Br. J. exp. Path. (1978) **59**, 473

EFFECT OF EXPERIMENTAL INFLAMMATION ON THE SYNTHESIS AND DISTRIBUTION OF ANTITHROMBIN III AND α_1-ANTITRYPSIN IN RABBITS

A. KOJ* AND E. REGOECZI

From the Department of Pathology, McMaster University Health Sciences Centre, Hamilton, Ontario, Canada L8S 4J9

Received for publication April 10, 1978

Summary.—Local inflammation, induced by s.c. injection of turpentine, evoked characteristic changes in the metabolism of antithrombin III, and α_1-antitrypsin. For a period of approximately 36 h, the plasma half-lives of both protease inhibitors were shortened to 70–74% of the respective preinjection values. Similar changes were also observed in the slope of iodine-labelled albumin, suggesting that increased capillary permeability was primarily responsible for the losses of labelled proteins from the circulation.

Incorporation of [^{3}H]- or [^{14}C]-leucine into albumin changed little during inflammation, but markedly increased values were measured for anti-thrombin III (3-fold), α_1-antitrypsin (4-fold) and, above all, for fibrinogen (7-fold) 24 h and 48 h after the injection of turpentine.

These changes in synthesis and elimination rates resulted in the following net balances: fibrinogen concentrations in plasma rose substantially during the early phase of inflammation; α_1-antitrypsin concentrations increased gradually but to a significantly lesser extent, peak concentrations being reached after a reverse trend in fibrinogen concentrations had become apparent; antithrombin III concentrations remained steady throughout at levels which were only marginally above the pre-treatment values.

THE concept that the biological integrity of a variety of tissues is intimately linked with the availability and proper functioning of serum protease inhibitors is relatively new. Well known examples are the high incidence of chronic obstructive lung disease in α_1-antitrypsin (α_1-AT) deficiency (see review by Kueppers, 1973), and thrombophilia accompanying antithrombin III (AT III) abnormalities (Egeberg, 1965; Marciniak, Farley and DeSimone, 1974; Sas *et al.*, 1974).

Extracellular protease activity, derived either from the activation of circulating zymogens or from the release of lysosomal enzymes (Reynolds, 1973), varies with the state of health, and this poses the question as to the protease/inhibitor balance under non-steady-state conditions. Increased protease activity, including granulocyte elastase, has long been recognized as an important mediator of the inflammatory reaction (Weissmann, Spilberg and Krakauer, 1969; Janoff, 1972). Furthermore, leucocyte proteases interact *in vivo* with serum protease inhibitors (Ohlsson, 1975). Therefore, it seemed interesting to investigate the synthesis and distribution of protease inhibitors during the development of a sterile abscess. Here we report our observations with rabbit α_1-AT and AT III.

* Present address: Department of Animal Biochemistry, Institute of Molecular Biology, Jagiellonian University, Grodzka 53, 31-001 Krakow, Poland.

MATERIALS AND METHODS

Animals.—Male New Zealand White rabbits, 3·1–4·5 kg body wt, were kept in individual metabolic cages and were provided with a pelleted diet and water *ad libitum*. In experiments with iodine-labelled proteins, 0·005% (w/v) NaI was added to the drinking water. Local inflammation was induced by a single s.c. injection of turpentine (0·2 ml/kg) in the scapular region.

Chemicals.—Turpentine oil was obtained from Rexell Drug Co. (Toronto, Ontario, Canada), heparin from Sigma Chemical Co. (St Louis, Mo., U.S.A.), DEAE–Sephadex and cross-linked Sepharose CL 4B from Pharmacia Fine Chemicals (Uppsala, Sweden), Arvin from Twyford Laboratories (Beckenham, Kent, U.K.), L-[^{3}H] leucine, L-[^{14}C] leucine, Na^{125}I, and Na^{131}I from New England Nuclear (Boston, Mass., U.S.A.), Quantafluor scintillation mixture from Mallinckrodt Inc. (St. Louis, Mo., U.S.A.), goat antiserum to whole rabbit plasma from Miles-Yeda (Kankakee, Ill., U.S.A.). All other chemicals were A.R. grade, where available.

Experimental design.—Three kinds of experiments were carried out to assess any change which may occur in the metabolism of α_1-AT and AT III during inflammation, namely: (1) determinations of protein concentrations in plasma, (2) measurements of the incorporation of radioactive leucine, and (3) studies of the disappearance of iodine-labelled proteins from the circulation. In each of these categories, data were also obtained on fibrinogen and albumin for comparison.

Measurement of the concentrations of AT III, α^1-AT, albumin and fibrinogen in plasma samples.—Blood samples were collected into acid–citrate–dextrose solution using 1 vol of the anticoagulant for 5 vol of blood. The total protein content of the resulting plasma was determined by the method of Lowry *et al.* (1951) using bovine serum albumin as a standard. The same measurement was also performed on small parallel samples taken into dry heparin and the dilution factor was calculated.

Concentrations of AT III and α_1-AT were measured either by quantitative crossed immunoelectrophoresis using goat antiserum to whole rabbit plasma or by rocket electrophoresis using monospecific guinea-pig antisera (Weeke, 1973). The reactivity of the commercial goat antiserum with AT III was originally inadequate, thus necessitating supplementation with the IgG fraction from a guinea-pig antiserum to rabbit AT III. Both methods were calibrated with homologous, pure AT III and α_1-AT (see below).

Albumin concentration was determined as follows. To 0·1 ml. plasma, a trace of rabbit [^{125}I]-albumin and 0·15 M NaCl were added to a final volume of 1 ml. After its radioactivity had been determined, the sample was passed through a column (0·6 cm × 3 cm) of Sepharose–Cibacron Blue (Travis *et al.*, 1976) equilibrated with 0·14 M NaCl in 0·005 M Tris/HCl, pH 8·8. The column was washed with 25 ml of the equilibrating buffer, then albumin was eluted by five 1 ml portions of 0·3 M NaSCN. From the protein content (Lowry *et al.*, 1951) and radioactivity of the eluate the albumin concentration in plasma was calculated.

Fibrinogen concentration was determined by a clotting assay as described elsewhere (Koj and McFarlane, 1968).

Comparison of the incorporation of radioactive leucine into AT III, α-$_1$AT, albumin and fibrinogen.—Control and turpentine-treated animals were given an i.v. injection of [^{3}H] leucine (25 μCi/kg) or [^{14}C] leucine (8 μCi/kg) in pyrogen-free saline and 5 h later enough blood was collected into acid–citrate–dextrose to yield approximately 20 ml citrated plasma. The blood was centrifuged at 3000 *g* for 5 min and the plasma clarified by further centrifugation at 10,000 *g* for 10 min. Specimens were processed either immediately or after storage at −20°C. Specific activities of the four proteins of interest were determined after their purification in a 4-stage fractionation procedure briefly outlined below.

Stage 1: Fibrinogen. Arvin (3 units in 5 ml of 0·9% NaCl) was added to the plasma (20 ml) and the mixture was left at room temperature. After 5 h, the clot was wound on to a glass rod and immersed overnight in 5 ml 0·9% NaCl. The washing was added back to the serum and the clot was rinsed 3 times in 0·9% NaCl (20 ml) before dissolution in 3 ml monochloroacetic acid (2% w/v). Insoluble material was removed by centrifugation and repolymerization of the fibrin was induced by the addition of 30 ml 0·05 M Tris/HCl, pH 8·8. The meshwork was brought to collapse by centrifugation at 3000 *g* for 5 min, the clot liquor decanted and the fibrin dissolved in 5 ml. 2% monochloroacetic acid. Finally, the solution was clarified by centrifugation.

Stage 2: AT III. The serum obtained in Stage 1 was saturated to 40% with a saturated solution of $(NH_4)_2SO_4$ and, after 2 h at room temperature, the precipitate was removed by centrifugation at 10,000 *g* for 10 min. The supernatant was exhaustively dialysed against 0·02 M sodium phosphate, pH 7·4, containing 0·15 M NaCl. A column (1 cm × 5 cm) of Sepharose–heparin (Miller-Andersson, Borg and Andersson, 1974) was equilibrated with the same buffer and the protein solution passed through it. The column was washed with 200 ml 0·02 M sodium phosphate, pH 7·4, containing 0·3 M NaCl before step-eluting AT III by 0·3 M lysine, pH 8·0. The displaced protein was

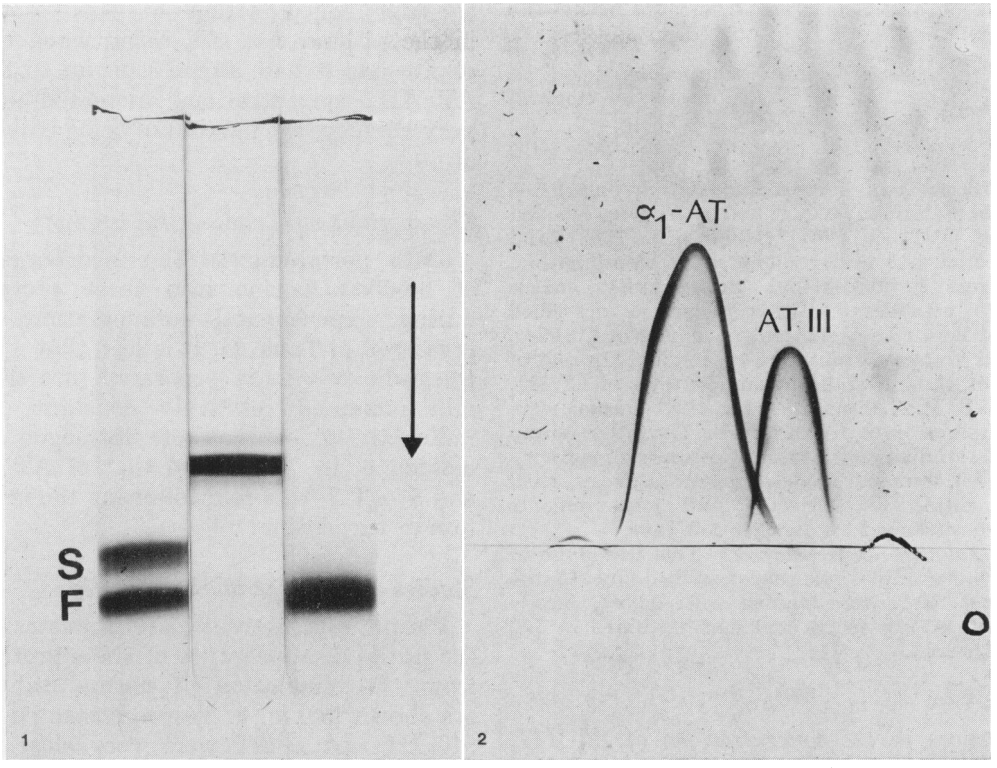

FIG. 1.—Electrophoretograms of rabbit α_1-AT (left), rabbit AT III (centre) and rabbit serum albumin (right) in 7·5% polyacrylamide gel. F and S are the fast and slow components of α_1-AT. Direction of migration is indicated by the arrow. Load: approx. 40 μg protein per gel.

FIG. 2.—Two-dimensional crossed immunoelectrophoresis of rabbit α_1-AT (4·6 μg) and rabbit AT III (2·8 μg) in agarose gel. Electrophoresis was for 100 min at 24 V/cm and 4°, followed by immunoelectrophoresis (5 V/cm, 15 h, 4°) against goat antiserum to whole rabbit plasma enriched with IgG from a guinea-pig serum to rabbit AT III. The small arcs, one on each side of the main peaks, are due to trace impurities.

concentrated by pressure dialysis against 0·005 M Tris/HCl, pH 8·0. The yield from 20 ml plasma was 2–3 mg AT III. Under conditions given elsewhere for α_1-AT (Koj *et al.*, 1978), 1 mg AT III inhibited 0·30–0·37 mg bovine trypsin. In alkaline polyacrylamide gels AT III electrophoresed as 3–4 closely spaced bands (Fig. 1), all of which were displaced by preincubation with thrombin. However, occasionally (and more often after turpentine treatment) small quantities of a contaminating protein were also detected (Fig. 2). This impurity was of a relatively slower anodic mobility and it did not interact with thrombin.

Stage 3: α_1-AT. The protein fraction which passed straight through Sepharose–heparin was combined with the first 10 ml washing fluid from this column. α_1-AT was isolated from this starting material by a four-step purification procedure, involving salt fractionation, chromatographies on DEAE–Sephadex at pH 8·8 and 6·5, and affinity chromatography on Sepharose–Cibacron Blue (see Method A in Koj *et al.*, 1978). The yield from 20 ml plasma was 6–7 mg α_1-AT. A preparation of α_1-AT is shown in Figs 1 and 2.

Stage 4: Albumin. This protein was obtained in a pure state (Fig. 1) during the isolation of α_1-AT by adsorption to a column of Sepharose–Cibacron Blue and subsequent elution by 0·3 M NaSCN as already described. Thiocyanate was removed by dialysis against 0·005 M Tris/HCl, pH 8·0.

Aliquots of the purified proteins were used for protein determination (Lowry *et al.*, 1951) and counting radioactivity in a Beckman LS-230 β-spectrometer. From the specific activity and the concentration of a protein in undiluted plasma the total amount of [^{3}H] or [^{14}C] activity incorporated into that protein present

in 1 ml of plasma was calculated using the formula:

$$\text{Activity} = \frac{\text{specific activity (dpm/mg)} \times \text{concentration (mg/ml)}}{\text{leucine content (res./mole)} \times \text{dose } (\mu\text{Ci})}$$

Experiments with iodine-labelled proteins.—Rabbit albumin, AT III and α_1-AT were labelled with [^{131}I] or [^{125}I] (McFarlane, 1958) and injected i.v. either alone or in combination. Plasma protein-bound radioactivity curves were established by a technique described elsewhere (Regoeczi, Hatton and Wong, 1974). Two groups of rabbits were studied. In one of them, the initial disappearance rates of AT III, α_1-AT and albumin from the plasma were compared over the first 5 h. The other group was studied until the plasma radioactivity curves assumed single-exponential decay, then turpentine was injected and measurements were continued for another 2–3 days.

Analytical electrophoresis.—This was done in polyacrylamide gels as described by Clarke (1964). Gels were stained with 0·05% Amido Black in 7% acetic acid and destained in 7% acetic acid.

RESULTS

Changes in the concentrations of AT III, α_1-AT, albumin and fibrinogen during inflammation

The relevant information is summarized in Table I and shows a varied response of the four proteins under investigation. Thus, concentrations of albumin changed little, except for a transient decrease at 24 h; fibrinogen, as expected, rose sharply with maximal values at 48 h; in contrast, increases in α_1-AT were less pronounced and spread over a longer period of time, so that concentration of this protein in the plasma was still rising when that of fibrinogen had already begun to fall; AT III concentrations increased only very slightly, the maximum being reached at 48 h.

Observations with radioactive leucine

Data pertaining to the incorporation of labelled leucine into these proteins during experimental inflammation are presented in Table II. It is seen that while the radioactivity incorporated into albumin remained relatively constant, the radioactivity content of fibrinogen increased 6- to 7-fold, and that of AT III and α_1-AT 3- to 4-fold following the injection of turpentine.

Studies with iodine-labelled proteins

Plasma radioactivity curves illustrating the initial disappearance of the 4 proteins from the circulation of normal rabbits are shown in Fig. 3. Values measured for AT III and α_1-AT were very close. In contrast, albumin-bound radioactivity decreased slightly more slowly and labelled fibrinogen considerably more slowly over the period of interest.

Examples for the effect of experimental inflammation on the plasma slopes of albumin, AT III and α_1-AT are shown in Fig. 4. It may be observed that turpentine brought about a moderate acceleration of the slope of each protein, lasting for approx. 36 h. After that, concentrations

TABLE I.—*Plasma Concentrations of Four Proteins at Intervals after the Subcutaneous Administration of Turpentine to Rabbits**

Time (h)	Albumin			Antithrombon III			α_1-Antitrypsin			Fibrinogen		
	n	mg/ml	%change	n	mg/ml	%change	n	mg/ml	%change	n	mg/ml	%change
0	4	39·1 (±1·9)	0	12	0·36 (±0·01)	0	15	1·08 (±0·05)	0	8	2·46 (±0·16)	0
24	4	36·0 (±1·7)	−7·9 (±4·7)	10	0·41 (±0·02)	+13·5 (±5·1)	11	1·28 (±0·07)	+18·5 (±5·4)	8	5·47 (±0·43)	+122·3) (±7·9)
48	4	38·7 (±0·6)	−1·1 (±1·5)	6	0·47 (±0·05)	+29·7 (±11·2)	7	1·55 (±0·13)	+43·5 (±8·4)	4	8·29 (±0·44)	+236·9 (±5·3)
72	–	—	—	3	0·45 (±0·05)	+23·9 (±10·9)	3	1·90 (±0·17)	+75·9 (±8·9)	3	6·43 (±0·29)	+161·3 (±4·5)

* Numbers of animals studied are listed under "n". Values are means with standard errors in parentheses. % changes are expressed relative to the zero time values.

TABLE II.—*Plasma Concentrations and Radioactive Leucine Contents of Four Proteins at Intervals after the Subcutaneous Injection of Turpentine to Rabbits**

Time after turpentine (h)	Albumin		Fibrinogen			Antithrombin III			α_1-Antitrypsin		
	mg/ml	Activity† $\times 10^2$	mg/ml	Activity† $\times 10^2$	Ratio‡	mg/ml	Activity† $\times 10^2$	Ratio‡	mg/ml	Activity† $\times 10^2$	Ratio‡
0	39·1 (±1·8)	449·8 (±22·1)	2·56 (±0·10)	10·8 (±1·1)	2·4 (±0·2)	0·38 (<0·01)	11·6 (±1·1)	2·6 (±0·3	1·03 (±0·15)	27·5 (±2·9)	5·0 (±0·7)
24	34·2 (±1·2)	461·7 (±34·4)	6·09 (±0·71)	75·5 (±16·7)	16·5 (±1·0)	0·46 (±0·02)	31·8 (±3·7)	7·0 (±1·1)	1·19 (±0·08)	83·7 (±6·4)	18·2 (±1·0)
48	38·0 (±0·9)	432·0 (±7·0)	8·28 (±0·44)	65·9 (±13·1)	16·3 (±1·5)	0·48 (±0·08)	36·5 (±5·0)	9·1 (±1·1)	1·39 (±0·18)	97·3 (±10·7)	24·4 (±1·9)

* Values are the means of four experiments and the standard errors of the means in parentheses.
† As defined in Material and Methods.
‡ Ratio is the "activity" of a protein expressed as a percentage of that of albumin (*e.g.* ratio AT III at 0 h: $11 \cdot 6 \times 100 \div 449 \cdot 8 = 2 \cdot 6$).

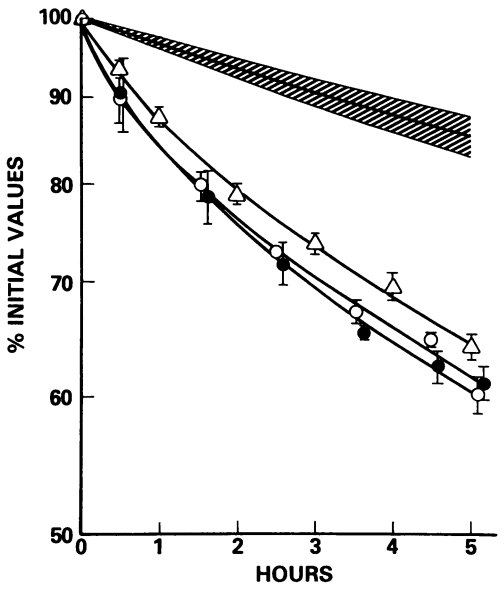

FIG. 3.—Disappearance of homologous, iodine-labelled proteins from the plasma volume of rabbits over the first 5 h of i.v. administration. AT III (○) and α_1-AT (●) were studied in the same recipients ($n=3$), while albumin (△) was studied in 3 other rabbits of comparable body weights. Dose: approx. 1 mg protein each. Plots represent mean protein-bound radioactivities per ml of plasma, the vertical bars are standard errors. The upper curve was computed for fibrinogen from an earlier study in 10 rabbits (Regoeczi, 1970*a*), the shaded area representing ± 1 s.d.

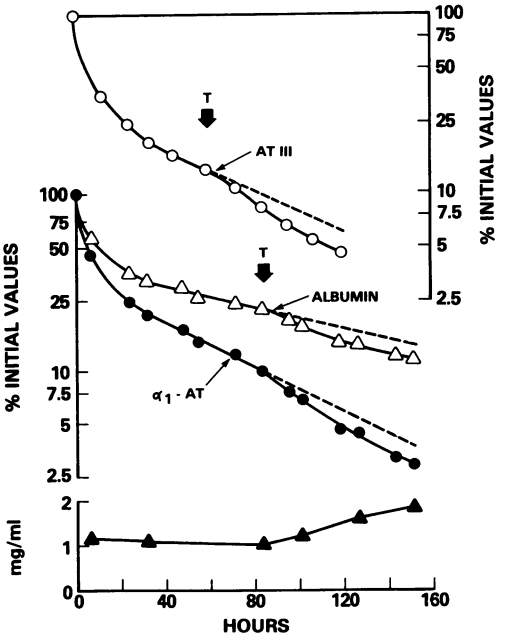

FIG. 4.—Effect of turpentine (T) on the plasma slopes of homologous [^{131}I]- AT III, [^{125}I]-albumin and [^{131}I]-α_1-AT in the rabbit. Albumin and α_1-AT were studied simultaneously in the same recipient, AT III in another rabbit. Plots represent protein-bound radioactivities per ml of plasma, the dashed lines the expected slopes. α_1-AT concentrations from the dual-label experiment are shown in the lower diagram (▲).

of the radioactive proteins remained slightly below expected values. During the period of accelerated disappearance, the half-lives of these proteins were shortened to 70–74% of the respective pretreatment values.

DISCUSSION

From the foregoing observations, the question posed in the introduction may be answered as follows. Local inflammation induced by the s.c. injection of turpentine promotes, for a limited duration, the transcapillary escape (and, perhaps, the subsequent catabolism) of both AT III and α_1-AT. The resulting imbalance is compensated for by concomitant changes in the synthesis rates of these proteins, whereby a decrease in the concentrations of both protease inhibitors is effectively prevented. The response of AT III synthesis to stimulation is such that the concentration of this protein in the plasma during acute inflammation remains almost steady at a level which is only marginally above normal. In contrast, α_1-AT synthesis clearly over-reacts, thus giving rise to the gradual accumulation of excess inhibitor in the plasma. The reason for the transient shortening of the half-lives of AT III and α_1-AT after turpentine injection is not altogether clear. Moderate alterations in capillary permeability may be inferred from the fact that the slope of plasma albumin was also affected. Similar plasma radioactivity curves were observed by Mouridsen (1969) with albumin after injecting histamine. On the

other hand, the plasma slope of the much larger fibrinogen molecule is not affected by the injection of turpentine (Regoeczi, 1970*b*). Some of the α_1-AT and AT III that disappeared could have interacted with proteases released from the damaged tissues, but to what extent is not apparent from the data.

Although the experiments described here were primarily concerned with the two serum protease inhibitors, albumin and fibrinogen were routinely included as reference proteins because they have been extensively studied in the past under comparable conditions (*e.g.* Koj, 1968; Koj and McFarlane, 1968). The early, substantial rise in plasma fibrinogen concentrations, together with the essentially unchanged rates of incorporation of a labelled amino acid into albumin, are in good agreement with previous observations and thus confirm the successful indication of the acute-phase reaction (Koj, 1974; Gordon, 1976). By perfusing livers obtained from rats at different times after tissue injury, Gordon and Koj (1968) found that maximal stimulation of α_1-globulin synthesis was considerably delayed relative to that of fibrinogen. From the present observations the same conclusion appears justifiable also for rabbit α_1-AT.

Data in Table II show that quantities of the labelled precursor incorporated into fibrinogen, AT III and α_1-AT during inflammation bore no direct relationship to the changes in the plasma concentrations of these proteins. If a precursor is incorporated from the same amino acid pool into two or more classes of plasma proteins simultaneously, quantities of the radioactivity appearing in these proteins should be proportional to their absolute synthesis rates. Prevalence of such a proportionality cannot be inferred from the present data for technical reasons. Nevertheless, as is apparent from Table III, the precursor radioactivity contents of the proteins under study (except for that of α_1-AT) compare favourably with their absolute catabolic rates as calculated from independent experiments.

TABLE III.—*Comparison of the Absolute Catabolic Rates of Albumin, Fibrinogen, AT III and α_1-AT with the Relative Quantities of Radioactivity Incorporated into them Following the Injection of Labelled Leucine**

Protein	Absolute catabolic rate		[^{3}H] or [^{14}C] Activity ratio
	μmoles. d^{-1}	Ratio	
Albumin†	17	—	—
Fibrinogen‡	0·36	2·1	2·4
AT III§	0·51	3·0	2·6
α_1-AT§	1·5	8·8	5·0

* [^{3}H] or [^{14}C] activity ratios are control values from Table II, absolute catabolic rate ratios are the absolute catabolic rate of a protein expressed as a percentage of that of albumin.

† Calculated from data by Reeve, Pearson and Martz (1963).

‡ Calculated from earlier data (Regoeczi, 1970*a*).

§ From unpublished studies in this laboratory.

Therefore, a likely explanation of the discrepancies between protein mass and radioactivity values in Table II is the net balance between fractional catabolic rates and the absolute synthesis rates, with fibrinogen as the obvious example. The fractional catabolic rate of this protein has repeatedly been shown to be independent of the mass of fibrinogen present (Atencio, Joiner and Reeve, 1969*a*; Regoeczi, 1970*a*), implying that rising absolute synthesis rates are paralleled by rising absolute catabolic rates. Consequently, to double plasma concentreation the liver secretes fibrinogen at well over twice the normal rate (Reeve and Chen, 1973). The 7-fold increase in the synthesis rate and 3-fold rise in plasma fibrinogen concentration observed here are in keeping with the values reported earlier by Koj (1968) and those measured by Atencio *et al.* (1969*b*) after injecting ACTH in rabbits.

The situation with the two serum protease inhibitors is different in several aspects. First of all, their synthesis rates are stimulated less during inflammation (see Ratios in Table II); secondly, their normal fractional catabolic rates are

approximately twice the fractional catabolic rate of fibrinogen (E. Regoeczi and A Koj, unpublished); thirdly, unlike fibrinogen, both inhibitors are subject to increased transcapillary losses during the early phase of the inflammatory reaction. Acting in combination, these three factors would clearly tend to diminish (α_1-AT)—*viz.*, minimize (AT III) the impact of increased synthesis rates on the plasma concentrations of the protease inhibitors.

AT III is an important inhibitor of a number of procoagulant enzymes (Rosenberg, 1977), and the overall impression from the present experiments is that the mass of AT III available at any one time in the circulation is controlled more tightly than that of α_1-AT. Whether this is a purposeful arrangement, aimed at preventing hypocoagulability due to excess AT III, remains to be explored in the future.

These investigations were supported by the Canadian Heart Foundation by an award to A. K. and by the Medical Research Council of Canada by a grant. We wish to thank Dr J Gauldie for the generous supply of antisera, Dr M. W. C. Hatton for Sepharose–heparin, and Miss B. Ausrotas and Mrs P. Taylor for skilled technical assistance.

REFERENCES

ATENCIO, A. C., JOINER, K. & REEVE, E. B. (1969*a*) Experimental and Control Systems Studies of Plasma Fibrinogen Regulation in Rabbits. *Am. J. Physiol.*, **216**, 764.

ATENCIO, A. C., CHAO, P.-Y., CHEN, A. Y. & REEVE, E. B. (1969*b*) Fibrinogen Response to Corticotropin Preparations in Rabbits. *Am. J. Physiol.*, **216**, 773.

CLARKE, J. T. (1964) Simplified Disc (Polyacrylamide Gel) Electrophoresis. *Ann. N.Y. Acad. Sci.*, **121**, 428.

EGEBERG, O. (1965) Inherited Antithrombin Deficiency Causing Thrombophilia. *Thromb. Diath. haemorrh.*, **13**, 516.

GORDON, A. H. (1976) The Acute Phase Plasma Proteins. In *Plasma Protein Turnover*. Eds. R. Bianchi, G. Mariani and A. S. McFarlane. London: MacMillan Press Ltd. p. 381.

GORDON, A. H. & KOJ, A. (1968) Changes in the Rates of Synthesis of Certain Plasma Proteins following Tissue Damage Due to Talc Injection. *Br. J. exp. Path.*, **49**, 436.

JANOFF, A. (1972) Human Granulocyte Elastase. *Am. J. Path.*, **68**, 579.

KOJ, A. (1968) The Measurement of Absolute and Relative Synthesis Rates of Fibrinogen *in vivo*. *Biochem. biophys. Acta*, **165**, 97.

KOJ, A. (1974) Acute-phase Reactants. In *Structure and Function of Plasma Proteins*. Ed. A. C. Allison, Vol. 1. London and New York: Plenum Press. p. 73.

KOJ, A., HATTON, M. W. C., WONG, K.-L. & REGOECZI, E. (1978) Isolation and Partial Characterization of Rabbit Plasma α_1-Antitrypsin. *Biochem. J.*, **169**, 589.

KOJ, A. & MCFARLANE, A. S. (1968) The Effect of Endotoxin on Plasma Albumin and Fibrinogen Synthesis Rates as Measured by the ^{14}C-Carbonate Method. *Biochem. J.*, **108**, 137.

KUEPPERS, F. (1973) α_1-Antitrypsin. *Am. J. hum. Genet.*, **25**, 677.

LOWRY, O. H., ROSEBROUGH, N. J., FARR, A. L. & RANDALL, R. J. (1951) Protein Measurement with the Folin Phenol Reagent. *J. biol. Chem.*, **193**, 265.

MARCINIAK, E., FARLEY, C. H. & DESIMONE, P. A. (1974) Familial Thrombosis Due to Antithrombin III Deficiency. *Blood*, **43**, 219.

MCFARLANE, A. S. (1958) Efficient Trace-labelling of Proteins with Iodine. *Nature, Lond.*, **182**, 53.

MILLER-ANDERSSON, M., BORG, H. & ANDERSSON. L.-O. (1974) Purification of Antithrombin III by Affinity Chromatography. *Thromb. Res.*, **5**, 439.

MOURIDSEN, H. T. (1969) The Extravascular Retention of Albumin in Wound Tissue and its Contribution to the Postoperative Hypoalbuminaemia in Rabbits. *Clin. Sci.*, **37**, 431.

OHLSSON, K. (1975) Alpha-1-Antitrypsin and Alpha-2-Macroglobulin Interaction with Human Neutrophil Collagenase and Elastase. *Ann. N.Y. Acad. Sci.*, **256**, 409.

REEVE, E. B. & CHEN, Y. (1973) Studies with a Mass Balance Method of Measuring Fibrinogen Synthesis. In *Ciba Foundation Symposium* 9 (New Series) Amsterdam: Elsevier. p. 91.

REEVE, E. B., PEARSON, J. R. & MARTZ, D. C. (1963) Plasma Protein Synthesis in the Liver: Method for Measurement of Albumin Formation *in vivo*. *Science, N.Y.*, **139**, 914.

REGOECZI, E. (1970*a*) Fibrinogen Catabolism: Kinetics of the Catabolism following Sudden Elevation of the Pool with Exogenous Fibrin. *Clin. Sci.*, **38**, 111.

REGOECZI, E. (1970*b*) Abnormal Fibrinogen Metabolism. In *Plasma Protein Metabolism*. Eds. M. A. Rothschild and T. Waldmann. New York: Academic Press. p. 459.

REGOECZI, E., HATTON, M. W. C. & WONG, K.-L. (1974) Studies of the Metabolism of Asialotransferrins: Potentiation of the Catabolism of Human Asialotransferrin in the Rabbit. *Can. J. Biochem.*, **52**, 155.

REYNOLDS, J. J. (1973) Connective Tissue Catabolism and the Role of Lysosomal Enzymes. In *Lysosomes in Biology and Pathology*. Eds. J. T. Dingle and H. B. Fell, Vol. 2. Amsterdam: North Holland Publishing Co. p. 163.

ROSENBERG, R. D. (1977) Chemistry of the Hemostatic Mechanism and its Relationship to the Action of Heparin. *Fed. Proc.*, **36**, 10.

SAS, G., BLASKÓ, G., BÁNHEGYI, D., JÁKÓ, J. & PÁLOS, L. V. (1974) Abnormal Antithrombin III (Antithrombin III "Budapest") as a Cause of

a Familial Thrombophilia. *Thromb. Diath. haemorrh.*, **32**, 105.

TRAVIS, J., BOWEN, J., TEWKSBURY, D., JOHNSON, D. & PANNELL, R. (1976) Isolation of Albumin from Whole Human Plasma and Fractionation of Albumin Depleted Plasma. *Biochem. J.*, **157**, 301.

WEEKE, B. (1973) Crossed Immunoelectrophoresis. In *A Manual of Quantitative Immunoelectrophoresis, Methods and Applications*. Eds. N. H. Axelson, J. Kroll and B. Weeke, *Scand. J. Immun.*, **2**, Suppl. 1. p. 47.

WEISSMANN, G., SPILBERG, I., KRAKAUER, K. (1969) Inflammation Induced by Leucocyte Lysosomes. In *Inflammation Biochemistry and Drug Interaction*. Eds. A. Bertelli and J. C. Houck. Amsterdam: Excerpta Medica Foundation. p. 12.