Journal of Clinical Investigation
Vol. 45, No. 1, 1966

Studies of the Metabolism and Distribution of Fibrinogen in Healthy Men with Autologous ^{125}I-labeled Fibrinogen *

Y. TAKEDA †

(From the Department of Medicine, University of Colorado School of Medicine, Denver, Colo.)

A few studies of the metabolism and distribution of fibrinogen in healthy men have been reported (1–3), but because of the lability of fibrinogen and the methods employed the earlier of these do not appear to have been entirely successful. Recent advances in the preparation of fibrinogen-^{131}I (4, 5) and in the mathematics of its kinetic behavior (6) have made possible more reliable and extensive studies. Observations have therefore been made on 12 healthy male volunteers with autologous fibrinogen labeled with ^{125}I.

Methods

The subjects, mainly graduate students or janitors, were healthy male volunteers, ranging from 16 to 54 years in age. They took 10 drops of saturated KI solution three times a day for the 3 days preceding the experiment and 10 drops once daily during the 7 to 9 days of the experiment. The preparation of fibrinogen for labeling was carried out by a minor modification of the method of Atencio, Burdick, and Reeve (4) as follows: About 20 ml plasma, separated from the subject's heparinized blood, was diluted with an equal volume of 0.09 M sodium citrate and recentrifuged for 10 minutes to remove any remaining platelets and red cells. The diluted plasma was mixed with 4 M $(NH_4)_2SO_4$ (saturated) in a volume ratio of 3 to 1, left at room temperature for 5 minutes, and spun for 5 minutes at 2,000 rpm, and the supernatant was discarded. The precipitate, after washing three times with a total of 120 ml of 1 M $(NH_4)_2SO_4$, was dissolved in 15 ml of 0.005 M sodium citrate, reprecipitated with 5 ml of 4 M $(NH_4)_2SO_4$, and washed once with 40 ml 1 M $(NH_4)_2SO_4$. The fibrinogen was then dissolved in 4 ml of 0.005 M sodium citrate, refrigerated at 4° C for 3 hours, and spun down at 4° C for 20 minutes to remove cold insoluble material. At this stage the clottability of fibrinogen was usually 95% or greater. If less than 95%, the fibrinogen was reprecipitated and again washed with 1 M $(NH_4)_2SO_4$. The clottability was measured as described elsewhere (4). The fibrinogen was then labeled with ^{125}I in a ratio of 0.5 or less atoms iodine per molecule of fibrinogen by the iodine monochloride method (4, 5, 7), after which free ^{125}I was removed by repeated precipitation and washing with 1 M $(NH_4)_2SO_4$. The fibrinogen-^{125}I was dissolved in 4 ml of 0.005 M sodium citrate, refrigerated for 3 hours, and centrifuged at 3,000 rpm for 20 minutes to remove any cold insoluble material. The amount of free ^{125}I in the fibrinogen-^{125}I preparations was less than 1% of the total radioactivity. The preparation was sterilized by filtration through a Selas filter (FMB-52-03) [1] with a positive pressure of 5 pounds per square inch. One ml of 6 mg per ml human serum albumin [2] in 0.005 M sodium citrate was first passed through the filter and then the fibrinogen-^{125}I mixed with 1 ml of the same albumin solution. The clottability of fibrinogen-^{125}I did not significantly differ from that of the unlabeled fibrinogen (4).

The interval between the withdrawal of plasma for preparation of fibrinogen for labeling and the injection of fibrinogen-^{125}I was 24 hours. Five to 15 μc of fibrinogen-^{125}I in a volume of 4 to 5 ml containing about 5 mg of fibrinogen was injected intravenously into the subjects. The first blood sample was withdrawn 15 minutes and subsequent samples ¼, ½, 1, 2, 3, 4, 5, 6, and 7, and sometimes 8 and 9 days after the injection. Samples were heparinized. Twenty-four-hour urine collections were made daily throughout the experiments. Plasma and urine samples were assayed for radioactivity in a well scintillation counter with spectrometer and a sensitivity of about 7×10^5 cpm per μc of ^{125}I above the background of 50 cpm. The plasma volume was obtained from the total fibrinogen-bound radioactivity injected divided by the counts per minute per milliliter plasma of the 15-minute sample. The plasma fibrinogen concentration was measured daily by the isotope dilution method of Atencio and co-workers (4). Many plasma samples were analyzed for radioactivity unbound to fibrinogen. For this 2 ml plasma was mixed with 1 ml of 30% trichloroacetic acid

* Submitted for publication July 14, 1965; accepted October 6, 1965.

This work was supported by research grant HE-02262 from the National Heart Institute and stimulated by research grant FR-051 from the U. S. Public Health Service. The work was done during the tenure of an Advanced Research Fellowship of the American Heart Association.

A preliminary report was presented to the Sixteenth Autumn Meeting of the American Physiological Society.

† Address requests for reprints to Dr. Y. Takeda, University of Colorado Medical Center, 4200 E. Ninth Ave., Denver, Colo. 80220.

[1] Microbiological Associates, Bethesda, Md.

[2] Normal human serum albumin U.S.P., Merck Sharp & Dohme, Philadelphia, Pa.

TABLE I

Body weight and plasma data

Subject	Age	Height	Weight	Plasma volume	Plasma fibrinogen	Total plasma fibrinogen	Hematocrit
	years	*cm*	*kg*	*ml/kg*	*mg/ml*	*mg/kg*	%
YT	37	173	72.7	34.1	3.34	114	51
LR	22	177	65.9	36.3	3.59	130	49
CJ	26	179	69.0	35.0	3.06	107	50
MM	23	180	90.9	31.7	4.58	145	52
AT	29	189	79.5	36.3	4.05	147	51
BS	17	180	68.2	37.5	3.05	114	49
ML	25	191	75.0	39.9	3.26	130	51
FG	27	185	77.2	32.8	4.12	135	52
RU	48	168	67.2	27.7	3.79	105	51
WF	54	165	77.2	35.7	3.96	141	50
AP	20	174	72.9	35.5	3.04	108	49
CU	21	179	65.9	44.8	3.33	149	47
Mean		178	73.5	35.6	3.60	127	50
SD				±3.5	±0.50	±17	±1

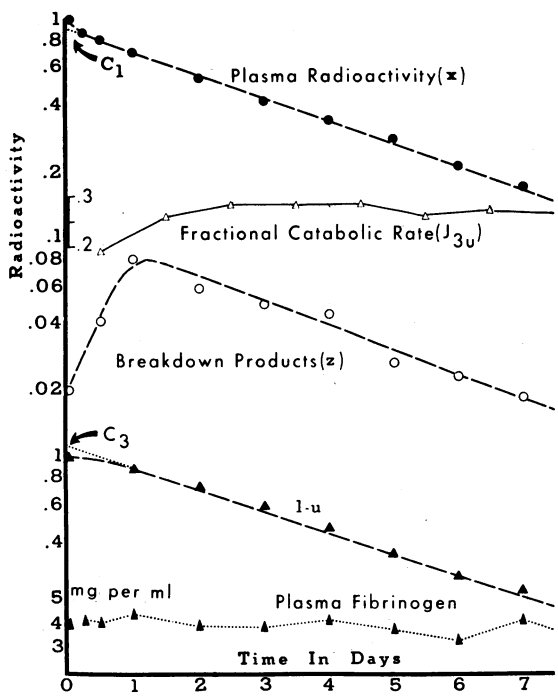

FIG. 1. IN VIVO BEHAVIOR OF IV ADMINISTERED FIBRINOGEN-^{125}I IN SUBJECT RU. x, z, and 1 − u represent the radioactivities of the plasma fibrinogen-^{125}I, the breakdown products of fibrinogen-^{125}I in the iodide space, and the total radioactivity remaining in the body at time t, respectively, and are expressed as fractions of the total radioactivity initially injected. C_1 is the intercept of the slower component of x with the ordinate at zero time, and C_3 is the intercept of the 1 − u curve with the ordinate at zero time. j_{3u} is calculated from x and u (6). The plasma fibrinogen concentrations are given in terms of milligrams per milliliter.

and after refrigeration for 30 minutes spun at 3,000 rpm for 30 minutes at 4° C. The supernatant was assayed for radioactivity. All measured radioactivities were corrected for decay by reference to duplicate standards, prepared from dilutions of the fibrinogen-^{125}I with nonradioactive carrier plasma in 0.9% NaCl. The microhematocrit was determined on all blood samples.

The mathematical model used for analysis of the data is fully described elsewhere (6). The differential equations describing the model and their solutions provide the following rate constants: the fractional catabolic rates of plasma fibrinogen j_{3p} and j_{3u} were calculated from $j_{3p} = (C_1/a + C_2/b)^{-1}$, and j_{3u} from $j_{3u} = u_{2,3}/\xi_{2,3}(t_3 - t_2) \cdot (k_5 - a)/k_5$, where C_1, a, C_2, and b are the parameters of a function (x) describing the behavior of plasma fibrinogen-^{125}I, $u_{2,3}$ is the total radioactivity excreted in the urine during the time interval t_2 to t_3, $\xi_{2,3}$ is the mean plasma radioactivity during the same interval, and k_5 is the daily fractional excretion rate of the breakdown products of fibrinogen-^{125}I through the kidney. Previous observations (8) showed that the mean value of k_5 was 2.02 per day. The fractional transcapillary transfer rate of plasma fibrinogen j_1 was calculated from $j_1 = C_1a + C_2b - j_{3p}$. The catabolic and transcapillary fibrinogen fluxes were obtained from $j_{3p}\bar{x}$ and $j_1\bar{x}$, respectively, where $\bar{x}$ is the mean total fibrinogen in the plasma. The amount of interstitial fibrinogen, $\bar{y}$, was calculated as described previously (9, 10).

Results

Body weight and plasma data. Table I summarizes measurements of the body weight, plasma volume, plasma fibrinogen concentration, the total plasma fibrinogen, and the hematocrit values. The plasma volume ranged from 27.7 to 44.8 with a mean value of 35.6 ml per kg body weight. The hematocrit values averaged 50% with a range of

47 to 52. The plasma fibrinogen concentration fluctuated around the mean from day to day, and averaged 3.60 mg per ml with a range of from 3.04 to 4.58. The degree of fluctuation varied from one subject to another. It was large in some and practically undetectable in other subjects (Figure 1). The quantities of fibrinogen ($\bar{x}$) within the plasma compartment averaged 127 mg per kg body weight with a range of from 105 to 149. The clottability of the fibrinogen-^{125}I preparations varied from 95 to 98 with a mean of 96% (Table II).

The function describing the plasma fibrinogen-^{125}I radioactivity, x, and the cumulative excretion of radioactivity, u. The plasma radioactivities of all samples were expressed as fractions of the 15-minute sample, and these data were analyzed by the method of least squares with the IBM 709 digital computer (11). The data were closely described by a two-exponential equation of the form, $x = C_1e^{-at} + C_2e^{-bt}$. The closeness of the fit is demonstrated by the small values of the "variance of the fit" (11) as given in Table II. The values for the parameters of x are also given in Table II, which shows that in the 12 subjects studied the average value is $x = 0.82e^{-0.207t} + 0.18e^{-3.57t}$. The results of a typical experiment are shown in Figure 1. The half-life of the slower component, a, of x varied from 2.78 to 3.65 with a mean value of 3.36 days (Table II).

The cumulative urinary excretions of radioactivity in all subjects were expressed as fractions of the total radioactivity initially injected. Table III shows that the radioactivity excreted during the first 24 hours varied from 12.5 to 16.4 with a mean of 14.5% excluding subjects AT and WF. The total radioactivity excreted by the seventh day ranged from 63.5 to 82.2 with a mean of 72.5%. In subject YT the total radioactivity excreted amounted to 89.5% by the ninth day. A typical

TABLE II

*Tracer data on the in vivo behavior of fibrinogen-^{125}I and the calculated rate constants**

Subject	Clottability of fibrinogen-^{125}I	$x = C_1e^{-at} + C_2e^{-bt}$				s^2	$t_{\frac{1}{2}}$ for a	j_1	j_{3p}	j_{3u}
		C_1	a	C_2	b					
	%						*days*	*day*$^{-1}$	*day*$^{-1}$	*day*$^{-1}$
YT	95	0.89 ±0.01†	0.200 ±0.004	0.11 ±0.01	2.39 ±0.00	1×10^{-4}	3.47	0.219	0.222	0.242
LR	95	0.77 ±0.02	0.201 ±0.006	0.23 ±0.02	2.68 ±0.41	8×10^{-5}	3.45	0.515	0.256	0.265
CJ	98	0.83 ±0.04	0.214 ±0.010	0.17 ±0.04	3.61 ±1.96	5×10^{-4}	3.24	0.540	0.251	0.250
MM	97	0.76 ±0.01	0.198 ±0.005	0.24 ±0.01	3.12 ±0.33	5×10^{-5}	3.50	0.644	0.255	0.237
AT	98	0.83 ±0.01	0.220 ±0.005	0.17 ±0.01	3.89 ±0.69	7×10^{-5}	3.15	0.582	0.262	‡
BS	95	0.84 ±0.01	0.249 ±0.005	0.16 ±0.01	9.26 ±3.24	8×10^{-5}	2.78	1.394	0.295	0.295
ML	96	0.82 ±0.05	0.190 ±0.01	0.18 ±0.05	2.10 ±0.82	3×10^{-4}	3.65	0.307	0.227	0.202
FG	95	0.80 ±0.05	0.203 ±0.01	0.20 ±0.05	2.11 ±0.84	3×10^{-4}	3.41	0.333	0.251	0.232
RU	95	0.82 ±0.03	0.220 ±0.01	0.18 ±0.03	2.30 ±0.65	2×10^{-4}	3.15	0.372	0.222	0.252
WF	96	0.87 ±0.02	0.206 ±0.008	0.13 ±0.02	5.68 ±2.72	2×10^{-4}	3.36	0.682	0.236	‡
AP	95	0.79 ±0.01	0.190 ±0.006	0.21 ±0.01	3.59 ±0.49	9×10^{-5}	3.65	0.667	0.237	0.227
CU	97	0.82 ±0.02	0.196 ±0.008	0.18 ±0.02	2.13 ±0.49	1×10^{-4}	3.54	0.320	0.234	0.219
Mean	96	0.82 ±0.02	0.207 ±0.007	0.18 ±0.02	3.67 ±1.16	1×10^{-4}	3.36 ±0.25	0.548 ±0.309	0.246 ±0.016	0.242 ±0.026

* In the two-exponential equation, $x = C_1e^{-at} + C_2e^{-bt}$, C_1, a, C_2, and b are parameters of the function of time (t) describing the behavior of plasma fibrinogen-^{125}I; $t_{\frac{1}{2}}$ is the half-life of the slower component, a, of x. j_1 is the fractional transcapillary transfer rate of plasma fibrinogen per day. j_{3p} and j_{3u} are the fractional catabolic rates of plasma fibrinogen per day. j_{3p} is calculated from x and j_{3u} from x and the urinary radioactivity (6). s^2 is the "variance of the fit" (11).

† Standard deviation.

‡ j_{3u} values were not calculated for subjects AT and WF because their urine collections were inadequate.

TABLE III

*Cumulative urinary excretion of radioactivity (u) expressed as fractions of the total radioactivity injected**

Subject	C_3	a_u	s^2	Days 1	2	3	4	5	6	7	8	9
YT	1.10 ±0.02†	0.239 ±0.006	2×10^{-4}	0.150	0.306	0.453	0.570	0.655	0.738	0.801	0.846	0.895
LR	1.06 ±0.01	0.212 ±0.002	3×10^{-5}	0.146	0.297	0.434	0.541	0.631	0.701	0.766		
CJ	1.03 ±0.01	0.201 ±0.003	4×10^{-5}	0.164	0.306	0.439	0.548	0.619	0.688	0.753		
MM	1.00 ±0.02	0.168 ±0.005	2×10^{-4}	0.157	0.309	0.414	0.508	0.581	0.641	0.677		
AT‡				0.136	0.256	0.346	0.423	0.496	0.542	0.602		
BS	1.08 ±0.01	0.253 ±0.002	1×10^{-5}	0.158	0.344	0.490	0.603	0.691	0.761	0.822		
ML	1.00 ±0.01	0.180 ±0.003	9×10^{-5}	0.138	0.275	0.375	0.454	0.524	0.592	0.635		
FG	1.02 ±0.01	0.172 ±0.003	5×10^{-5}	0.130	0.280	0.382	0.478	0.569	0.634	0.693		
RU	1.08 ±0.01	0.209 ±0.003	8×10^{-5}	0.125	0.278	0.421	0.540	0.630	0.689	0.739		
WF‡				0.052	0.152	0.238	0.278	0.329	0.360	0.392		
AP	1.00 ±0.02	0.173 ±0.006	2×10^{-4}	0.144	0.312	0.405	0.488	0.590	0.646	0.688		
CU	1.01 ±0.02	0.158 ±0.007	4×10^{-4}	0.137	0.290	0.359	0.437	0.556	0.610	0.677		
Mean	1.04 ±0.02	0.197 ±0.004	1×10^{-4}	0.145	0.300	0.417	0.516	0.605	0.671	0.725	0.846	0.875

* C_3 is the intercept of the 1 − u curve with the ordinate at zero time. a_u is the rate of decline per day of the 1 − u curve. s^2 is the "variance of the fit" (11).

† Standard deviation.

‡ Calculations were not made in subjects AT and WF because their urine collections were inadequate.

urinary radioactivity curve is shown in Figure 1. When 1 minus the cumulative urinary excretion of radioactivity, u, is plotted against time, after a day or two in all experiments 1 − u is described by the exponential curve $1 - u = C_3e^{-a_ut}$. The parameters C_3 and a_u were determined by the method of least squares by the IBM 709 digital computer (11). The values of C_3, a_u, and "the variance of the fit" are given in Table III, which demonstrates that observed 1 − u is closely fitted by the exponential equation $1 - u = C_3e^{-a_ut}$.

The radioactivity unbound to fibrinogen in the body, z. This radioactivity was calculated from the counts per minute per milliliter of the supernatant from plasma treated with trichloroacetic acid and corrected for the dilution by the trichloroacetic acid and distribution in the iodide space. The latter was taken as 31% of body weight (8). The radioactivity so calculated was expressed as a fraction of the total radioactivity injected. A typical z curve is shown in Figure 1. Mean values for z with their standard deviations for seven subjects at 0, ¼, ½, 1, 2, 3, 4, 5, 6, and 7 days after injection of fibrinogen-^{125}I were 0.02 ± 0.01, 0.03 ± 0.01, 0.05 ± 0.02, 0.09 ± 0.03, 0.08 ± 0.01, 0.05 ± 0.01, 0.05 ± 0.01, 0.04 ± 0.01, 0.03 ± 0.01, and 0.03 ± 0.01, respectively. Thus z rises rapidly, reaches its maximal value on the first day, and thereafter declines at an exponential rate, paralleling the rate of decline of the plasma fibrinogen-^{125}I radioactivity. However, the radioactivity unbound to fibrinogen in the plasma never exceeded 2% of the total plasma fibrinogen radioactivity.

Calculation of the fractional rates and fibrinogen fluxes. It was shown by Atencio, Bailey, and Reeve (6) that whether or not the animal fibrinogen system was in a steady state, as evidenced by fluctuations or lack of fluctuations of plasma fibrinogen concentration, the fractional rates for fibrinogen breakdown and transcapillary transfer and fibrinogen fluxes could be calculated from the tracer data provided these showed certain characteristics. First, the plasma fibrinogen-^{125}I radioactivity x must be described by an exponential equation of the form $x = C_1e^{-at} + C_2e^{-bt}$; second, after a day or two the curve describing 1 − u must be described by an exponential equation of the

form $1 - u = C_3e^{-a_u t}$ with a_u close in numerical value to a. Further characteristics are that the data describing the downslope of the z curve should be described by $z = C_ze^{-a_z t}$ and that a_z should also be close to a. Another requirement is that the observed values of 1 − u, z, and the extravascular fibrinogen radioactivity (y) should closely agree with those predicted by the model of fibrinogen metabolism (6). Table II demonstrates that the plasma fibrinogen-^{125}I data are well fitted by a two-exponential curve and Table III that 1 − u is closely fitted by a single exponential curve. Further, the Tables show that a and a_u are very near in magnitude, the mean value for a being 0.207 and for a_u 0.197 in the subjects in whom valid comparisons can be made. This agreement is excellent considering the inherent errors in urinary collection and the possibilities of loss of ^{125}I through sites other than the urine (12). Analysis of the data for z shows that the mean value for a_z is 0.198, which is very near the mean value for a in the same subjects. Also, it was seen, as discussed later, that the observed values of 1 − u, z, and y agreed very closely with those predicted by the model (6). It is therefore concluded that fibrinogen-^{125}I in man and rabbit behaves very similarly and the same kinetic analysis may be applied to both. The equations used for calculating the fractional rates and fibrinogen fluxes are given in the Methods, and more fully elsewhere (6).

The fractional rates, j_1 and j_3, and the fibrinogen fluxes, $j_1\bar{x}$ and $j_3\bar{x}$. Table II shows that the fraction of plasma fibrinogen filtered through the capillaries per day, j_1, averaged 0.548 with a range of from 0.219 to 1.394, and the fraction of plasma fibrinogen catabolized per day, j_{3p}, ranged from 0.222 to 0.295 with a mean value of 0.246 per day. The fractional catabolic rate calculated from x and the urinary radioactivity, j_{3u}, averaged 0.242 per day with a range of 0.202 to 0.295 and is in close agreement with j_{3p}. The transcapillary fibrinogen flux, $j_1\bar{x}$, ranged from 24.9 to 159.4 with a mean value of 69.0 mg per kg body weight per day. The catabolic flux, $j_{3p}\bar{x}$, averaged 31.3 mg per kg per day with a range of from 23.3 to 38.5 (Table IV).

Interstitial fibrinogen, $\bar{y}$, and its passage time distribution through the interstitial fluids, $F(T)$. Elsewhere (9, 10) the interstitial albumin is pictured as flowing through an innumerable number of tubes with varying passage times, and the passage time distribution [F(T)] of these tubes, namely that of interstitial albumin, is derived with the plasma albumin specific activity curve. This being the case, the total amount of interstitial albumin can be calculated from $j_1\bar{x}\int_0^\infty F(T)dT$, where $j_1\bar{x}$ is the inflow of albumin into the interstitial fluids (9, 10). By these methods the interstitial fibrinogen $\bar{y}$ was calculated, which averaged 24.0 mg per kg body weight with a range of from

TABLE IV

*Fibrinogen fluxes and interstitial fibrinogen**

Subject	$j_1\bar{x}$	$j_{3p}\bar{x}$	$\bar{y}$	$\bar{y}/\bar{x}$	$F(T) = e^{-dT}$ d
	mg/day/kg	*mg/day/kg*	*mg/kg*		
YT	24.9	25.3	11.6	0.102	2.15
LR	67.1	33.4	31.8	0.244	2.11
CJ	57.8	26.9	19.1	0.178	3.03
MM	93.5	37.0	38.6	0.266	2.42
AT	85.5	38.5	26.2	0.178	3.27
BS	159.4	33.7	20.3	0.178	7.82
ML	39.9	29.5	22.8	0.175	1.75
FG	45.0	33.9	25.9	0.192	1.73
RU	39.1	23.3	20.3	0.193	1.93
WF	96.4	33.4	19.4	0.137	4.97
AP	71.9	25.6	25.0	0.232	2.88
CU	47.7	34.9	26.9	0.180	1.78
Mean	69.0	31.3	24.0	0.188	2.99
SD	±35.3	±5.0	±6.8	±0.044	±1.78

* $j_1\bar{x}$ is the amount of intravascular fibrinogen filtered through the capillaries per day. $j_{3p}\bar{x}$ is the amount of intravascular fibrinogen catabolized per day. $\bar{y}$ is the interstitial fibrinogen. $\bar{y}/\bar{x}$ is the ratio of the interstitial to the intravascular fibrinogen. F(T) is the function describing the passage time distribution of interstitial fibrinogen.

11.6 to 38.6. The ratio of the interstitial to the intravascular fibrinogen ranged from 0.102 to 0.266 with a mean value of 0.188 (Table IV). When the plasma fibrinogen radioactivity curve is a two-exponential function, the passage time distribution of $\bar{y}$ through the interstitial fluids is a single exponential function, $F(T) = e^{-dT}$ (9, 10), and Table IV shows that d varied from 1.73 to 7.82 with a mean value of 2.99. The values of $F(T_1)$ for a given value of T_1 represent the fraction of the transcapillary fibrinogen flux, $j_1\bar{x}$, that requires a passage time greater than T_1 to pass through the interstitial fluids (9, 10). The calculation showed that on the average 30% of the fibrinogen influx returned to the vascular system in $\frac{1}{8}$ day after entry, 49% by $\frac{1}{4}$ day, 72% by $\frac{1}{2}$ day, 84% by $\frac{3}{4}$ day, and 90% by 1 day. However, the calculation of the fraction of $\bar{y}$ that requires passage times through the interstitial fluids greater than $\frac{1}{8}$, $\frac{1}{4}$, $\frac{1}{2}$, $\frac{3}{4}$, and 1 day (9, 10) showed that they were 94, 83, 60, 43, and 29%, respectively. Thus, the major part of the fibrinogen flux passes rapidly through the interstitial fluids, but the major fraction of the interstitial fibrinogen is in slower passage through the interstitial fluids.

Discussion

The present studies were carried out in residents of Denver at an altitude of 5,280 feet. The average plasma fibrinogen concentration was 3.60 ± 0.5 mg per ml. This is considerably higher than the average value of 2.5 mg per ml for residents at sea level (13). The isotope dilution method used for making the measurements has been thoroughly studied elsewhere (4), and the raised plasma fibrinogen levels in healthy Denver residents have been confirmed by another method (13).

The user of iodinated proteins for metabolic studies always has to provide evidence that the iodination has not altered the metabolism of the protein. In animal experiments comparisons can be made with proteins "biologically" labeled (5) or with "screened" proteins (5), but this is not possible in man. The following arguments, however, indicate that the ^{125}I-labeled fibrinogens used were satisfactory. Preparations made in similar circumstances (3, 14) behave satisfactorily in rabbits. The fibrinogen preparations averaged 96% in clottability, and ultracentrifugal analysis (Figure 2) showed a high degree of purity. Protein denaturation leads to lowered values of

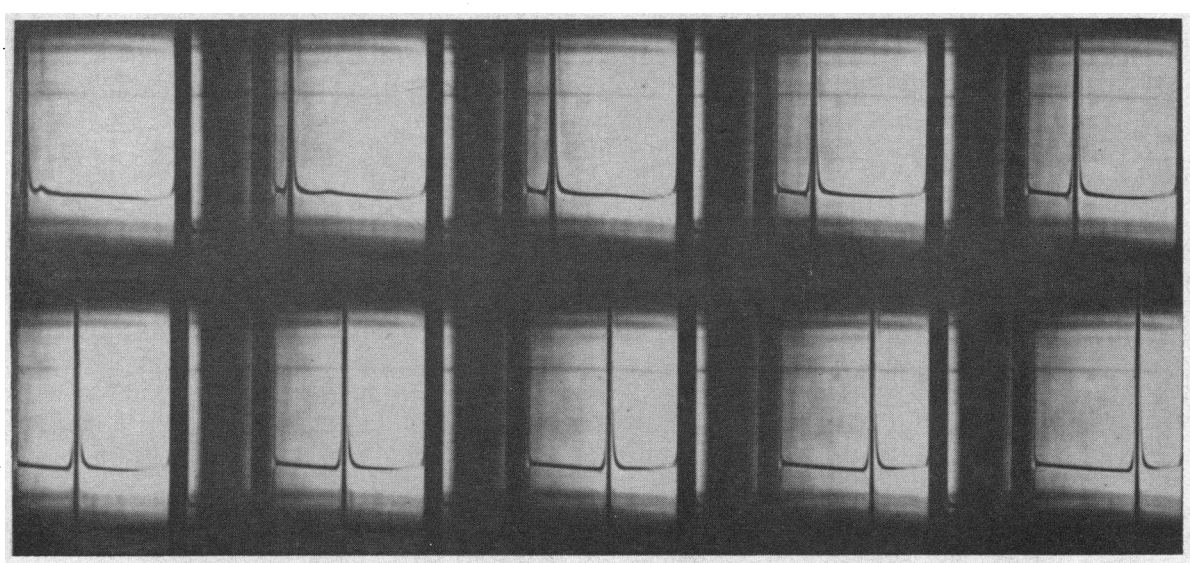

FIG. 2. ULTRACENTRIFUGAL PATTERNS OF THE FIBRINOGEN PREPARATION. The fibrinogen analyzed was 95% clottable with a concentration of 1.2 g per 100 ml. The solvent was 0.005 M sodium citrate. The analysis was carried out in a Spinco model E ultracentrifuge at 59,780 rpm and 20° C. The pictures were taken at 8-minute intervals (from left to right) for the total period of 80 minutes. There are small amounts of impurities in the first and second pictures. The calculation showed that the sedimentation coefficient of the impurity was 37.2, which is much greater than 7.63 for fibrinogen.

C_1, early accumulation of free ^{125}I in the plasma with resulting high z values, and early urinary excretion of ^{125}I at an increased rate (15). None of these findings were seen in the present study. C_1 averaged the high value of 0.82 (Table II), plasma free ^{125}I never exceeded 2% of the total plasma fibrinogen radioactivity, and j_{3u}, which is equivalent to Berson's degradation rate (15), was minimal during the first day and thereafter remained constant (Figure 1). Another test may be used with the fibrinogen-^{125}I. It has been claimed that plasma volume measured with fibrinogen-^{125}I is significantly less than when measured with albumin-^{131}I (16). Fibrinogen-^{131}I that gives higher values for plasma volume than albumin-^{131}I may therefore be considered questionable in conjunction with our unpublished observations. In the present study plasma volume measured with fibrinogen-^{125}I averaged 35.6 ml per kg body weight and was significantly less ($.05 > p > .02$) than the average value of 38.3 ml per kg obtained with albumin-^{131}I on a similar group of subjects in a previous study (10).

Previous studies using fibrinogen-^{131}I and other methods have reported varying results. Madden and Gould (1) reported a study of fibrinogen metabolism in three presumably healthy subjects using ^{35}S-labeled amino acids and gave a mean half-life of 5.6 days. Volwiler and his associates (2) also made a study in eight healthy subjects using ^{35}S-labeled amino acids and gave a mean half-life of 3.5 days. Half-lives alone are insufficient to define fibrinogen metabolism. Gitlin and Borges (17) reported studies on two children with congenital afibrinogenemia in whom they injected nonradioactive fibrinogen intravenously and followed its behavior in the plasma immunochemically. They found about 0.5 for C_1 and a mean half-life of 4 days. However, the metabolism of a plasma protein in a subject congenitally lacking it may not reflect its metabolism in a normal subject. Christensen (18), Hammond and Verel (19), and MacFarlane, Todd, and Cromwell (3) have published studies of fibrinogen metabolism in healthy and sick subjects using fibrinogen-^{131}I, but in some of the studies signs of denaturation of the fibrinogen-^{131}I preparations seem more or less evident. Thus, Christensen gave about 0.5 for the average value of C_1 and a mean half-life of 4.3 days in subjects suffering from headache, neurosis, neurasthenia, gastritis, epilepsy, or asthenia. Hammond and Verel reported about 0.45 for the average value of C_1 and a mean half-life of 5.1 days in six patients, and their fibrinogen-^{131}I gave much higher values of plasma volume than those obtained with albumin-^{131}I. The long half-lives suggest the presence of labeled globulins, since altered fibrinogen-^{131}I does not show an increased half-life (6). The fibrinogen-^{131}I used by McFarlane and co-workers seems the most satisfactory. In four normal subjects the mean value of C_1 was 0.77 and the half-life about 3 days, compared with our means of 0.82 and 3.36 days. Their mean values of 0.31 per day for fractional catabolic rate and 41.2 mg per kg per day for catabolic flux of fibrinogen are, however, rather higher than our values of 0.25 and 31.3 mg per kg per day. Amris and Amris (20) reported studies of fibrinogen-^{131}I metabolism in three subjects suffering from neurosis or intervertebral disc prolapse that agree closely with ours.

The present study does not provide clues as to how fibrinogen is catabolized, namely, whether it is broken down directly or after fibrin formation, or by both paths. However, mathematical considerations described below provide some information. Elsewhere (6) the fibrinogen system is described by six differential equations, but when assumed that the fibrin-^{131}I [w(t)] and the fibrinogen-^{131}I at the catabolic site [v(t)] were negligibly small or nonexistent, or their respective fractional catabolic rates j_5 and k_6 were very large if both w(t) and v(t) were present in significant amounts, the fibrinogen system can be described by four differential equations. The validity of these assumptions may be tested by comparing the observed values of the breakdown products of fibrinogen-^{125}I (z), the cumulative radioactivities excreted in the urine (u), and the extravascular fibrinogen-^{125}I (y) obtained from $y = 1 - x - z - u$ with those values predicted by the four differential equations describing the simplified model. The analysis showed that the observed values of z and y agreed closely with those predicted by the equations, and as shown in Figure 3 the agreement between the observed and predicted values of u was excellent, justifying the assumptions made above. Particular attention should be paid to the close agreement between the observed and predicted values of u, since had there been significant

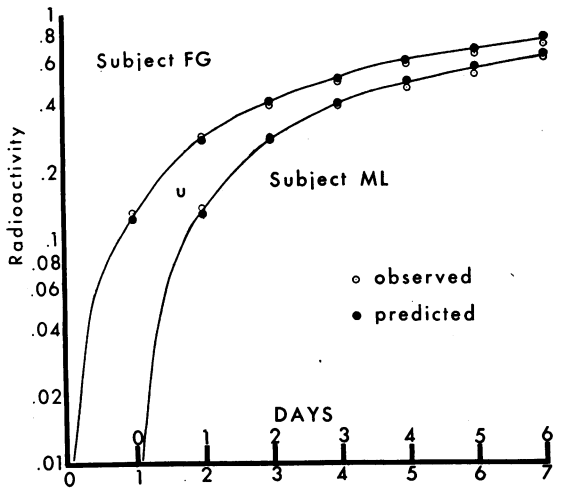

FIG. 3. COMPARISON OF OBSERVED AND PREDICTED VALUES OF U IN SUBJECTS FG AND ML. u is the cumulative radioactivities excreted in the urine. For further details see text.

amounts of fibrin-^{125}I formation with a slow catabolic rate there should be a delay in their excretion in the urine compared with the predicted values of u. Thus, these results do indicate that in healthy individuals fibrinogen is either catabolized directly with insignificant fibrin formation, or, if significant amounts of fibrin are formed, these are broken down very rapidly. Also, the close agreement between the observed and predicted values of z, y, and u implies that fibrinogen is broken down intravascularly, since in this model fibrinogen is assumed to be catabolized in the vascular compartment, although it does not eliminate the possibility that it may be broken down at sites in rapid exchange with the vascular compartment. This thesis is particularly strengthened in view of the close agreement between the observed and predicted values of u, for if the catabolism of fibrinogen-^{125}I had taken place in extravascular compartments slowly exchanging with the plasma, the urinary excretion of the breakdown products of fibrinogen-^{125}I would have been significantly delayed compared with the predicted values of u.

Summary

Fibrinogen averaging 96% clottability was prepared from the subjects' own plasma by repeated salt fractionation with ¼ saturated ammonium sulfate and labeled with ^{125}I in a ratio of 0.5 or less atoms iodine per molecule of fibrinogen. With this preparation, studies were made of the metabolism and distribution of fibrinogen in 12 healthy male subjects.

The results of the present studies were as follows: The average value for the plasma volume was 35.6 ± 3.5 ml per kg; for the plasma fibrinogen concentration, 360 ± 50 mg per 100 ml; for the intravascular fibrinogen, 127 ± 17 mg per kg; for the interstitial fibrinogen, 24.0 ± 6.8 mg per kg; for the slower half-life of fibrinogen-^{125}I, 3.36 ± 0.25 days; for the transcapillary transfer rate of fibrinogen, 69.0 ± 35.3 mg per kg per day; and for the catabolic rate of fibrinogen, 31.3 ± 5.0 mg per kg per day.

These studies are compared with those of previous investigators, and criteria of satisfactory behavior of fibrinogen-^{131}I are examined. The kinetic data indicate that in healthy individuals fibrinogen is either catabolized directly with insignificant fibrin formation or that if significant amounts of fibrin are formed these are broken down almost instantaneously. Also it was shown that fibrinogen is catabolized intravascularly or at sites in rapid exchange with the vascular compartment.

Acknowledgments

My sincere thanks are due to Dr. E. B. Reeve for his unfailing aid and encouragement and to Laurie Stuart for her technical assistance.

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