Turnover of factor X and of prothrombin in rabbits fed on a standard or cholesterol-supplemented diet

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The turnover of prothrombin and of factor X was investigated in rabbits fed on a 1% -cholesterol-supplemented or a standard diet by studying the evolution of radioactivity in blood and in plasma from these animals after the intravenous injection of either ^{125}I -rabbit factor X or ^{125}I -bovine prothrombin. For factor X, half-lives and fractional pool sizes were similar for the two groups of rabbits in the extravascular, intravascular and plasma compartments. However, the equivalent plasma fractional pool size for the two groups of rabbits was only 73% of that in the intravascular compartment. The fractional catabolic rate for the hypercholesterolaemic rabbits [0.064 ± 0.007 (of the intravascular pool)/h] was not significantly different from that in the rabbits fed on the standard diet (0.074 ± 0.008 /h). However, the absolute catabolic rate, and therefore the rate of synthesis, was significantly higher (1.261 ± 0.141 mg/day per kg body wt. of rabbit) in the rabbits fed on the cholesterol-supplemented than that in the rabbits fed on the standard diet (0.705 ± 0.019 mg/day per kg). The prothrombin half-lives and fractional pool sizes were similar for the two groups of rabbits in the extravascular and the intravascular compartments. The fractional catabolic rate for the hypercholesterolaemic rabbits [0.041 ± 0.003 (of the plasma pool)/h] was not significantly different from that in the rabbits fed on the standard diet (0.035 ± 0.003 /h). However, the absolute catabolic rate and therefore the rate of prothrombin synthesis was significantly higher (3.96 ± 0.48 mg/day per kg body wt.) in the rabbits fed on the cholesterol-supplemented than that in the rabbits fed on the standard diet (2.24 ± 0.12 mg/day per kg).

INTRODUCTION

The prospective Northwick Park Heart Study has examined the possible role of the haemostatic system in the development of ischaemic heart disease and has shown that high levels of factor VII coagulant activity (VIIc) are associated with disease incidence in men (Meade *et al.*, 1980, 1986; Meade, 1983). The association of VIIc with plasma lipid concentrations has been a consistent feature and points to the plasma lipids as determinants of VIIc (Dalaker *et al.*, 1985; Miller *et al.*, 1985, 1986; Scarabin *et al.*, 1985). The increase in VIIc associated with hyperlipidaemia has been attributed to an increase in the reactivity of factor VII rather than to a change in the concentration of this protein in plasma (Miller *et al.*, 1985). Factor VII normally circulates in plasma as a single-chain protein, and it is unique among the zymogens that participate in blood coagulation in that the single-chain protein has 0.8% of the activity of the two-chain form (αVIIa) (Nemerson, 1983; Radcliffe & Nemerson, 1976). Moreover, the zymogen can be converted into αVIIa by activated factors (βXIIa , IXa and Xa) generated by the intrinsic pathway of the coagulation system (Kiesel *et al.*, 1977; Radcliffe *et al.*, 1977; Seligsohn *et al.*, 1978).

In an attempt to investigate the sequence of events leading to the activation of factor VII *in vivo* and to describe the consequences of this increase in the flux of activated factors within the coagulation pathway, we

have previously studied VIIc and the activity of various coagulation factors in rabbits fed on a 1% -cholesterol-supplemented diet (Mitropoulos *et al.*, 1987). It has been shown that the increase in plasma cholesterol concentration, in response to the diet, is associated with a considerable increase in VIIc. This increase is due to a higher ratio of $\alpha\text{VIIa}/\text{VII}$ rather than to a higher concentration of the single-chain form of the protein. Moreover, the plasma concentrations of factor X and of prothrombin rose to 150–200% of those found in rabbits fed on a standard diet.

The aim of the present study was to study the turnover of prothrombin and of factor X in hypercholesterolaemic rabbits in order to see whether the elevated concentration of these proteins is due to an increased synthetic rate or to a decreased rate of removal of the proteins from the circulation.

MATERIALS AND METHODS

Animals and their treatment

Male New Zealand White rabbits (University Park Farms, Oxford, U.K.) weighed 2.5 kg on delivery. The rabbits were kept in cages with feed and water *ad libitum*, in a room maintained at 23 °C on a 12 h light/12 h dark cycle. All rabbits were fed on the standard diet for 1 week before they were randomly separated into two groups, each consisting of six animals. One group was fed on the

Abbreviations used: VIIc, factor VII coagulant activity; αVIIa , two-chain form of factor VII; VIIt, factor VII total functional protein; IIc, prothrombin coagulant activity; Xc, factor X coagulant activity; ^{125}I -ester, *N*-succinimidyl 3-(4-hydroxy-5-[^{125}I]iodophenyl)propionate.

standard diet, the other on the standard diet supplemented with 1% cholesterol (Special Diets Services Ltd., Witham, Essex, U.K.). For blood sampling rabbits were anaesthetized with Hypnorm (Crown Co., Lamberhurst, Tunbridge Wells, Kent, U.K.), injected intramuscularly (0.1 ml/kg). Blood samples (2–4 ml) were drawn from the peripheral ear vein with a butterfly-21 needle into a syringe containing 20% (of blood volume) of a 6% trisodium citrate solution. All the animals were treated in the same manner with regard to blood sampling and timing. For routine measurements of plasma cholesterol, triacylglycerols and the concentration of the various clotting factors, blood sampling was between 10:00 and 11:00 h at intervals of 5–7 days. After the injection of radiolabelled proteins, blood sampling (1.6–2.0 ml) was at the frequency indicated in the text. Citrated blood was centrifuged at room temperature at 3000 *g* for 15 min and the plasma was removed with a plastic Pasteur pipette.

Coagulation assays

All coagulation assays were performed in an H. Amelung KC10 Coagulometer (American Hospital Supply, Didcot, Oxon., U.K.) at 37 °C. The plasmas to be assayed were diluted appropriately using 0.05 M-Tris/HCl buffer (pH 7.5)/0.1 M-NaCl. A plot of log (clotting time) against the log dilution was constructed for each plasma. The results are expressed as the average ($\pm$ S.D.) percentage change of activity in each group as compared with that in a standard rabbit plasma. The preparation or supply of various factor-deficient plasmas, of rabbit brain thromboplastin, of Russell's-viper (*Vipera russelli russelli*) venom/cephalin mixture, and of tiger-snake (*Notechis scutatus scutatus*) venom/cephalin mixture was as reported previously (Mitropoulos *et al.*, 1987).

Assay of amidolytic activities

Factor X amidolytic activity was measured in the appropriately diluted plasmas on activation of factor X by purified Russell's-viper-venom factor X activator (Williams & Esnouf, 1962) in the presence of phospholipid (platelet substitute) and 1.5 mM-CaCl₂ in 0.05 M-Tris/HCl/0.1 M-NaCl buffer, pH 7.9 (Aurell *et al.*, 1977). The mixture was incubated for 6 min at 37 °C, then S-2222 (see below) (0.17 mM) was added and the initial rate of absorbance increase at 405 nm was measured. Activity is expressed as units/ml in the original plasma (1 unit is the factor Xa activity that results in release of 1 mol of *p*-nitroaniline/min (assuming an $\epsilon_{405} = 10 \text{ mM}^{-1} \cdot \text{cm}^{-1}$) or as mg of factor X/ml of plasma, calculated from the relation of purified rabbit factor X and its amidolytic activity. Prothrombin amidolytic activity was measured in diluted plasmas on conversion of factor II into thrombin with Ecarin (Sigma, Poole, Dorset, U.K.) in 0.05 M-Tris/HCl/0.1 M-NaCl buffer, pH 7.9 (Axelsson *et al.*, 1976). The mixture was incubated for 4 min at 37 °C, then S-2238 (0.2 mM) was added and the initial rate of absorbance increase at 405 nm was measured. Activity is expressed as units/ml or as mg of prothrombin/ml in the original plasma, calculated from the relation between purified bovine prothrombin and its amidolytic activity. The synthetic substrates (S-2222 and S-2238) were obtained from Flow Laboratories (Irvine, Ayrshire, Scotland, U.K.).

Factor VIII assays

The VIII assay was a modification of a previous method (Silverberg *et al.*, 1977) and employed [*sialyl*-³H]bovine factor X prepared as described previously (Mitropoulos *et al.*, 1987). This assay involves clotting of the plasma in the presence of high concentration of tissue factor. In the resultant serum, used in the assay, it is likely that all VII has been converted into α VIIa (Rao *et al.*, 1985). The concentration of α VIIa present in the serum is then measured from the rate of activation of excess of [³H]bovine factor X. VIII measures the concentration of functional VII rather than the activation state of this factor.

Purification and radiolabelling of proteins

Factor X was isolated from rabbit plasma by DEAE-Sephadex chromatography of a barium citrate eluate (Esnouf *et al.*, 1973). Further purification was achieved by a second chromatography on DEAE-Sephadex. The non-reduced protein was resolved by SDS/polyacrylamide-gel electrophoresis into a major and a minor band, the latter attributable to Xa. Coagulant activity was 80–100 units/mg of protein, and 2.5% of the activity was due to Xa (measured in the absence of Russell's-viper venom). Amidolytic activity was 55 units/mg of protein and, in the absence of Russell's-viper venom X activator, activity was 1.7 units/mg. Rabbit factor X was ¹²⁵I-labelled with the Bolton & Hunter (1973) reagent. A 300 μ g sample of the protein (600 μ g/ml) in 0.1 M-borate buffer, pH 8.5, was added to 500 μ Ci of ¹²⁵I-ester (2000 Ci/mmol) at 0 °C for 15 min. The protein was isolated on a 5.0 ml column of Sephadex G-25 equilibrated in 0.02 M-Tris/HCl/0.15 M-NaCl, pH 7.4. After determination of protein, radioactivity, coagulant and amidolytic activities, the pooled ¹²⁵I-factor X fractions were diluted with non-radio-labelled factor X, in the same buffer, to a concentration of 220 μ g of protein/ml. Approx. 23% of the labelled ester was incorporated into the isolated protein (1 mol of ¹²⁵I-ester/95 mol of factor X), corresponding to 185 d.p.m./ng of the diluted factor X. Coagulant activity and amidolytic activities were not affected by radiolabelling.

Prothrombin was isolated from bovine plasma by DEAE-Sephadex chromatography of a barium citrate eluate (Esnouf *et al.*, 1973). Further purification was achieved by re-chromatography on DEAE-Sephadex followed by fast protein liquid chromatography on Mono Q. The protein behaved as single band on SDS/polyacrylamide-gel electrophoresis, and it had 51 units of amidolytic activity/mg. This was ¹²⁵I-labelled with the Bolton & Hunter (1973) reagent. A solution of the protein (1.4 mg/0.8 ml) in 0.1 M-borate buffer, pH 8.5, was incubated with 500 μ Ci of the ¹²⁵I-ester (2000 Ci/mmol) at 4 °C for 15 min. The protein was isolated by filtration on a 10 ml column of Sephadex G-25 equilibrated and eluted with 0.02 M-Tris/HCl/0.15 M-NaCl buffer, pH 7.4. The pooled ¹²⁵I-prothrombin fractions were diluted with non-radiolabelled prothrombin, in the same buffer, to 400 μ g/ml. Approx. 30% of the labelled ester was incorporated into the isolated protein (1 mol of ¹²⁵I-ester per 248 mol of prothrombin), corresponding to 240 d.p.m./mg. The amidolytic and coagulant activities were not affected by radiolabelling.

Metabolic studies

Three rabbits fed on the cholesterol-supplemented diet were weight-matched with three rabbits fed on the standard diet on the day 85 from the beginning of treatment. At zero time, 1 ml of ^{125}I -factor X was injected into the left ear peripheral vein, and 1–2 min after the injection a blood sample was taken from the right ear peripheral vein. For the studies involving prothrombin, three rabbits fed on the cholesterol-supplemented diet and three rabbits fed on the standard diet were used 40 days from the beginning of treatment. The rabbits from the two groups had similar weights, and at zero time were injected with 0.5 ml of ^{125}I -prothrombin as described above for the factor X studies. For both the studies, blood samples were taken at various time intervals, as indicated in the text. A portion of the blood sample was assayed for radioactivity and the rest was used to prepare the plasma. Portions of these plasmas were assayed for VIIc, VIIt, factors X and II coagulant and amidolytic activities and also for radioactivity. The blood volume was the product of the radioactivity injected and that which was recovered per ml of blood at zero time. The plasma volume was calculated from the blood volume and the average volume of plasma separated from the blood samples obtained during the experiment.

Analysis of tracer data

The tracer data from blood and plasma were fitted with a sum of two exponential terms:

$$X(t) = C_1 e^{-a_1 t} + C_2 e^{-a_2 t}$$

Terms are defined below. This analysis makes it possible to represent the metabolism of the labelled protein by a two-compartment model and to calculate metabolic parameters such as the fractional catabolic rate constant, transcapillary rate constants and the fractional pool size of each compartment (Berman & Schoenfeld, 1956; Matthews, 1957). Thus, from the radioactivity injected and that found at a given time, the fractions (X_t) of the injected radioactivity remaining in blood or the plasma were calculated and semilogarithmic plots of such fractions versus the time from the injection were constructed for each protein (see Figs. 1 and 2 below): the log-linear portion of the radioactivity curve is

extrapolated to the ordinate to obtain the intercept C_1 . The slope of this line is $-a_1$. By subtracting the extrapolated line from the original curve $[X(t) - C_1 e^{-a_1 t}]$, a new line is obtained:

$$C_2 e^{-a_2 t}$$

Linear-regression analysis of a plot of the logarithm (base e) of the corrected fraction versus time (see Figs. 1 and 2) gave intercept C_2 and the slope of this term, $-a_2$. From the slopes $-a_1$ and $-a_2$ the $t_{1/2}$ for each compartment is calculated, and slopes and intercepts were used to calculate fractional catabolic rate constants.

RESULTS

Both groups of rabbits, each involving three animals, had been on their respective diets for 85 days when the experiment on the turnover of factor X started. Table 1 summarizes the plasma concentration of cholesterol and triacylglycerols, amidolytic activities for factors X and II, VIIc and VIIt, and coagulant activities for factors II and X in the plasmas obtained during the study from these rabbits. Factor X amidolytic activities were considerably higher in the rabbits fed on the cholesterol-supplemented than in those fed on the standard diet. The results of the assays of the factor X amidolytic activity in plasmas obtained at various time intervals during the 3 days of the experiment were similar for each rabbit, and therefore rabbits in both groups were assumed to be in a steady state as regards factor X during the experimental period. In agreement with previous results (Mitropoulos *et al.*, 1987), the increase in plasma cholesterol associated with the administration of dietary cholesterol is also associated with considerable increase in VIIc (Table 1).

Turnover of factor X in rabbits fed on the standard or on the cholesterol-supplemented diet

The loss of radioactivity from the blood after an intravenous injection of ^{125}I -factor X into normal and cholesterol-fed rabbits (Fig. 1) shows an initial rapid decline, followed by a slower log-linear phase which continued for 3 days (the last time at which a sample was taken). Interpreted in terms of a two-compartment model (Matthews, 1957), the initial phase reflects mixing of labelled protein molecules with non-labelled factor X in the extravascular compartment and their concurrent

Table 1. Rabbits and concentrations or activities in their plasmas during the study of the turnover of factor X

The results are averages $\pm$ S.D. for the three rabbits in each group. The value for each rabbit is the mean of at least eight determinations on the plasma samples obtained during the experiment.

Rabbits and plasma concentration or activity	Standard diet	Cholesterol-supplemented diet
Weight (kg)	3.99 $\pm$ 0.11	3.54 $\pm$ 0.06
Plasma volume (ml/kg)	30.8 $\pm$ 0.9	36.6 $\pm$ 0.62
Cholesterol (mg/100 ml)	17.1 $\pm$ 1.9	1164 $\pm$ 153
Triacylglycerols (mg/100 ml)	11.8 $\pm$ 1.9	11.4 $\pm$ 1.0
VIIc (% of standard-plasma value)	138 $\pm$ 29	305 $\pm$ 67
VIIt (% of standard-plasma value)	99 $\pm$ 33	142 $\pm$ 39
IIc (% of standard-plasma value)	122 $\pm$ 8	122 $\pm$ 14
II amidolytic (units/ml)	2.9 $\pm$ 0.3	5.6 $\pm$ 0.8
Xc (% of standard plasma value)	118 $\pm$ 16	152 $\pm$ 40
X amidolytic (units/ml)	0.514 $\pm$ 0.015	0.883 $\pm$ 0.100

Table 2. Turnover of ^{125}I -labelled rabbit factor X in rabbits fed the standard or the 1%-cholesterol-supplemented diet

Treatment	Extravascular compartment		Intravascular compartment		Fractional catabolic rate (h^{-1})	Plasma compartment	
	Half-life (h)	Pool size (%)	Half-life (h)	Pool size (%)		Half-life (h)	Pool size (%)
Standard diet	2.0 ± 0.6	45.5 ± 2.3	15.5 ± 1.5	56.2 ± 3.4	0.074 ± 0.008	19.7 ± 1.3	41.3 ± 2.2
Cholesterol-supplemented diet	1.6 ± 0.3	36.0 ± 11.7	6.6 ± 1.6	58.2 ± 2.1	0.064 ± 0.007	22.6 ± 0.8	42.3 ± 2.8

Fig. 1. Turnover of factor X in a rabbit fed on the standard diet

The blood radioactivity (●) after an intravenous injection of $18 \mu\text{Ci}$ of ^{125}I -labelled rabbit factor X is plotted against time. The log-linear portion of the curve ($t_{1/2} = \ln 2/a_1 = 16.7 \text{ h}$) was back-extrapolated to zero time; the lower curve (○, $t_{1/2} = \ln 2/a_2 = 2.9 \text{ h}$) was derived by subtracting the extrapolated line from the observed values. The slopes and the intercepts of these lines were used to calculate the fractional catabolic rate (FCR) from the equation:

$$\text{FCR} = \left(\frac{C_1}{a_1} + \frac{C_2}{a_2} \right)^{-1}$$

removal from the system by catabolism; the log-linear phase reflects removal of the equilibrated ^{125}I -factor X from the system. The half-life of the log-linear phase was $15.5 \pm 1.5 \text{ h}$ and $16.6 \pm 1.6 \text{ h}$ correspondingly for the rabbits fed on the standard and those fed on the cholesterol-supplemented diet (Table 2). The kinetic parameters of the extravascular compartment were determined from the line obtained by subtracting the extrapolated portion of the log-linear phase from the observed values (Fig. 1). Half-lives of this phase were $2.0 \pm 0.6 \text{ h}$ and $1.6 \pm 0.3 \text{ h}$ respectively for the rabbits fed on the standard and on the cholesterol-supplemented diet. Of the total exchangeable factor X, the extravascular pool accounted for $45.5 \pm 2.3\%$ in rabbits fed on the standard and $36.0 \pm 11.7\%$ in the rabbits fed on the cholesterol-supplemented diet. The corresponding values for the intravascular pools are $56.2 \pm 3.4\%$ and $58.2 \pm 2.1\%$ (Table 2). Since the sum of the fractional pool sizes is close to unity for both groups of rabbits,

kinetics of factor X metabolism can be analysed on the basis of the two compartments. The calculation (Matthews, 1957) of the fractional catabolic rates of factor X in the intravascular compartment from the data in Table 2 gives $0.074 \pm 0.008\%$ for the control animals and $0.064 \pm 0.007\%$ for the rabbits fed on the cholesterol-supplemented diet.

The half-life of ^{125}I -factor X in the plasma, obtained from the results presented in Fig. 1, was $19.7 \pm 1.3 \text{ h}$ for the group of rabbits fed on the standard diet compared with $22.8 \pm 1.8 \text{ h}$ for the three rabbits fed on the cholesterol-supplemented diet. Subtraction of the extrapolated portion of the log-linear phase from the observed initial values does not give a straight line, which suggests that the intravascular space contains a compartment in addition to the plasma compartment. The size of the plasma compartment determined from the log-linear phase is $41.3 \pm 2.2\%$ and $42.3 \pm 2.8\%$ respectively for the control and experimental group of rabbits. These values are lower than the corresponding values determined for the intravascular pool from the blood radioactivity data (Table 2).

The plasma factor X pool size for each rabbit was derived from the amidolytic assays (Table 1). The factor X concentration of the intravascular compartment was calculated from the fractional pool size of the two compartments and the concentration of factor X in the plasma. The absolute catabolic rate of factor X in each rabbit was determined from the intravascular pool size, and the fractional catabolic rate was determined from the blood radioactivity data. The mean absolute catabolic rate, and therefore the rate of synthesis, for the three rabbits fed on the cholesterol-supplemented diet is considerably higher than the value determined for the three rabbits fed on the standard diet (Table 3).

Turnover of prothrombin in rabbits fed on the standard and on the cholesterol-supplemented diet

The catabolism of prothrombin was investigated by injecting intravenously ^{125}I -bovine prothrombin into three rabbits fed on the standard and three rabbits fed on the cholesterol-supplemented diet. All animals had been on their respective diets for 40 days at the start of the experiment. Blood was taken at various time intervals, and radioactivity in these samples and the plasmas was assayed. Table 4 summarizes the plasma concentration of cholesterol and triacylglycerols, the amidolytic activities for factors II and X, VIIc and VIIt and the coagulant activities of factors II and X. Fig. 2 shows a typical blood radioactivity decay curve. From such plots the kinetics of prothrombin metabolism in the extravascular and intravascular compartments were calculated for the

Table 3. Intravascular pool size and absolute catabolic rate of factor X in rabbits fed the standard or the 1%-cholesterol-supplemented diet

Treatment	Pool size (mg/kg body wt. of rabbit)		Absolute catabolic rate (mg/day per kg body wt. of rabbit)
	Plasma compartment	Intravascular compartment	
Standard diet	0.292 ± 0.008	0.397 ± 0.011	0.705 ± 0.019
Cholesterol-supplemented diet	0.597 ± 0.067	0.821 ± 0.092	1.261 ± 0.141

Table 4. Rabbits and concentrations or activities in their plasmas during the study of the turnover of prothrombin

The results are averages ± s.d. for three rabbits in each group. The value for each rabbit is the mean of at least eight determinations on the plasma samples obtained during the experiment.

Rabbits and plasma concentrations or activities	Standard diet	Cholesterol-supplemented diet
Weight (kg)	3.587 ± 0.078	3.440 ± 0.178
Plasma volume (ml/kg)	31.7 ± 0.6	36.7 ± 2.8
Cholesterol (mg/100 ml)	81.3 ± 25.4	1335.2 ± 217
Triacylglycerols (mg/100 ml)	17.7 ± 4.4	19.0 ± 3.7
VIIc (% of standard-plasma value)	136 ± 36	611 ± 195
VIIIt (% of standard-plasma value)	113 ± 20	141 ± 45
IIC (% of standard-plasma value)	118.3 ± 24.2	95.6 ± 16.4
II amidolytic (units/ml)	3.7 ± 0.2	6.6 ± 0.8
Xc (% of standard-plasma value)	116.9 ± 14.7	139.3 ± 23.0
X amidolytic (units/ml)	0.47 ± 0.04	0.78 ± 0.16

Fig. 2. Turnover of prothrombin in a rabbit fed on the standard diet

The blood radioactivity (●) after an intravenous injection of 20 μ Ci of 125 I-labelled bovine prothrombin is plotted against time. The log-linear portion of the curve ($t_{1/2} = \ln 2/a_1 = 42.8$ h) was extrapolated to zero time; the other curve (○; $t_{1/2} = \ln 2/a_2 = 4.9$ h) was derived by subtracting the extrapolated line from the observed values. The slopes and the intercepts of these lines were used to calculate the fractional catabolic rate (see the legend to Fig. 1 and the Materials and methods section).

rabbits in the two groups (Table 5). These data were used to calculate the fractional catabolic rate and gave 0.041 ± 0.003 of the intravascular pool/h for the rabbits fed on the standard diet and 0.035 ± 0.003 /h for the rabbits fed on the cholesterol-supplemented diet.

The plasma radioactivity data obtained from the same experiment were also plotted as in Fig. 2. The half-lives and the intercepts of the log-linear phase were similar for the two groups of rabbits and close to the values for the intravascular compartment determined from the blood radioactivity decay curves (Table 5). The absolute size of the plasma prothrombin pool was determined from the amidolytic activity and is an average value derived from nine assays on the plasmas from each rabbit obtained at various time intervals during the experiment. In the case of prothrombin, intravascular and plasma compartments are of equal size. Therefore, the rate of prothrombin synthesis was determined from the concentration of this protein in plasma and from the fractional catabolic rate derived from the blood radioactivity decay curves. The mean absolute catabolic rate for the rabbits fed on the cholesterol-supplemented diet was considerably higher than that calculated for the three rabbits fed on the standard diet (Table 6).

DISCUSSION

The increased concentrations of factor X and of prothrombin in the plasmas of hypercholesterolaemic rabbits shown previously (Mitropoulos *et al.*, 1987) and

Table 5. Turnover of ^{125}I -labelled bovine prothrombin in rabbits fed the standard or the 1%-cholesterol-supplemented diet

Treatment	Extravascular compartment		Intravascular compartment		Fractional catabolic rate (h^{-1})	Plasma compartment	
	Half-life (h)	Pool size (%)	Half-life (h)	Pool size (%)		Half-life (h)	Pool size (%)
Standard diet	4.9 ± 0.4	62.8 ± 8.4	39.8 ± 3.0	34.6 ± 7.5	0.041 ± 0.003	33.1 ± 5.2	35.1 ± 4.6
Cholesterol-supplemented diet	6.1 ± 0.3	56.9 ± 2.1	41.4 ± 4.0	33.7 ± 2.5	0.035 ± 0.003	41.1 ± 3.4	34.5 ± 2.0

Table 6. Intravascular pool size and absolute catabolic rate of prothrombin in rabbits fed on the standard or on the 1%-cholesterol-supplemented diet

Treatment	Intravascular pool size (mg/kg body wt. of rabbit)	Absolute catabolic rate (mg/day per kg body wt. of rabbit)
Standard diet	2.28 ± 0.12	2.24 ± 0.12
Cholesterol-supplemented diet	4.71 ± 0.57	3.96 ± 0.48

confirmed in the present paper could be a consequence of the increased ratio of αVIIa to VII in these animals. This would be consistent with the lag period between the increase in VIIc and the increase in the concentrations of factor II and X (Mitropoulos *et al.*, 1987) or the delay between the fall in VIIc and the return of the plasma concentration of prothrombin and factor X to normal levels that results from the withdrawal of the cholesterol-supplemented diet (K. A. Mitropoulos, unpublished work). Thus, on administration of the cholesterol-supplemented diet, a new steady state between αVIIa and VII is induced that dictates a new steady-state concentration for prothrombin or factor X. The increases in prothrombin and factor X concentrations were apparent on day 10 and continued at the same levels for at least 100 days from the beginning of the experiment. The increase in the concentration of these proteins could be due either to an increase in their synthetic rate or to a decrease of their absolute catabolic rate. The present results demonstrate that the absolute catabolic rates of both factor X and prothrombin are considerably higher in the hypercholesterolaemic rabbits than in the rabbits fed on the standard diet.

The present studies assume that the modification of bovine prothrombin or of rabbit factor X, associated with ^{125}I -labelling with the Bolton & Hunter (1973) reagent, does not influence the metabolism of these proteins (Jesty & Morrison, 1986) after their injection into the rabbits. Rates of synthesis, of catabolism and of transcapillary transport of prothrombin have been shown to vary among the species previously studied. Thus the half-life of this protein in the intravascular compartment was 2.8–3.1 days in man (Shapiro & Martinez, 1969; Collen *et al.*, 1978), 1.77 days in cows (Takeda, 1970), 1.7 days in dogs (Owen *et al.*, 1979), and 0.43 day in rats (Koj *et al.*, 1984). The fractional catabolic rates determined in the present study for the rabbits fed the standard diet (0.980/day) or for the rabbits fed the cholesterol-supplemented diet (0.84/day) are close to those previously determined in dogs (Owen

et al., 1979) or calves (Takeda, 1970). Although we were unable to use the autologous prothrombin in the present study, we do not think that this would influence the comparison between the two groups of rabbits. However, the kinetic data we have obtained might not reflect the true turnover of the rabbit prothrombin. As has previously been shown (van Dam-Mieras & Hemker, 1982) the half-life of factor X in the intravascular compartment was considerably shorter, and the fractional catabolic rate higher, than for prothrombin.

The increased synthesis of factor X and of prothrombin in the rabbits fed on the cholesterol-supplemented diet strongly suggests an increased rate of thrombin generation in these rabbits, presumably as a consequence of the increased ratio of αVIIa to VII. This will be consistent with previously obtained results demonstrating that the incubation of rat hepatoma cells in the presence of fragments derived from the *N*-terminus of prothrombin, released on the conversion of prothrombin into thrombin, has increased the synthesis of prothrombin and of factor X by these cells (Graves *et al.*, 1981, 1982). Since prothrombin is the only vitamin K-dependent coagulation factor that loses its γ -carboxy-glutamic acid-containing peptides on activation to thrombin, this feedback-control mechanism is not limited to the synthesis of prothrombin, but could be relevant to the synthesis of all vitamin K-dependent proteins. Consistent with this are our previous results (Mitropoulos *et al.*, 1987) and the present results for the factor VIIa assays, which suggest an increase in the concentration of VII in the hypercholesterolaemic rabbit that is, however, not sufficient to explain the increase in VIIc observed in these animals.

The major part of the excess cholesterol in the hypercholesterolaemic rabbit is associated with large particles (very-low- and intermediate-density lipoprotein fractions), and there is in these animals hyperchylomicronaemia (Mitropoulos *et al.*, 1987). It is likely that dietary lipid triggers higher flux within the intrinsic pathway of blood coagulation through the increased

plasma concentration of negatively charged lipoprotein particles and, in turn, β XIIa, IXa or Xa are responsible for the activation of VII. The increased ratio of α VIIa to VII is likely to be responsible for the increased rate of thrombin formation in the hypercholesterolaemic rabbit, whereas the increase in the concentration of vitamin K-dependent factors is secondary and due to the increased rate of release of prothrombin fragments and to a positive feedback control of synthesis in liver. In rabbit or man, under conditions where there is an increased ratio of α VIIa to VII, the anticoagulation pathways (including protein C), can take care of the increased flux within the coagulation pathway. Since antithrombin III has little effect on α VIIa (Godal *et al.*, 1974; Broze & Majerus, 1980), small increases in the flux of the intrinsic system will cause a significant change in the ratio of α VIIa to VII expressed as increased VIIc in plasma. Further, as both single- and double-chain forms of this protein interact with tissue factor with equal avidity (Zur *et al.*, 1982), it follows that, at limited concentrations of tissue factor and at a low ratio of α VIIa to VII, the single-chain protein can decrease the effective activity of the present α VIIa. However, when this ratio is increased or when there is high local concentration of tissue factor (e.g. rupture of an atheromatous plaque, injury etc.), this is likely to result in a marked increase in the rate of thrombin synthesis and therefore the formation of thrombi. This is relevant to the thrombotic element of ischaemic heart disease and may explain the positive correlation between VIIc and the incidence of ischaemic heart disease in man (Meade *et al.*, 1986).

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