ON THE [^{35}S]SULPHATE INCORPORATION IN FIBRINOPEPTIDE B FROM RABBIT FIBRINOGEN*

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SUMMARY

Two peptides, tentatively named A and B, are released during the coagulation of rabbit fibrinogen. Inorganic sulphate labelled with ^{35}S has been shown to be utilized *in vivo* by the rabbit in the synthesis of tyrosine-O-sulphate in one of the fibrinopeptides from fibrinogen. The half-life of this group in fibrinogen is found to be 70 h.

N-terminal analysis of rabbit fibrinogen and fibrin as well as of the fibrinopeptides has been performed, and the results compared with those found in other species.

INTRODUCTION

Fibrinogen is transformed into fibrin by the action of thrombin and other enzymes. During this process, limited proteolysis of the fibrinogen molecule occurs and different peptides are liberated[1-10]. At least two peptides, tentatively named A and B, are released from bovine fibrinogen. The release of peptide A occurs at a higher initial rate than that of peptide B. Furthermore, it has been shown that the release of peptide A runs parallel with fibrin formation, whereas peptide B is released at a maximum rate when fibrin formation is near completion[6-7]. This indicates that if proteolysis is necessary for the subsequent polymerization, splitting off of peptide A alone is indispensable for initiating polymerization of bovine fibrinogen. It was, in fact, found that a coagulating enzyme, reptilase, from the venom of *Bothrops jararaca* could quantitatively transform fibrinogen into fibrin, but nevertheless only split off peptide A[8,11].

Bovine peptides A and B show a definite difference in amino acid composition as well as structure[6,8,9,10a,10b,12].

In 1954, BETTELHEIM[13] found that peptide B contained tyrosine-O-sulphuric acid, this being, in fact, the first occasion on which the compound in question was identified in a protein. This finding has since been confirmed[7,14]. Tyrosine-O-sulphuric acid is known to be a normal constituent of urine[15]. By the use of ^{35}S-labelled sulphate and electrophoretic methods, it has been shown that sulphur is incorporated into the plasma proteins of the rat, especially α_1-globulins and albumins[16-18]. Since ^{35}S was also associated with the fibrinogen band during electrophoresis, incorporation into this protein as well seemed to occur. It was suggested that, in this case, the ^{35}S

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incorporation was due mainly to an incorporation into the sulphate group of tyrosine-O-sulphate[18].

In the present study, of which a preliminary report was given elsewhere[19], additional work has been done on the basis of the aforementioned observations. Thus, ^{35}S-labelled fibrinogen and a labelled fibrinopeptide containing tyrosine-O-sulphate were isolated from the plasma of rabbits given injections of ^{35}S-labelled sulphate. The half-life of the sulphate group in the fibrinopeptide was also estimated. Moreover, this isotope study provided some new data on the fibrinopeptides of rabbit plasma.

MATERIAL AND METHODS

Preparation of fibrinogen and fibrinopeptides

Rabbits weighing between 2 and 3 kg were used as experimental animals. In the *in vivo* isotope experiments, 1 mC of carrier-free ^{35}S-labelled sulphate per kg body weight was injected intravenously into one of the lateral ear veins.

The blood was withdrawn from one of the carotid arteries through a cannula inserted into the vessel, after anaesthetizing the animals with ether. As anticoagulant 4 % trisodium citrate ($\cdot 2H_2O$) was used (1 vol. of citrate solution + 7.3 vol. of blood). Usually, 100 ml of citrated blood were obtained from each animal.

In several experiments, radioactive sulphate was injected into groups of 4–10 rabbits, which were killed by exsanguination 24 h after the injection. The blood of all the rabbits was pooled, and fibrinogen and fibrinopeptides isolated as described in the following.

In the experiment carried out in order to estimate the half-life of the sulphate group in the fibrinopeptide, 5 groups of rabbits, 3 in each group, were sacrificed at varying intervals (4, 12, 24, 50 and 100 h) after injection of the isotope. The blood from the rabbits in each group was pooled, and the fibrinopeptides prepared as described below.

An isotope experiment *in vitro* was performed as follows. 200 ml of citrated blood was collected from untreated rabbits and mixed with 4.8 mC of ^{35}S-labelled sulphate. The mixture was incubated for 1 h at 37° in an atmosphere of an oxygen mixture (93.5 % oxygen + 6.5 % carbon dioxide). Fibrinogen and fibrinopeptides were also isolated from this blood.

Blood was also collected from untreated rabbits, for preparation of a non-labelled fibrinogen on which some of the chemical analyses were performed.

After collection, the blood was centrifuged at 3,500 rev./min in a Corda centrifuge for 20 min. When the plasma had been siphoned off, it was fractionated according to COHN'S[20] method 6. The fibrinogen in fraction I was further purified according to the glycine method of BLOMBÄCK[21].

In the first experiments, the fibrinogen used (fraction I-0) had a coagulability of 82–90 %. In later experiments the fibrinogen was further purified (fraction I-2). This purified fibrinogen had a coagulability of 94–98 % as determined with a spectrophotometric method[22]. The same extinction coefficient ($E^{1\%}_{1\,cm}$) at 282 mμ was used in the protein determinations as that found for bovine fibrinogen. In fraction I-0, the yield of fibrinogen from plasma was between 72 and 82 % (mean 75 %), the corresponding yield in fraction I-2 being between 59 and 75 % (mean 66 %).

For the preparation of fibrinopeptides from the fibrinogen, the wet fraction I-0

or I-2 was dissolved in 0.3 *M* ammonium acetate (pH 7) to a protein concentration of between 1.0 and 1.5 %. An equal volume of water was added, and immediately thereafter a bovine thrombin solution (100 NIH units/ml) to a final concentration of 1 NIH unit/ml. The coagulation mixture was left standing at room temperature (+ 20 to 22°) for approximately 6 h, whereupon the fibrin clot was collected on a Büchner funnel and thoroughly washed with water. The yield of fibrin under these conditions was usually in good agreement with that obtained when coagulation was performed under standard conditions (*cf.* ref. [22]). In the half-life study a lower yield was, however, obtained in all groups.

After acidification of the clot supernatant with formic acid to pH 3.0, it was put on a 2 % cross-linked Dowex-50 column (200–400 mesh), which had been previously equilibrated with 0.1 *M* ammonium formate–formic acid buffer*, pH 3.0. The inner diameter of the column was 0.9 cm. The length of the column varied with the amount of fibrinogen used for coagulation. For clot supernatants from 0.2, 0.5 and 1 g of fibrinogen, respectively, the following column lengths were used: 30, 50 and 70 cm. The column was eluted with ammonium formate–formic acid buffers* at pH values below 4, and with ammonium acetate–acetic acid buffers* above pH 4, as described earlier[7]. The elution rate was 1–6 ml/h. The pooled peaks were lyophilized, and the buffer salt removed by increasing the temperature to 40–50° during 5–7 h. The residue was dissolved in water and again lyophilized. A light, white powder was then obtained.

The combined biuret–phenol reagent of LOWRY *et al.*[23] was used for tracing the peptides eluted. Alternatively, the ninhydrin method of MOORE AND STEIN[24] was applied.

N-terminal amino acids

For N-terminal analysis of the fibrinopeptides, as well as of fibrinogen and fibrin, EDMAN's phenylthiocarbamyl method[25] was used. The details of the technique applied for fibrinogen and fibrin[26] and for fibrinopeptides[27] have been described previously.

Radioactivity determinations

The radioactivity of fibrinopeptide or fibrinogen solutions was determined by counting in a Geiger-Müller counter (2.3 mg/cm^2 mica end-window G.M. tube), after plating on frosted aluminium plates 0.25 ml of effluents from the ion-exchange chromatography, or (in the turnover study) of each fibrinopeptide preparation dissolved in 4 ml of distilled water.

Identification of tyrosine-O-sulphate

In order to establish the presence of tyrosine-O-sulphate in the rabbit fibrinopeptide B and its labelling *in vivo* by ^{35}S sulphate, the following procedures were undertaken.

1. *Ultraviolet absorption curves of the B peptide before and after acid hydrolysis*[7,13]. Peptide B was dissolved in 0.1 *N* HCl at room temperature, and the absorption curve read in the region between 230 and 400 mμ, against 0.1 *N* HCl as blank. After increasing the HCl concentration to 1 *N*, samples were refluxed on the boiling water

* 0.1 *M* with respect to ammonium.

bath for 4 and 75 min, and the ultraviolet absorption curve recorded after cooling the samples in running tap water. The peptide concentration was about 0.4 mg/ml.

2. *Chromatography.* 2 mg of rabbit peptide B and 20 mg of bovine peptide B, respectively, were hydrolyzed in baryta water for 24 h at 125°[13]. After neutralization with dilute sulphuric acid to pH 7, the barium sulphate precipitate was removed by high-speed centrifugation. Amino acids and the excess of barium ions were removed by passage over a Dowex-50 × 8 (200–400 mesh) column (1.2 × 1.2 cm) in the hydrogen cycle. After neutralization with ammonium hydroxide, the sample was lyophilized and subsequently used for the identification of tyrosine-O-sulphuric acid.

The alkaline hydrolysate was subjected to two-dimensional ascending paper chromatography on Whatman No. 1 filter paper in the frame described by DATTA, DENT AND HARRIS[28]. The following solvents were used: (a) Phenol–water (400 g + 100 g). A beaker containing concentrated ammonia was placed in the tank. (b) Butanol–2 *M* ammonia (250 ml + 250 ml).

After drying the papers in a stream of hot air from an electric hair-drying fan, the chromatograms of the labelled peptide were subjected to autoradiography on Gevaert Curix X-ray film and exposed for 1 week in a wooden screw-press. The X-ray films were then developed with Gevaert G. 150[29]. The papers were examined under the light of high-pressure mercury lamp. They were also developed with ninhydrin, and compared with chromatograms of an authentic specimen of synthetic tyrosine-O-sulphate and the alkaline hydrolysate of the fibrinopeptide B prepared from ox blood.

3. *Electrophoresis.* A similar comparative study was made by means of high-voltage paper electrophoresis (0.075 *M** sodium acetate–acetic acid buffer, pH 5.5, 30 V/cm, 1 h) of the alkaline hydrolysates (see 2.) combined with autoradiography.

RESULTS

Two rabbit fibrinopeptides were isolated by means of chromatography on 2 % cross-linked Dowex-50. A typical chromatogram of the clot supernatant of 932 mg of purified fibrinogen is shown in Fig. 1. The fibrinogen was prepared from rabbits which prior to the exsanguination had received ^{35}S-labelled sulphate. Both peptides, tentatively named A and B**, gave colour with the combined biuret-phenol reagent[23], or with the ninhydrin reagent[24]. In this chromatogram, peptide B was eluted from the column with 0.1 *M* formate buffer at pH 3.7, whereas peptide A was eluted with 0.1 *M* acetate buffer at pH 4.4. In other chromatograms the peptides were eluted at slightly different pH values, depending on the batch of resin used. In this experiment, 5.7 mg of peptide A and 4.7 mg of peptide B were obtained. In another experiment, the corresponding figures from 194 mg of fibrinogen were 1.6 and 1.8 mg. If the yield of 40–50 % as calculated below is considered, the peptides would constitute roughly 2–4 % of the fibrinogen.

Traces of glycine, used as reagent in the purification of fibrinogen, were found as a contaminant of the clot supernatant. The glycine was eluted from the column with 0.1 *M* ammonium formate buffers of pH 3.0 or slightly higher, and thus clearly separated from the B peptide.

* 0.075 *M* with respect to sodium.

** The peptide containing tyrosine-O-sulphate has been denoted as peptide B, in view of its similarity in this respect to bovine peptide B. For the sake of simplicity the other peptide has been denoted as peptide A.

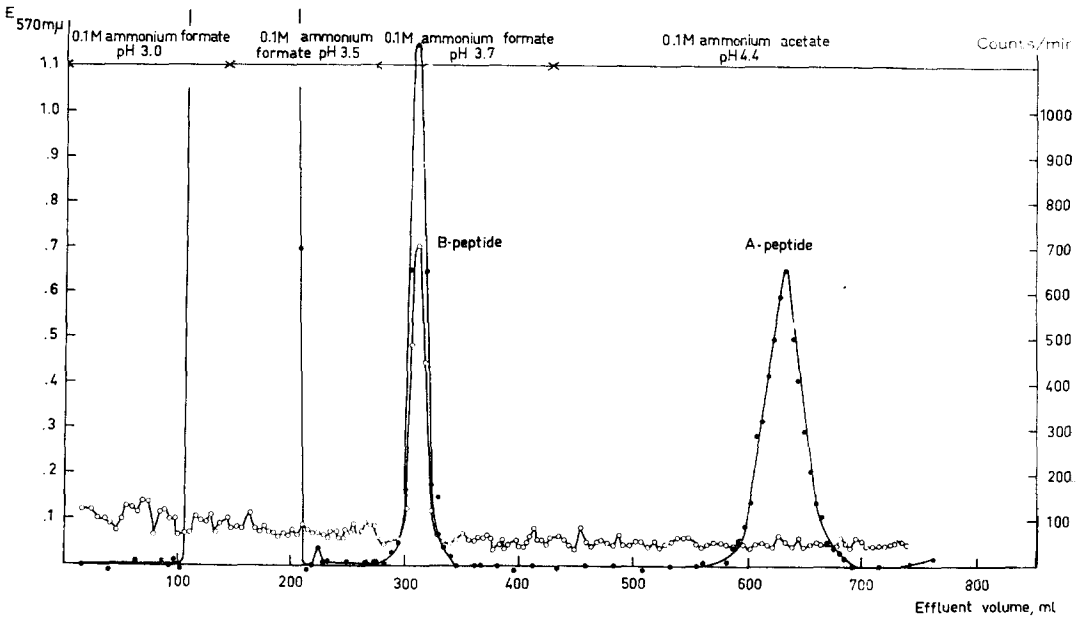

Fig. 1. Chromatogram of rabbit fibrinopeptides on 2 % cross-linked Dowex-50.

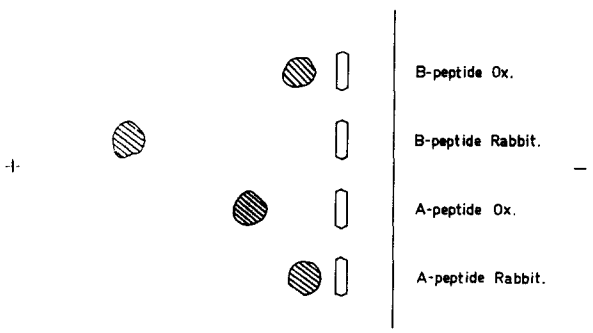

Fig. 2. Paper electropherogram of rabbit and ox peptides A and B. Electrophoresis was run in 0.1 *M* pyridine acetate buffer, pH 4.1, at + 8 to + 10° for 3 h, potential gradient about 20 V/cm. Whatman paper No. 3 MM was used. The spots were developed at 100° after spraying with a ninhydrin solution (0.2 % in acetone). The amounts of peptide applied to the paper were roughly 0.2–0.3 mg.

It is seen from Fig. 1 that the radioactivity was found exclusively in peptide B. No activity was present in peptide A. Variable amounts of inorganic sulphate were found in the solvent front of the chromatograms. In the *in vitro* isotope experiment, no incorporation of radioactivity occurred in fibrinogen or in fibrinopeptide B.

It can be inferred from the paper electropherograms shown in Fig. 2 that, at pH 4.1, peptide B had a stronger negative electric net charge than peptide A. Peptide B from rabbit fibrinogen differed markedly from the tyrosine-O-sulphate-containing bovine peptide B, in that it was much more acidic.

The ultraviolet absorption spectrum of rabbit fibrinopeptide B showed the same changes after hydrolysis as bovine peptide B. After acid hydrolysis, the typical

tyrosine peak appeared, indicating that tyrosine occurs in a conjugated form in rabbit fibrinopeptide B (Fig. 3).

After alkaline hydrolysis of peptide B, ^{35}S-labelled tyrosine-O-sulphate could be demonstrated by means of two-dimensional paper chromatography or high-voltage electrophoresis, combined with autoradiography (Figs. 4 and 5). In both cases, the zone of radioactivity on the chromatogram corresponded exactly to the ninhydrin-positive spot and the fluorescent zone. It had the same localization as the ninhydrin-positive spot and the fluorescent zone of alkaline hydrolysates of bovine fibrinopeptide B, or of an authentic specimen of tyrosine-O-sulphate.

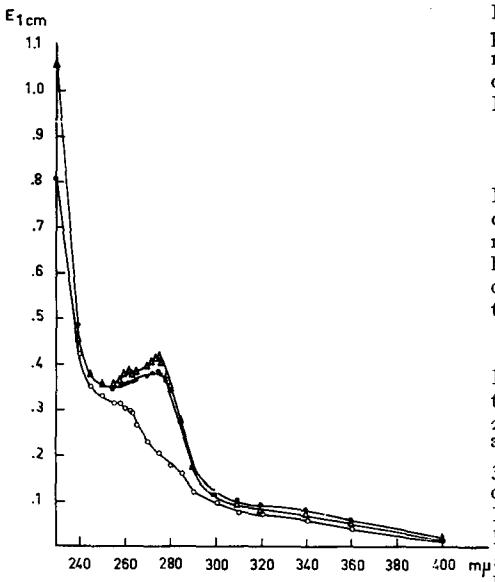

Fig. 3.

Fig. 3. Ultraviolet absorption properties of rabbit peptide B. O—O, B-peptide in 0.1 *N* HCl at room temperature. ●—●, B-peptide after hydrolysis for 4 min in 1 *N* HCl at 100°. △—△, B-peptide after hydrolysis for 75 min in 1 *N* HCl at 100°.

Fig. 4. Chromato-autoradiogram on alkaline hydrolysate of ^{35}S-labelled fibrinopeptide B from rabbit. The zone delineated by a solid line is ninhydrin-positive and fluorescent, and is completely congruent with the localization of the radioactivity (black zone). For further details see text.

Fig. 5. Electrophero-autoradiogram on a synthetic specimen of tyrosine-O-sulphate (1 and 6), 20 and 40 μg, respectively, of alkaline hydrolyzed ^{35}S-labelled fibrinopeptide B from rabbit (2 and 3), ^{35}S-labelled inorganic sulphate (4) and 40 μg of alkaline hydrolyzed, unlabelled fibrinopeptide B from ox (5). The zones delineated by a solid line are ninhydrin-positive and fluorescent, and are (in 2 and 3) completely congruent with the localization of the radioactivity (black zone). For further details see text.

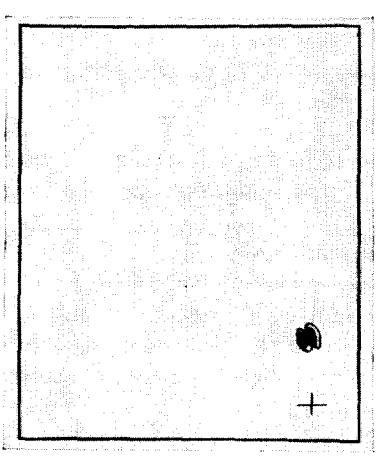

Fig. 4.

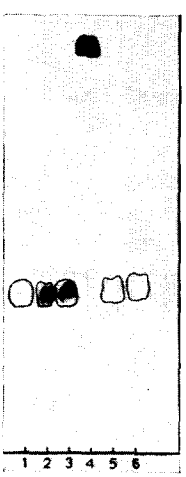

Fig. 5.

The results of the study of the half-life of the sulphate group of fibrinopeptide B are shown in Fig. 6, in which the specific radioactivity (counts/min/weight unit of peptide) is plotted against time after injection of the labelled sulphate. The maximum activity was recorded after 12–24 h, and was followed by a rapid decline. On the basis of the three last points of the decay curve, which approximately fits a 1st order reaction, the half-life of the sulphate group in the peptide was calculated to amount to 70 h. Curves of the same shape were obtained when the radioactivity was plotted against weight unit of fibrinogen or fibrin.

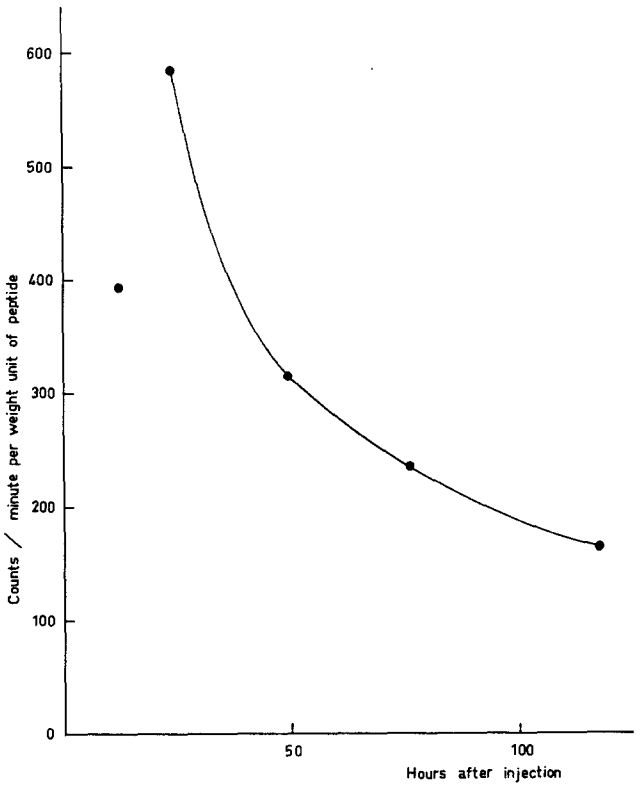

Fig. 6. ^{35}S incorporation into fibrinopeptide B in rabbit after a single injection of radioactive sulphate.

Table I shows the N-terminal amino acid pattern of rabbit fibrinogen and fibrin. Tyrosine, valine and alanine were found to be N-terminal in fibrinogen. The fibrinogen pattern thus differs from that of other species investigated, *i.e.*, ox, man, pig, goat, sheep, dog, and horse[26]. The only N-terminal residue common to all species is the tyrosyl residue. The most probable relation between the amino acids is two tyrosyl, two valyl and two alanyl residues in a 350,000 unit weight of protein. As far as the rabbit fibrin is concerned, it is interesting to note that the N-terminal pattern seems to be the same as that found in other fibrins, except that from horse[26]. The amount of tyrosyl residues, 0.56 mole per 10^5 g of protein, is in agreement with the amount found in the other species. The ratio of tyrosyl to glycyl residues in the fibrin was 1:2. The changes in the N-terminal pattern of fibrinogen and fibrin indicate that alanyl and

TABLE I

N-TERMINAL AMINO ACIDS IN RABBIT FIBRINOGEN AND FIBRIN

Figures in parentheses denote the relative amounts of different amino acids referred to tyrosine.

Protein	*Moles amino acid per 10^5 g protein*							
	Tyrosine		*Alanine*		*Valine*		*Glycine*	
	direct	*corr.**	*direct*	*corr.**	*direct*	*corr.**	*direct*	*corr.**
Fibrinogen	0.35	0.46 (1.0)	0.27	0.49 (1.06)	0.31	0.49 (1.06)	—	—
Fibrin	0.42	0.56 (1.0)	—	—	—	—	0.79	1.04 (1.9)

* Corrected with respect to the yields of the different amino acids. The figures of BLOMBÄCK AND YAMASHINA[26] were taken for tyrosine and glycine (77 and 76%, respectively). They found the yield of alanine to be 54%. The yield of alanine and valine determined in the same way by us was found to be 55 and 63%, respectively.

valyl peptides are split off from the fibrinogen during its conversion to fibrin. As is apparent from the analysis, two alanyl and two valyl peptides seem to be split off from the fibrinogen during its conversion to fibrin.

Analysis of the N-terminal amino acids in peptides A and B showed that valine was N-terminal in the former, and alanine in the latter. This affords definite evidence of the conclusion that these peptides isolated by chromatography are, in fact, those split off from fibrinogen during its transformation to fibrin. The over-all yield of the N-terminal valyl residues in the A peptide was calculated to be 50% of those found in the original fibrinogen. The yield of the alanyl residues in the B peptide was somewhat lower, or 40%.

DISCUSSION

In the present experiments, ^{35}S-labelled rabbit fibrinopeptide B was isolated, and ^{35}S-labelled tyrosine-O-sulphate was demonstrated in the peptide. These findings afford strong evidence that rabbit fibrinopeptide B derived from rabbit fibrinogen contains a tyrosine-O-sulphate residue, in agreement with its known occurrence in fibrinogen from cattle[8,13,14]. Our experiments have also shown that inorganic sulphate is utilized in the biosynthesis of tyrosine-O-sulphate in the rabbit *in vivo*. This is in conformity with the results of earlier studies on the biosynthesis of other ester sulphates reported by many workers[30–33].

The highly interesting problem of the site and mechanism of tyrosine-O-sulphate formation is still unsolved. Although many other ester sulphates have been synthesized in various *in vitro* systems of liver tissues, no report on the *in vitro* formation of tyrosine-O-sulphate has so far appeared[34].

The fate in the body of tyrosine-O-sulphate in fibrinopeptide B is not known, but the possibility exists that it is at least partially excreted in urine as tyrosine-O sulphate. This component is excreted in human urine in an amount of about 28 mg/day[15].

The question of the half-life of fibrinogen has been studied by several earlier

workers, using different isotope methods[35-39]. In one type of experiment, labelled amino acids, *e.g.* ^{35}S-labelled methionine, in pure form or as yeast or yeast hydrolysates, have been used as tracer substances. In other experiments, plasma protein fractions, including purified fibrinogen labelled with ^{14}C, ^{35}S or ^{131}I, have been administered to experimental animals or human volunteers.

As recently discussed by GERDES AND MAURER[39], both these techniques are open to some criticism. In the experiments with labelled amino acids, some re-utilization of amino acids seems to occur. This was clearly demonstrated by MADDEN AND GOULD[35] in experiments with [^{35}S]methionine. When heavy doses of unlabelled methionine were given simultaneously, the re-utilization of [^{35}S]methionine was decreased. When labelled proteins are given, the re-utilization of labelled material is considered to be low. On the other hand, it is an open question whether or not exogenous serum proteins, if injected intravenously, can be expected to have the same turnover rate as the corresponding proteins found in the body[39].

Despite these methodological difficulties, fairly good agreement seems to be present between the figures for the turnover rate of fibrinogen reported by different workers (Table II). In all species studied, the half-life of fibrinogen was fairly short compared with that of other plasma proteins. If, as has been suggested, continuous coagulation and fibrinolysis take place within the blood stream, this rapid consumption of the fibrinogen is not surprising[40].

In the present study on the turnover rate of the ester sulphate group of the fibrinopeptide B, ^{35}S-labelled sodium sulphate was given as tracer substance. A similar technique has been used in studies on the turnover rate of the ester sulphate groups in sulphated mucopolysaccharides[32, 33, 41]. On the basis of these ^{35}S sulphate experiments

TABLE II

HALF-LIFE OF FIBRINOGEN AS ESTIMATED BY DIFFERENT WORKERS

Methods	*Genus*	*Estimated half-life (h)*	*Reference*
[^{35}S]methionine	Man	134	MADDEN AND GOULD, 1952[35]
[^{35}S]cystine	Man	161	VOLWILER *et al.*, 1955[37]
[^{35}S]methionine [^{35}S]yeast [^{35}S]yeast hydrolysate	Man	161	GERDES AND MAURER, 1957[39]
[^{35}S]methionine [^{35}S]yeast	Dog	101	MADDEN AND GOULD, 1952[35]
[^{14}C]proteins	Rabbit	63	DOVEY *et al.*, 1954[36]
[^{131}I]fibrinogen	Rabbit	66	COHEN *et al.*, 1956[38]
[^{14}C]fibrinogen		69	
[^{35}S]plasma [^{35}S]yeast [^{35}S]yeast hydrolysate [^{35}S]methionine	Rabbit	55 86	GERDES AND MAURER, 1957[39]
[^{35}S]sulphate	Rabbit	70	Present authors

in mammals, the extent of re-utilization of labelled material liberated in the course of the experiment must be considered to be very low. Firstly, it now seems to be fairly well established that the incorporation of labelled sulphur given as sulphate in other sulphur compounds than ester sulphates is extremely low, *cf.* ref. [33]. Secondly, a rapid decrease to low figures of labelled sulphate is known to occur in blood and tissues during the first 24–28 h after a single injection of inorganic sulphate[32,33]. Thirdly, the bulk of ^{35}S sulphate retained in the tissues probably occurs in the ester group of mucopolysaccharides[32,33]. Since the latter have been shown to have a comparatively long half-life[33,41], the leakage of inorganic sulphate from these sources is considered to be small.

In the present study, estimation of the half-life of fibrinopeptide B is based on the three last points of the decay curve in the time interval between 24 and 100 h after injection of radioactive sulphate. On the basis of earlier rat experiments[32,33], it is suggested that, at this time, the inorganic sulphate peak has been passed, and the labelling of inorganic sulphate in blood and tissues is too low to interfere significantly with the shape of the last part of the fibrinopeptide decay curve. This suggestion is also supported by the fact that the latter roughly fits a 1st order reaction curve.

For the aforementioned reasons, we conclude that the figure found in the present study for the half-life of the fibrinopeptide B of rabbit fibrinogen is close to the real figure. Good agreement can be noted between this figure and those for the half-life of the whole fibrinogen molecule of rabbit plasma reported by earlier workers (Table II). This seems to indicate that the part of the molecule belonging to peptide B is synthesized concurrently with the main part of the molecule.

During the transformation of fibrinogen from various animal species to fibrin, different species-specific peptides are split off. Thrombin seems to be an enzyme with a high specificity of action. It can be recalled that, out of the thousands of bonds in fibrinogen, only a few are split by thrombin. This enzyme has been found to hydrolyze tosylarginine methyl ester[42], and it has consequently been suggested to have a specificity for certain arginyl linkages in proteins. As far as bovine fibrinogen is concerned, both peptides A and B have arginine as C-terminal residue[9,10,12]. Since the initial rate of release of peptide A from fibrinogen is the same as the rate at which glycyl residues appear in the fibrin[8], it seems likely that thrombin splits arginyl–glycine linkages in this protein. Since the rabbit peptides A and B also contain arginine, it is fairly probable that an arginyl–glycine linkage is split here as well. Thus, thrombin would appear to have a trypsin-like specificity of action. However, when acting on fibrinogen the proteolysis brought about by thrombin is more limited than that by trypsin.

It has newly been demonstrated that only the splitting off of peptide A from bovine fibrinogen is indispensable for initiating polymerization. The release of peptide A runs parallel with fibrin formation, whereas peptide B is released at a maximum rate when fibrin formation is near completion[7,8]. Although the physiological importance of the release of bovine peptide B is not yet elucidated, recent experiments indicate that the release of fibrinopeptide B from bovine fibrinogen may induce a side-to-side aggregation of the fibrin units[43]. The rate at which the rabbit fibrinopeptides are split off is not yet known, but it would not be surprising if, here too, the A and B peptides were split off at different rates.

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