Behaviour of commercially prepared I-125 fibrinogen in metabolic studies

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SYNOPSIS *In-vivo* studies of fibrinogen metabolism were carried out using an I-125 labelled preparation of fibrinogen supplied commercially by the Radiochemical Centre, Amersham for use in the diagnosis of deep venous thrombosis. These studies show that the material is suitable for obtaining the main metabolic parameters of clinical interest, though degradation products may be present initially or appear during storage. If suitable corrections are applied the results agree well with those of other authors.

Fibrinogen labelled with I-125 is used clinically in two ways. The first involves injection of labelled material and subsequent external monitoring of radioactivity at the site of interest to detect incorporation of fibrinogen into a blood clot. For example, this technique is used in the diagnosis of deep venous thrombosis (Negus *et al*, 1968) and in detecting rejection episodes in renal transplant recipients (Salaman, 1972). In the second technique, fibrinogen metabolism is studied by repeated plasma sampling using the labelled fibrinogen as a metabolic tracer. This method has been employed in the elucidation of microangiopathic haemolytic anaemia (Baker *et al*, 1968), hepatic failure (Flute *et al*, 1969), and certain types of renal failure (Wardle, 1973). It has also been used to monitor the effects of certain fibrinolytic drugs (Hickman, 1970).

¹²⁵I-labelled fibrinogen is readily available, for example from the Radiochemical Centre, Amersham, Bucks. This material was developed for fibrinogen monitoring using the first technique but the manufacturers specifically state that it has not been shown to be suitable for the second technique (Radiochemical Centre, 1974). This is because the only requirement for incorporation into a clot is the clottability of the fibrinogen while a true metabolic tracer must behave in exactly the same way as the material being studied.

When Amersham fibrinogen was first introduced, it was used for metabolic studies (Jeyasingh *et al*, 1970; Hickman, 1971; Wardle, 1973) and the results agreed well with those obtained with freshly-

prepared fibrinogen (Takeda, 1966). However, the manufacturing process was altered in view of the risk of transmission of viral hepatitis (Zuckerman, *et al*, 1971), and subsequently the metabolic handling of the material deteriorated (Hickman, unpublished work). The present preparation of ¹²⁵I fibrinogen has been available since 1972 (Gravett, 1974, personal communication) but no reports have been made so far on its metabolic behaviour.

In view of the problems attached to the separation and iodination of fibrinogen under sterile conditions in the laboratory, it is obviously more convenient to use a manufactured preparation if this is suitable. As part of a continuing study into certain haematological aspects of respiratory failure, the metabolic behaviour of Amersham fibrinogen was fully investigated in a small number of human subjects.

Materials and Methods

¹²⁵I-LABELLED FIBRINOGEN

The labelled fibrinogen was provided by the Radiochemical Centre, Amersham, Bucks from their routine stock. The fibrinogen, from donors rigorously screened for hepatitis, is prepared by precipitation and stored by freeze-drying (British Pharmacopoeia, 1973) until iodination using a strictly controlled modification (Gravett, 1974) of the chloramine-T method (Greenwood *et al*, 1963).

The material is supplied in ampoules containing not more than 1.2 mg of iodinated fibrinogen which is reconstituted with sterile water. The radioactivity injected is approximately 100 μ Ci, depending on the time from iodination. This material is claimed

to remain suitable for detection of deep-vein thrombosis for a period of six weeks from manufacture.

Five different fibrinogen batches were used and have been numbered in sequence. For each batch there was an inevitable delay of eight days between iodination and injection due to sterility checks after manufacture, packaging, and dispatch procedures. The fibrinogen was stored after receipt below -20°C for periods of up to 92 days from iodination before reconstitution and immediate injection.

SELECTION OF SUBJECTS

Eighteen subjects were investigated and all gave their informed consent to undergo the study. This total consisted of nine patients with respiratory failure, as defined by the usual blood gas criteria (Campbell, 1965), two with myocardial infarction, and seven healthy controls. Three of the patients were simultaneously screened for deep-vein thrombosis (Negus *et al.*, 1968) with negative results. With one exception (BF, aged 36) the patients were aged between 50 and 65 years, while the controls were men between 25 and 35 years.

PROCEDURE

Thyroid uptake of radioactive iodine was blocked by the oral administration of 150 mg KI daily for 48 hours before and for 10 days after injection of the labelled fibrinogen. Samples of venous blood were taken 15 minutes after injection, at least twice more on the first day, and then once or twice daily for seven to 10 days.

PLASMA RADIOACTIVITY

The method was that of Regoeczi (1967) as modified

by Hickman (1971). The radioactivity in the samples was measured in a well-type scintillation counter before (count I) and after (count II) removal of the fibrin clot with a stainless steel needle. The activity in the clottable fraction of the plasma was represented by count I – count II and the percentage clottability of the preparation by $\frac{\text{Count I} - \text{Count II}}{\text{Count II}} \times 100$. The mean percentage

error in the clottable radioactivity of 50 randomly selected pairs of duplicate samples was 1.7% (range 0.2–4.9%).

^{125}I fibrinogen survival was obtained from serial studies of the activity in the 'clottable' fraction of the plasma. All values after the initial 15-minute estimation were expressed as a percentage of the initial value, and the results were plotted graphically on a log-linear scale. The results were analysed according to the compartmental model of Matthews (1957).

Results

IN-VIVO CLOTTABILITY

The average clottability, defined as the mean of all clottability measurements for a given subject, remained between 87% and 96% (see table I, column 7). Figure 1 shows how the clottability of the 15-minute sample varied with time after iodination for different batches. It is evident that the clottability varied from batch to batch, but the samples used within 24 days of iodination had a higher clottability than the older samples. Figure 2 shows the day-to-day variation of clottability for individual subjects after injection, and measurements are shown for two different batches.

Subject	Sex		Batch No.	Age ¹	Half-life $T_{\frac{1}{2}}$ (days)	Average Clottability (%)	Intercepts			Rate Constants		
							C_1	C_2	C_3	b_1	b_2	b_3
JS	P	M	1	9	3.1	—	46	30	24	0.22	1.36	7
TH	C	M	1	78	4.0	87	38	32	30	0.17	1.5	14
LS	P	M	1	78	3.6	89	40	35	25	0.19	1.36	14
CC	P	M	1	92	3.8	88	35	34	31	0.18	1.66	13
BF	P	M	1	92	4.8	85	30	—	—	0.14	—	—
JH	C	M	2	10	5.1	94	62	38	—	0.14	1.67	—
DM	P	M	2	10	5.0	95	76	24	—	0.14	1.67	—
MJ	C	M	2	23	4.5	94	57	43	—	0.15	2.00	—
MF	C	M	2	69	4.5	92	35	27	38	0.51	0.91	7
LT	P	F	3	12	3.0	94	63	37	—	0.23	2.25	—
TF	C	M	3	12	3.5	95	68	32	—	0.20	4.35	—
LH	P	F	3	16	3.1	94	62	38	—	0.22	1.67	—
DE	C	M	4	10	3.9	96	71	29	—	0.18	1.54	—
CB	P	M	4	11	3.8	—	59	41	—	0.18	1.58	—
CS	P	M	4	12	3.8	—	63	37	—	0.18	1.05	—
HP	P	M	5	19	4.8	96	76	24	—	0.14	0.91	—
AD	C	M	5	24	3.7	96	76	24	—	0.19	2.50	—
ET	P	F	5	24	3.4	96	70	30	—	0.20	2.50	—

Table I Plasma clottable radioactivity: results for patients and controls

C = control; P = patient

¹Age = no. of days after iodination of the fibrinogen

Fig 1 Clottability of first sample (%) as a function of time since iodination (days) for different batches.

Fig 2 Variation of in-vivo clottability (%) with time after injection (days) for different batches.

FIBRINOGEN SURVIVAL

Table I summarizes the results. It was found that, when plotted graphically on a log-linear scale, some of the curves depicting the decline in the clottable radioactivity of the plasma could be analysed into two exponentials and others into three exponentials. The simpler method of visual curve-fitting was used in preference to a computer analysis because Tytgat has shown the two methods to be comparable in accuracy (Tytgat, 1971; Collen *et al*, 1972).

Figures 3 and 4 are representative of the two types of curve. In fig 3 the clottable radioactivity, shown by the solid line, can be described accurately by the sum of two exponentials with intercepts

Fig 3 Variation of clottable radioactivity (%) with time after injection (days) for subject DM. Analysis into the sum of two exponentials.

C_1 and C_2 and rate constants b_1 and b_2 respectively. In fig 4 the curve is described by the sum of three exponentials, with intercepts C_1 , C_2 , and C_3 and rate constants b_1 , b_2 , and b_3 respectively. Table I lists these parameters for the 18 subjects. The half-life ($T_{1/2}$) of the slowest decaying component is also listed.

Fig 4 Variation of clottable radioactivity (%) with time after injection (days) for subject MF. Analysis into the sum of three exponentials.

Discussion

COMPARTMENTAL ANALYSIS

Freshly iodinated ^{125}I fibrinogen, injected into healthy subjects, gives rise to plasma clottable radioactivity curves which are best described by the sum of two exponentially decaying components: $C_1\exp(-b_1t) + C_2\exp(-b_2t)$ —see fig 3. Matthews (1957) has shown that a plasma radioactivity curve of this form can be explained in terms of a three-compartment model. This model describes the interchange of fibrinogen between the plasma and an extravascular compartment, combined with catabolism and excretion of fibrinogen into the urine. Atencio *et al* (1965) have described a more complicated model along similar lines. The appearance of a third exponential in the plasma radioactivity curve, as in certain of the studies shown in table I, can be attributed either to the presence of a fourth compartment (Matthews, 1957) or to the existence of separately metabolized components in the nominally pure fibrinogen (Regoecki, 1971). The latter explanation is more likely in view of the effect of sample age.

CHANGE IN DECAY CURVES WITH SAMPLE AGE

Stored fibrinogen can be expected to deteriorate slowly (Regoecki and Stannard, 1969; Radiochemical Centre, 1974). The appearance of a third exponential in the decay curve in experiments using stored fibrinogen might therefore be attributed to this deterioration rather than to the presence of an additional compartment. Using fibrinogen stored for periods *in vitro* before iodination, Regoecki and Stannard (1969) showed that the clottable radioactivity consisted of a small fraction (5-6%) which disappeared rapidly from the plasma while the remainder behaved as metabolically normal fibrinogen. The results of the present study are consistent with this interpretation but the rapidly catabolized fraction reached, in certain cases, values of 30% or greater (see fig 4).

In some cases the very rapid disappearance of the third exponential could not be observed directly because of the limited number of data points. Its existence, however, could be inferred from a lowering of the intercept C_1 of the slow component. Fig 5 demonstrated the relationship between the intercept C_1 and the age of the fibrinogen. 'Old' material (more than 60 days after iodination) produced a clearly marked third exponential which (with one exception—JS) was not seen in recently iodinated material. As the material aged, the intercept C_1 decreased even before a third exponential could be distinguished. This decrease is presumably due to an increasing degradation with time, while

Fig 5 Distribution of the slow component intercept C_1 (%) with time since iodination (days) for different batches.

the initial scatter of C_1 may be due to manufacturing variation. The results of JS suggest that some degradation had occurred in that batch despite freshness of the material. Nevertheless the purity of the fibrinogen, as measured by clottability, remained high, and the metabolic rate constant, calculated after correction (*vide infra*) using the three-compartmental model, was in agreement with the results of other authors.

EFFECT OF SAMPLE AGE ON CLOTTABILITY

The difference between apparently pure and partially degraded fibrinogen samples can be seen in an effect on the clottability as well as in the appearance of a third exponent in the plasma radioactivity curve. If clottability is plotted against time after injection, a difference can be seen between stored and newly iodinated samples (see fig 2). In an attempt to quantify this variation, the average rate of change of clottability with time during the first 48 hours after injection ('initial clottability change'—in percent/day) was measured for each subject and plotted as a function of the slow component intercept C_1 . Fig 6 shows that a near-linear relationship exists between these two parameters for almost all the subjects examined. Since C_1 has been shown to decrease with sample age, initial clottability must also decrease with sample age, confirming the hypothesis of gradual inactivation of the fibrinogen.

Animal experiments by Regoecki (1967) have suggested that an initial clottability rise is to be expected even with material of reasonably high clottability (for example, greater than 93%). This initial rise can be explained in terms of differences in diffusing ability between the fibrinogen and the inevitable small amount of contaminating protein. This rise is still present in material showing three

Fig 6 Relation between initial clottability change (percent per day) and slow component intercept C_1 (%).

exponentials on the plasma clottable radioactivity curve. Thus it appears that the degradation products, whatever their nature, are still clottable but have a higher diffusing ability than 'pure' fibrinogen because they rapidly disappear from the plasma curve. It can be seen from fig 6 that two of the patients showed an initial decrease in clottability which might be explained by the presence of an undiagnosed deep-vein thrombosis or other fibrinogen-consuming site.

METABOLIC RATE CONSTANTS

The metabolic rate constants which can be calculated from the three-compartment model are the fractional catabolic rate (K_{12}), the fractional transcapillary transfer or efflux rate (K_{13}), and the fractional transcapillary reflux rate (K_{31}) (Matthews, 1957). Table II lists these parameters for 17 of the 18 subjects: the experimental scatter for one patient (BF) was too large to permit calculation of the parameters. For those subjects whose graph of clottable radioactivity against time showed a third

exponential component, the intercepts C_1 and C_2 were modified to C_1' and C_2' :

$$C_1' = C_1/(C_1 + C_2)$$

$$C_2' = C_2/(C_1 + C_2)$$

and the modified values were used in the analysis instead of C_1 and C_2 . This correction is necessary to eliminate the effect of the third component, which plays no part in the fibrinogen metabolism and only acts as a contaminant.

A systematic dependence of the rate constants on the purity of the material, as measured by the intercept C_1 , might be expected *a priori*. This is because it is possible to show from Matthews' analysis that, for example, the change dK_{12} in the parameter K_{12} due to a changed C_1 in the intercept C_1 is given by

$$dK_{12} = -(K_{12})^2 \cdot dC_1/b_1.$$

Thus, taking typical values (Tytgat, 1971) of $K_{12} = 0.24$, $C_1 = 0.67$, and $b_1 = 0.17$, a 20% decrease in C_1 (from 67% to 54%) produces a 19% increase in K_{12} (from 0.24 to 0.295). This anticipated systematic dependence of the rate constants is not evident in fig 7, which shows the rate constants obtained in the present study plotted against the slow-component intercept C_1 . The horizontal dotted lines depict the upper and lower limits (± 1 standard deviation) to the range calculated by Tytgat (1971). The normal ranges for K_{12} and K_{13} calculated by Takeda (1966) are very similar.

It is clear that the rate constants calculated for the control subjects in the present study agree well with Tytgat's and Takeda's values. Table III lists the average values and standard deviations for K_{12} , K_{13} , and K_{31} obtained in the present study and by previous authors. The difference in value for K_{31} between Tytgat and the present study (1.02 against 1.33) is due entirely to the value of 2.99 obtained from one control (TF). This unusually high value for the reflux rate constant appears to be genuine and is associated with a high value for K_{13} .

The other parameters listed in table III are the half-life ($T_{1/2}$) and the slow-component intercept C_1 . Because the half-life of the plasma radioactivity curve bears an approximately inverse relationship to the fibrinogen catabolic rate and is an easily

	Subject																
	JS	TH	LS	CC	JH	DM	MJ	MF	LT	TF	LH	DE	CB	CS	HP	AD	ET
Patient/control	P	C	P	P	C	P	C	C	P	C	P	C	P	P	P	C	P
K_{12}	0.91	0.89	0.81	0.94	1.09	1.29	1.20	0.58	1.50	2.99	1.12	1.15	1.01	0.70	0.72	1.45	1.01
K_{13}	0.33	0.29	0.32	0.32	0.21	0.18	0.26	0.23	0.33	0.28	0.32	0.24	0.29	0.27	0.18	0.25	0.27
K_{31}	0.34	0.49	0.42	0.62	0.51	0.34	0.69	0.25	0.65	1.26	0.45	0.33	0.47	0.26	0.15	0.29	0.62

Table II Metabolic parameters for patients and controls in the present study

Author	No. studied	$T_{1/2}$ (days)	C_1	K_{21} (/day)	K_{12} (/day)	K_{13} (/day)
Takeda (1966)	12	3.36 ±0.25	0.82 ±0.02	— —	0.246 ±0.016	0.548 ±0.309
Regoezci and Stannard (1969)	1	3.90	—	—	—	—
Hickman (1971)	13 ¹	4.00 ±0.54	0.77 ±0.09	— —	0.225 ±0.02	—
Tytgat (1971)	35	4.14 ±0.56	0.67 ±0.08	1.02 ±0.39	0.24 ±0.04	0.60 0.26
Present study	7	4.17 ±0.55	0.64 ±0.08	1.33 ±0.70	0.25 ±0.03	0.55 ±0.33

Table III Metabolic parameters of healthy subjects: comparison with previous studies

¹Contrast group of patients

Fig 7 Metabolic rate constants versus intercept C_1 (%).

obtained measurement requiring no calculation, it is the most frequently quoted parameter in studies of fibrinogen metabolism. It can be seen in the table that the present study shows values for $T_{1/2}$ which are consistent with those of other authors, although Takeda's value (1966) is much lower. There seems to be some correlation between the intercept C_1 and the half-life. The highest value of C_1 is associated with the shortest half-life and *vice-versa*.

It is worth noting that the 'double-half-life' phenomenon described by Regoezci (1971) was not observed in this study, although the effect is reported to be more frequent in improperly stored or overstored preparations. As described, this phenomenon takes the form of a sudden change in the slope of the plasma clottable radioactivity curve with an apparent abrupt change in the half-life. Regoezci attributes this to a genuine change in the catabolic rate constant and has put forward two

possible explanations which are hypotheses related to the metabolic behaviour of the reticulo-endothelial cells. Nevertheless the similarity between fig 4 (which was analysed into the sum of three exponentials) and Regoezci's illustrative example is quite striking. It is probable that some of the apparent observations of this effect may be the result of small numbers of samples, combined with the effects of random errors.

In conclusion it appears that ready-prepared ¹²⁵I fibrinogen, at least from this source and when used within four weeks of iodination, is adequate for obtaining data on fibrinogen metabolism. Nevertheless Regoezci's comments (1967, 1971) regarding the desirability of using freshly prepared and iodinated fibrinogen for metabolic studies still remain valid.

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