabbit B9. Linear regression of the data for $t > 0.5$ days produced the continuous line.

Fig. 6. Model of normal antithrombin III behaviour *in vivo*. AT within the vascular system distributes between the plasma (A_p) and endothelial binding sites. A pool of the latter (A_w) serves both to activate AT for proteinase reaction and as a receptor mediating AT transport to extravascular space (A_e). Catabolism of AT occurs during transport of AT through the endothelial cell cytoplasm (cross-hatched portion of A_e). AT is returned to the plasma via the lymphatic system.

nism: after receptor association, AT-containing endocytotic vesicles may enter the endothelial cell cytoplasm, and then, instead of being transported to the extravascular surface (Renkin, 1980), some of these vesicles may combine with lysosomes, leading to breakdown of the AT. This paradigm is particularly appealing, because it at least partially explains the more rapid catabolism of AT_{vh} .

Plots like the one shown in Fig. 5 were also constructed for the other five rabbits injected with

Table 2. AT_{vh} and AT_h fractional rates of catabolism and the fractions in plasma (A_p), non-circulating vascular (A_w) and extra-vascular (A_e) compartments

j_T is the fraction of the total-body AT catabolized daily (Carlson *et al.*, 1984); j_3 is the fraction of plasma AT catabolized daily; $j_{3.5}$ is the fraction of vascular AT (plasma + non-plasma vascular) catabolized daily (Carlson *et al.*, 1984). The kinetic constants were used to calculate the fractions of AT_h and AT_{vh} in A_p , A_w and A_e . The bottom set of data were obtained by dividing AT_{vh} data by AT_h data.

Molecular form of AT	Rabbit no.	j_T	j_3	$j_{3.5}$	A_p	A_w	A_e
AT_{vh}	R14	0.4129	1.89	0.933	0.218	0.224	0.558
	B7	0.3994	1.81	0.998	0.221	0.180	0.599
	B8	0.4033	1.84	1.23	0.219	0.109	0.672
	B9	0.4119	1.96	1.07	0.210	0.175	0.615
	B10	0.3520	1.94	0.931	0.181	0.197	0.622
	B11	0.3725	1.85	1.14	0.202	0.126	0.672
	B12	0.3603	1.85	0.829	0.194	0.240	0.566
	Mean ($n = 6$)	0.3832	1.88	1.03	0.204	0.171	0.624
	$\pm$ S.D.	± 0.0227	± 0.06	± 0.13	± 0.014	± 0.044	± 0.038
	Mean ($n = 7$)	0.3875	1.88	1.02	0.206	0.179	0.615
	$\pm$ S.D.	± 0.0235	± 0.05	± 0.13	± 0.014	± 0.044	± 0.042
AT_h	B7	0.2734	0.759	0.505	0.360	0.181	0.459
	B8	0.2683	0.776	0.567	0.346	0.127	0.527
	B9	0.2789	0.806	0.523	0.346	0.187	0.467
	B10	0.2516	0.748	0.442	0.336	0.233	0.431
	B11	0.2474	0.738	0.524	0.335	0.137	0.528
	B12	0.2601	0.751	0.501	0.346	0.173	0.481
	Mean	0.2633	0.763	0.510	0.345	0.173	0.482
	$\pm$ S.D.	± 0.0113	± 0.023	± 0.037	± 0.008	± 0.035	± 0.035
AT_{vh}/AT_h	B7	1.46	2.38	1.98	0.614	0.994	1.31
	B8	1.50	2.37	2.17	0.633	0.858	1.28
	B9	1.48	2.43	2.05	0.607	0.936	1.32
	B10	1.40	2.60	2.11	0.539	0.845	1.44
	B11	1.51	2.51	2.17	0.603	0.920	1.27
	B12	1.39	2.47	1.65	0.561	1.39	1.18
	Mean	1.46	2.46	2.02	0.593	0.990	1.30
	$\pm$ S.D.	± 0.05	± 0.08	± 0.18	± 0.032	± 0.185	± 0.08

both labelled proteins. From linear regression the slopes of the linear portion of these curves averaged 0.713 ± 0.068 for the six animals. This reflects the relative rates of catabolism of the forms of AT, and agrees well with the average AT_v/AT_{vh} ratios of j_T values of 0.688 ± 0.023 (see Table 2). Furthermore, when these curves were extrapolated to $t = 0$, a ratio for $^{125}\text{I-AT}_h/^{131}\text{I-AT}_{vh}$ of 1.35 ± 0.13 was obtained. Assuming the model in Fig. 6, this value gives the ratio of the fractions of vascular AT_{vh} and AT_h in the receptor pool, A_w , and it is essentially identical with the 1.36 ± 0.14 value calculated from the sizes of the two vascular compartments.

In addition to AT, isoforms of other proteinase inhibitors with different charges and molecular masses are present in plasma. Thus two forms of α_1 -antitrypsin, have been found (Koj *et al.*, 1978), which differ in physical and biological properties (Regoez *et al.*, 1980) like the two forms of AT. Also, two forms of α_2 -antiplasmin (Clemmensen *et*

al., 1981) and an inhibitor of activated protein C (Suzuki *et al.*, 1983) can be isolated from plasma. Studies of the behaviours of these molecules *in vivo* have not yet been carried out.

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