Evaluation of Mathematic Models to Assess Platelet Kinetics

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Twelve mathematic methods used to calculate the mean platelet survival time were compared by determining the "goodness of fit" of the models to the platelet survival curves of 15 reference subjects and 54 patients. Platelets were labeled with [^{111}In]oxine. The linear (LN), exponential, weighted mean, multiple hit (MH), Dornhorst (DH), Meuleman (ML), alpha order (AO), and polynomial (PO) mathematic models were investigated. The goodness of fit for the exponential model was determined by the nonlinear least squares method (EP), and also by the linear least squares method on logarithmically transformed data (EX) as is recommended. The modified weighted mean (MWM) and the usual weighted mean method (WM) obtained with these exponential models were tested. The Dornhorst (DH10) and Meuleman (ML10) models, where the potential age-dependent platelet survival times were kept constant at 10 days, were also evaluated. The goodness of fit results, expressed as % s.d. indicated that the LN (5.2%), EX (5.0%), EP (4.4%), WM (3.7%), DH10 (3.7%), and ML10 (3.7%) models all fitted the data significantly worse than the MWM, MH, DH, ML, AO, and PO models (range 3.2–3.3%). The mean platelet survival time determined with the MH model differed significantly from the results with the DH, ML, and AO models. The results of mean platelet survival time calculated with different mathematic models cannot, therefore, be compared directly. The models that fitted the platelet survival curve well varied slightly in sensitivity to noise as is indicated by the coefficient of variation of the mean platelet survival time estimates for the reference subjects (range 7.9–12.0%). Fitting data to at least two mathematic models has definite advantages. Data on which the calculations are based are probably invalid if the following are true: (a) if the mean platelet survival time estimated with the alpha order model is shorter than that estimated with the EP, MWM, or MH models, or (b) the mean platelet survival time estimated with either the DH, ML, AO, or PO models, is longer than the LN, MWM, or MH estimate of the mean platelet survival time. We conclude that the mean platelet survival time can be reliably estimated by fitting the data to either the MWM method (if limited computing facilities are available) or the MH model. Confidence in the result will be increased if considered in conjunction with the finding obtained with one other model; in those cases where the platelet survival time is very short, the alpha order model is recommended. In other instances the results of the MWM method or MH model should be compared with that obtained with either the DH, ML, AO, or PO models.

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Shortening of the platelet life span is generally accepted as an important reflection of in vivo platelet activation. This measurement has been especially valuable for the understanding of the role of platelets in (a) various diseases characterized by thromboembolic phenomena or thrombocytopenia, (b) the evaluation of the efficacy of drug therapy in these diseases, and (c) for the detection of some prethrombotic states. The accurate measurement of platelet life span is a prerequisite for the adequate investigation of these disorders.

The available methods for isolating or labeling a cohort of platelets of the same age are unsatisfactory and cannot be used in clinical studies. Therefore, a population of platelets representative of those in the circulation is isolated, labeled with a radionuclide, and reinjected. The mean survival time of the labeled platelets is indirectly estimated from the ratio of the

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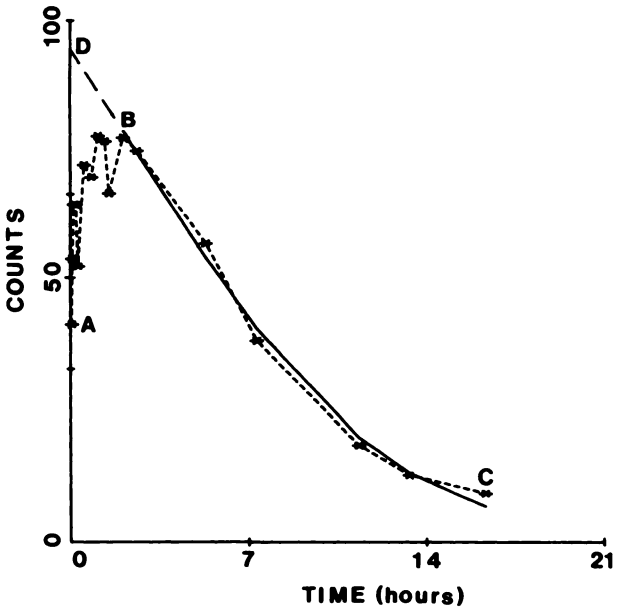

FIGURE 1
Calculation of mean platelet survival time is demonstrated. Period A to B represents equilibrium phase. Mathematic model is fitted from B to C and regression curve, indicated by solid line, is generated. This curve is back extrapolated from B to D as indicated by dotted line. At D y-intercept and initial slope can be determined

y-intercept and the initial slope of the platelet survival curve (*1,2*). This curve is constructed from the radioactivity of the labeled platelets remaining in the circulation. This is measured in blood samples taken at appropriate times after the reinjection of the labeled platelets. The y-intercept and the initial tangent of the survival curve cannot be directly measured because the stability of the early part of the curve is disturbed by the redistribution of platelets between the circulation and the splenic platelet pool, and by the temporary accumulation of platelets in the liver. The latter, ascribed to the "collection injury," varies in severity. The labeled and circulating platelets only equilibrate after 1–24 hr. This results in a plateau of the early phase of the platelet survival curve (*3,4*). Only the later part of the survival curve will reflect the true rate of clearance of platelets from the circulation. The y-intercept and slope of the curve can be determined only by back extrapolation of this part of the curve (Fig. 1). Although extrapolation may be performed visually, this method is subject to errors and bias. Mathematic models have therefore been developed to facilitate accurate fitting of the platelet survival curve to the data by using statistic methods. The regression constants generated by the mathematic models permit more precise estimation of the initial parameters. It should be noted that these mathematic models are not required to be based on valid physiologic assumptions. They solely function to improve the "goodness of fit" of the platelet survival curve to the data points.

Although many such models have been applied to the study of platelet kinetics in health and disease, little information is available on their comparative value or on their suitability under specific pathologic conditions (*5–9*). The present study focuses on these questions. It also specifically provides information on the estimate of mean survival of autologous platelets labeled with indium-111- (^{111}In) oxine, a radionuclide complex which has specific advantages for the study of platelet kinetics (*3,10*).

MATERIALS AND METHODS

Subject Selection

Platelet survival was determined in 15 reference subjects and on 54 patients with a variety of disorders. The patient group consisted of four patients with valvular disease of the heart, 17 with previous renal transplantation, 14 with idiopathic thrombocytopenic purpura, two with diabetes mellitus, 14 with heterozygous Type IIa familial hypercholesterolemia, and three with aortic aneurysms. All subjects were in a steady state of platelet production and destruction during the course of investigation. This was reflected by a constant blood platelet count.

Platelet Labeling

Platelets were labeled with [^{111}In]oxine as previously described (*9–11*). Eighty-five milliliters of blood were collected in a polystyrene syringe containing 15 ml acid citrate dextrose and NIH formula A (ACD) as anticoagulant. Platelet rich plasma (PRP) was obtained by centrifugation of the blood at 180 *g* for 15 min. Platelets trapped in the red cell layer were harvested by washing four times: blood was centrifugated at 750 *g* for 3 min, the supernatant removed, and the cells resuspended in Plasmalyte B electrolyte solution.* After acidifying to pH 6.2–6.5 with ACD, platelets were sedimented by centrifuging at 800 *g* for 30 min. The platelet pellet was resuspended in physiologic saline and incubated for 30 min with [^{111}In]oxine. The labeled platelets were washed with, and finally resuspended in, autologous platelet-poor plasma.

Labeled platelets were used for in vivo studies only if in vitro aggregation with ADP and collagen was normal (*3,9,10*). A dose not exceeding 500 μCi (18.5 MBq) of ^{111}In was administered.

Following injection of the labeled platelets, blood samples were obtained at 5, 10, 15, 30, 70, and 90 min, and daily thereafter. In patients with short mean platelet survival times, blood sampling was appropriately spaced so that at least eight valid data points were available for curve fitting. Blood samples of 3 ml were directly collected in the counting vials, the mass determined, and water added to a constant final volume of 4 ml. The samples were counted in a well scintillation counter with the pulse-height analyzer set to include the 172 and 247 keV peaks of ^{111}In. The measured count rate was corrected for background and mass. Plasma ^{111}In activity was determined in all cases, and was <5%.

MATHEMATIC FUNCTIONS AND METHODS TO FIT PLATELET SURVIVAL CURVES

The following mathematic functions (see full description in Appendix) were fitted to the data points reflecting the rate of clearance of platelets from the circulation:

Linear Function (LN) (*11,12*)

Exponential Function (*11,12*)

Two methods were used to fit the data to an exponential function. In the first method (EX) the regression constants for the exponential model were calculated as recommended by the ICSH; the data were logarithmically transformed and the curve fitted with the linear least squares method (*11*). In the second method (EP), the curves were fitted with the Newton-Gauss method, an iterative nonlinear least squares technique (*13*). The latter method was investigated because the ICSH recommends that the discrepancies between the fitted line and data points be calculated without logarithmic transformation of the data. The nonlinear least squares technique minimizes these discrepancies (*11,13*).

Weighted Mean Function (*11,12*)

This was calculated by two methods, dependent on the method used to fit the data to an exponential function. The first method (WM) was according to the recommendations of the ICSH where the exponential regression curve is constructed after transforming the data logarithmically (*11*). For the second, a modified weighted mean method (MWM), the exponential regression constants were obtained by fitting the data with a nonlinear least squares technique. The variance of the linear, exponential, and modified weighted mean methods was calculated. If the variance of either the linear or exponential models was less than that of the modified weighted mean method, the result of the method that fitted the data best was used as the estimate of the mean platelet survival time by the modified weighted mean method. This modification was introduced to minimize the influence of noise when data points were distributed perfectly linearly or exponentially.

Alpha Order Function (AO) (*14*)

This function was fitted to the survival curve data by the Newton-Gauss iterative nonlinear least squares technique (*13*). Initial estimates of regression constants for the iteration procedure were selected as follows. The initiation constant k_0 was set to unity and alpha, "a," replaced by the weighted mean estimate of the variance shape factor (*12*). The rate constant "k" was calculated substituting the alpha order mean platelet survival time, T_a, by the weighted mean estimate, T_w (see Appendix):

$$k = (1/T_w)\,[1 + (1 - a)k_0].$$

Polynomial Function (PO)

Only the second degree polynomial function was used. The higher degree polynomial functions were found to be too sensitive to noise and outliers.

Multiple Hit Function (MH) (*11*)

The curve fitting technique described by the ICSH was used. Initial values for iteration were obtained from the linear and exponential curve fitting procedures (*11*).

Dornhorst Function (*11,15*)

The curves were fitted with the Newton-Gauss iterative nonlinear least squares technique (*13*). Mean platelet lifespan was determined by two methods. First (DH10), the potential age-dependent platelet survival time was kept constant at the value determined for normal subjects, 10 days. In the second method (DH), the potential age-dependent platelet survival time as determined for the specific patient was used.

The iterative nonlinear curve fitting technique requires initial estimates of the potential age-dependent platelet survival time and the rate constant, "k." The first was taken to be 10 days, and the initial estimate of k was calculated by solving the following equation by the Newton-Raphson method (*16*) (see Appendix):

$$T_d = [1 - \exp(-kT)]/k.$$

The weighted mean estimate of the platelet survival time, T_w, was used in the initial value of T_d, the Dornhorst estimate of the mean platelet survival time.

Meuleman Function (*17*)

Curve fitting of the Meuleman function (ML and ML10) was performed as was described for the Dornhorst function.

CURVE FITTING PROCEDURE

A computer program in Fortran, executed on a nuclear medicine digital imaging system, was developed to fit survival curves to the mathematic models. The survival curves were entered by keyboard and stored on the system database. Curve fitting, with any of the above models, was menu driven. It allowed graphic display of the curves to select the range of points for curve fitting. The data points were selected visually as follows. The first point included was that reflecting the highest blood radioactivity after equilibrium had been reached. All subsequent data points with radioactivity >10% that of the initial counts were included. The goodness of fit of the data points to each of the mathematic models was visually evaluated by inspecting the graphic display of the survival and regression curves on the computer terminal. Print-out of results, including all relevant information such as regression constants, mean platelet survival time, and indication of goodness of fit could be selected by menu. A hard copy of measured and regression survival curves could then be recorded with a four-color plotter. A separate graphics package was developed for use with the system database.

COMPARISION OF MATHEMATIC MODELS

The different mathematic models were compared by calculating the root mean square of the difference between the measured and regression survival curve points. This value, reflecting the goodness of fit, was expressed as a percentage of the y-intercept and designated as the % s.d. The survival curves with different count rates could thus be compared.

The % s.d. and mean platelet survival time calculated for the different models were compared with Hotteling's multivariate T-squared statistic to test the hypothesis of equality of means (*16,18*). The patients were considered to be a random sample from a population and a log-transformation applied

TABLE 1
Percentage Standard Deviation (PSD) of Platelet Survival Curve Points Around Fitted Curve

Model	Whole group (69) PSD	Whole group (69) s.d.	Reference subjects (15) PSD	Reference subjects (15) s.d.
Linear	5.2	3.4	2.9	1.3
Exponential (EP)	4.4	1.8	4.8	1.3
Exponential (EX)	5.0	2.4	6.1	2.6
Modified weighted mean	3.4	1.9	2.6	1.2
Weighted mean	3.7	2.3	2.6	1.2
Multiple hit	3.3	1.8	2.5	1.4
Dornhorst	3.3	1.9	2.5	1.3
Dornhorst (DH10)	3.7	1.8	2.9	1.3
Meuleman	3.4	1.9	2.5	1.3
Meuleman (ML10)	3.7	1.8	2.9	1.3
Alpha order	3.2	1.8	2.5	1.4
Polynomial	3.3	1.9	2.5	1.3

to the % s.d. and mean platelet survival time data. The multivariate model incorporates different observations that may be dependent, on the same patient. In those instances where the means differed significantly, multiple comparisons of the means were used to identify those differences which were responsible for the rejection of the hypothesis of equality of means.

The influence of experimental error ("noise") on the estimate of mean platelet survival was determined by the coefficient of variation (CV) in the normal subjects.

RESULTS

Evaluation of Mathematic Models: Goodness of Fit

Goodness of fit achieved by the different mathematic models were evaluated by calculating the % s.d. The results of the mean % s.d. for the reference subjects, and for the whole group (patients and reference subjects) determined for all the mathematic models are given in Table 1. The range was 3.2–5.2% for the whole group and 2.5–6.1% for the reference subjects. The differences in the % s.d. of data fitted with the different models are given in Table 2. The % s.d. of the LN model and the fitting of the data to the exponential model both by a linear least squares fit of logarithmically transformed data (EX) or by the linear least squares method (EP) was significantly ($p < 0.01$) larger than that of the other models: the differences exceeded 0.6%. These models are therefore not suitable for the calculation of the mean platelet survival time. Also, the mean % s.d.s obtained by the Dornhorst model where the age-dependent survival time is held constant at the normal value (DH10), were significantly larger than the MH ($p < 0.01$), DH ($p < 0.01$), ML ($p < 0.05$), AO ($p < 0.05$), and PO ($p < 0.05$) models. The % s.d. associated with the Meuleman model, with a constant age-dependent survival time (ML10), was larger than that of the MH ($p < 0.01$), DH ($p < 0.05$), and AO ($p < 0.05$) models. The % s.d. obtained with the WM model was significantly larger ($p < 0.01$) than that obtained with the MH and DH models. The % s.d. of the MWM and DH models differed significantly ($p < 0.05$). The good correlation of the % s.d.s associated with those mathematic models which did not differ significantly is shown in Fig. 2.

In the reference group, the % s.d. of the exponential model, obtained by both methods of curve fitting (EX and EP), and the other models differed significantly (Table 3). The exponential models, therefore, fitted the data poorly.

Platelet Survival Time

We next investigated whether the mean platelet survival times estimated with those mathematic models that fitted the survival curves equally well differed. The mean platelet survival time (ST), standard deviation (s.d.), and coefficient of variation (CV) are presented in Table 4. The estimate of the mean platelet survival time for the reference subject group varied from 204.2 ± 24.5 hr for the DH model to 227.7 ± 17.7 hr for the MH model. The mean platelet survival time estimated by the MH model was significantly longer when compared with that measured with the DH ($p < 0.01$), ML ($p < 0.01$), and AO models ($p < 0.05$) (Table 5). The estimate with the WM method was also significantly longer ($p < 0.05$) than the DH and ML models. In the reference subjects there was also a significant difference ($p < 0.01$) between the mean platelet survival time calculated with the MH method com-

TABLE 2
Differences in PSD for Mathematic Models Investigated in Patient Group and Reference Subjects (N = 69)

Model	EP	EX	MWM	WM	MH	DH	DH10	ML	ML10	AO	PO
LF	0.9	0.2	1.8†	1.5†	1.9†	1.9†	1.6†	1.9†	1.6†	2.0†	2.0†
EP		−0.6†	1.0†	0.7†	1.1†	1.1†	0.7†	1.0†	0.7†	1.2†	1.1†
EX			1.6†	1.3†	1.7†	1.7†	1.3†	1.7†	1.4†	1.8†	1.7†
MWM				−0.3	0.1	0.1*	−0.3	0.1	−0.2	0.2	0.1
WM					0.4†	0.4†	0.0	0.3	0.0	0.5	0.4
MH						−0.0	−0.4†	0.0	−0.3†	0.1	0.0
DH							−0.4†	−0.0	−0.3*	0.1	0.0
DH10								0.3*	0.0	0.5*	0.4*
ML									−0.3	0.2	0.1
ML10										0.4*	0.4
AO											−0.1

* Difference significant at $p < 0.05$.
† Difference significant at $p < 0.01$.

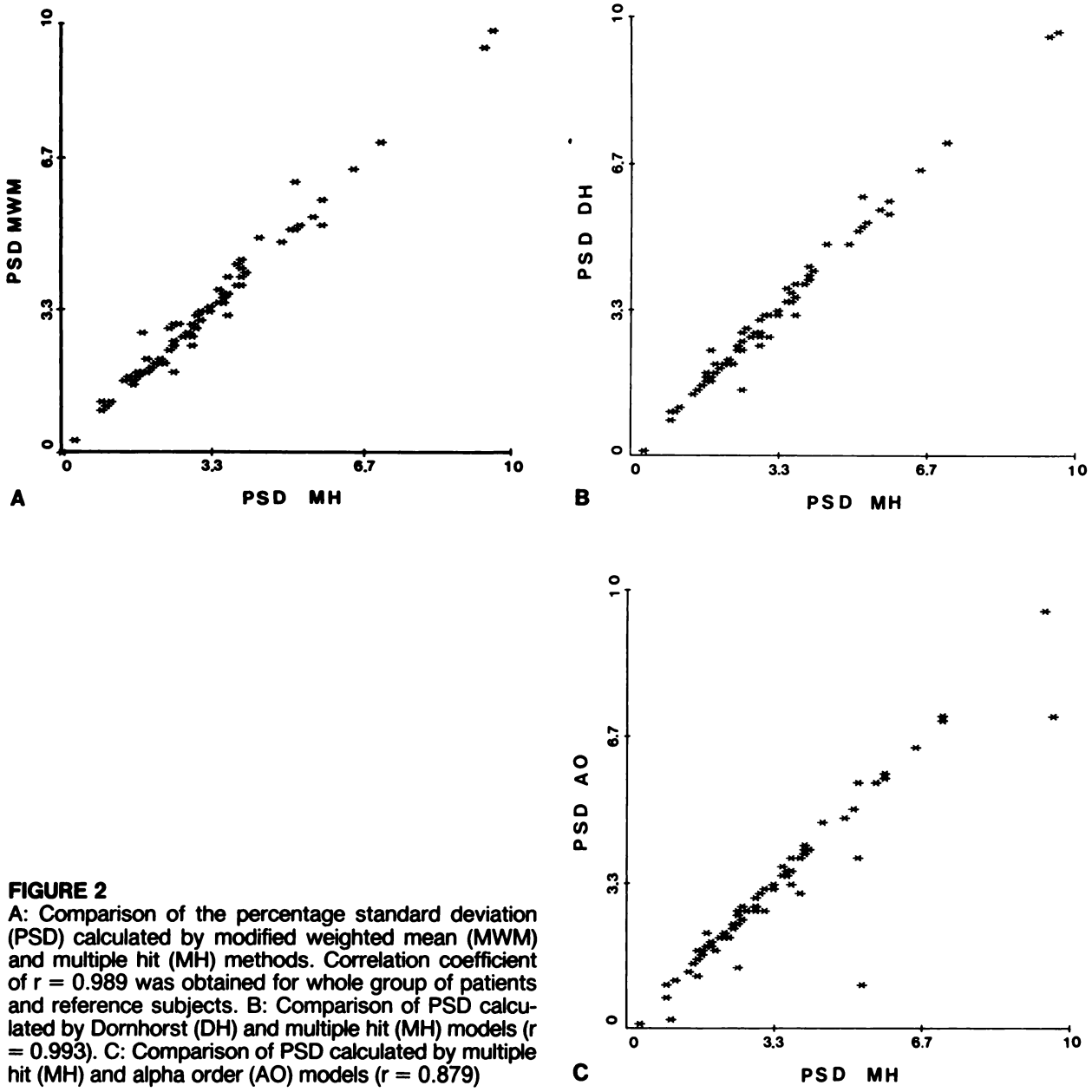

FIGURE 2
A: Comparison of the percentage standard deviation (PSD) calculated by modified weighted mean (MWM) and multiple hit (MH) methods. Correlation coefficient of r = 0.989 was obtained for whole group of patients and reference subjects. B: Comparison of PSD calculated by Dornhorst (DH) and multiple hit (MH) models (r = 0.993). C: Comparison of PSD calculated by multiple hit (MH) and alpha order (AO) models (r = 0.879)

pared to that with the DH, ML, and the PO models (Table 6). The estimated mean platelet survival time with the AO model also differed significantly ($p < 0.05$) from that with the DH and ML models. The estimated mean platelet survival time with the DH and ML models were nearly identical.

The range of the mean platelet survival time (CV) observed in the reference subjects was used as an approximate indicator of the sensitivity of the mathematic models to noise. The CV for the MH model was 7.9% (Table 4). The range of the CV for the MWM, DH, ML, PO, and AO models was 9.9–12%.

There was good correlation (r = 0.986) of mean platelet survival times calculated with MH and MWM models (Fig. 3). In addition, the MH and DH models correlated well (r = 0.962), despite a deviation from the linear regression line in some instances where the mean platelet survival time fell within the normal range (Fig. 4). This deviation was not observed when the DH, ML, AO, and PO models were correlated (Fig. 5). Deviations from the linear regression line within the normal range was accompanied by another finding: the estimate of the mean platelet survival time with the DH, ML, AO, and PO models was longer than that obtained by fitting the data to the LN model. This finding was reflected by a slightly concave shape of the platelet survival curve.

DISCUSSION

Although the method of labeling platelets with chromium-51 has been widely used, the estimation of platelet life span with ^{111}In-labeled platelets has been validated and is now generally regarded as superior (*4,9, 10*). The latter radionuclide also permits quantitative imaging of the in vivo distribution of platelets. This has some important advantages when determining the

TABLE 3
Differences in PSD for Mathematic Models Investigated in Reference Subjects (N = 15)

Model	EP	EX	MWM	WM	MH	DH	DH10	ML	ML10	AO	PO
LF	−1.9*	−3.2†	0.3	0.3	0.4	0.4	−0.0	0.4	−0.0	0.4	0.4
EP		−1.3†	2.2†	2.3†	2.3†	2.3*	1.9†	2.3*	1.9†	2.3*	2.3*
EX			3.5†	3.5†	3.6†	3.6†	3.2†	3.6†	3.2†	3.6†	3.6†
MWM				0.0	0.1	0.1	−0.3	0.1	−0.3	0.1	0.1
WM					0.1	0.1	−0.3	0.1	−0.3	0.1	0.1
MH						0.0	−0.4	0.0	−0.4	0.0	0.0
DH							−0.4	0.0	−0.4	−0.0	−0.0
DH10								0.4	0.0	0.4	0.4
ML									−0.4	−0.0	−0.0
ML10										0.4	0.4
AO											0.0

* Difference significant at $p < 0.05$.
† Difference significant at $p < 0.01$.

mean platelet survival time. Monitoring of the extent of the early accumulation of labeled platelets in the liver permits more precise evaluation of the "collection injury." Also, the size of the exchangable splenic platelet pool may be estimated accurately by quantitative imaging. These advantages were exploited in our study.

The confidence in the accuracy of the estimate of platelet life span is dependent on accepting a number of assumptions and satisfying certain basic prerequisites: platelet production and destruction must be in a steady state, the platelet population isolated for labeling must be fully representative of those in the circulation and a subpopulation of either younger or older platelets should not be harvested, and in vivo elution of the label must not be excessive. In the present study, platelets were labeled with a validated method (*9,10*). Only those patients in a steady state of platelet destruction and production were included. There was no evidence of severe in vitro damage to platelets: This was reflected by an adequate in vitro platelet aggregation response, normal recovery of labeled platelets in the circulation, and the transient excess accumulation of platelets in the liver after reinjection of ^{111}In-labeled platelets was <5%.

One of the major problems in estimating the mean platelet survival time is the decision to include or exclude initial data points on which the calculation of the rate of clearance of platelets from the circulation will be based. We did not use an automatic mathematic method but relied on visual inspection of the selected data points and correlating this with the data obtained from the in vivo quantification of platelet distribution. Excessive collection injury can readily be recognized by visual inspection and correlation with quantitative data of organ radioactivity. The signs of collection injury are as follows: The early plateau of the platelet survival curve is prolonged or fluctuates markedly, and radioactivity in the liver does not reach a plateau or remains higher than expected (*9,10*). Care was also taken to include a sufficient number of appropriately spaced data points for fitting the survival curve.

Although the ICSH has recommended the weighted mean, multiple hit, and Dornhorst models for the estimate of platelet life span (*11*), other mathematic models have their proponents (*19–22*). We have slightly modified the weighted mean method recommended by the ICSH (*11*). We determined the regression constants by

TABLE 4
Estimation of Mean Platelet Survival Time (hr) with Different Mathematic Models

	Whole group (69)		Reference subjects (15)		
Model	ST	s.d.	ST	s,d,	CV
Linear	176.9	72.5	232.6	15.2	6.5
Exponential (EP)	97.8	42.1	122.7	15.4	12.5
Exponential (EX)	87.5	37.1	99.9	15.9	15.8
Modified weighted mean	144.0	74.3	214.3	23.0	10.7
Weighted mean	145.0	69.0	215.4	19.1	8.9
Multiple hit	149.4	75.6	222.7	17.7	7.9
Dornhorst	142.4	78.2	204.2	24.5	12.0
Meuleman	143.1	78.5	204.7	24.1	11.7
Alpha order	146.2	87.6	212.4	21.0	9.9
Polynomial	147.3	78.4	206.8	22.6	10.9

TABLE 5
Differences in Mean Platelet Survival Time Obtained with Mathematic Models in Reference Subjects and Patient Group (N = 69)

Model	WM	MH	DH	ML	AO	PO
MWM	−1.0	−5.4	−1.6	0.9	−2.1	−3.2
WM		−4.4	2.6*	1.9*	−1.2	−2.3
MH			7.0†	6.3†	3.3*	2.2
DH				−0.7	−3.8	−4.9
ML					−3.0	−4.1
AO						−1.1

* Difference significant at $p < 0.05$.
† Difference significant at $p < 0.01$.

Table 6
Differences in Mean Platelet Survival Time of Reference Subjects Estimated with Mathematic Models (n = 15)

Model	WM	MH	DH	ML	AO	PO
MWM	−1.1	−8.4	10.1	9.6	2.0	7.5
WM		−7.3	11.2	10.7	3.0	8.6
MH			18.5†	18.0†	10.3	15.9†
DH				−0.5	−8.1*	−2.5
ML					−7.6*	−2.0
AO						5.6

* Difference significant at $p < 0.05$.
† Difference significant at $p < 0.01$.

nonlinear least squares calculation rather than by a linear least squares fit of logarithmically transformed data. The nonlinear curve fitting technique improved the goodness of fit for the exponential model significantly. We also preferred to adopt the result with either the linear or the exponential models rather than that of the modified weighted mean model if the % s.d. of data points from the fitted line of the latter was greater than that of either of the former. These modifications, which are logical and do not increase the complexity of the calculations, improved the goodness of fit of the modified weighted mean method (Table 2). It is, therefore, preferred over the ICSH weighted mean method.

On the basis of our results we could divide the ability of the models to fit the data into three groups. The linear and exponential models fitted the platelet survival curves of the patients badly. The poor fit of the data by the regression curves resulted in an inaccurate estimate of the mean platelet survival time. This finding is in agreement with results reported by Paulus (*20*). Estimates of the mean platelet survival time were in many cases in error by 50–300%. These methods can obviously not be recommended. Our results also indicate

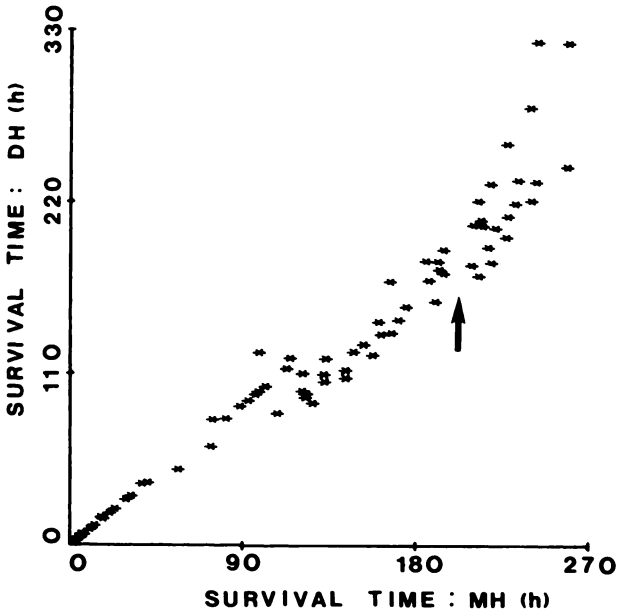

FIGURE 4
Comparison of mean platelet survival time estimated by Dornhorst (DH) and multiple hit (MH) models (r = 0.962). There was deviation of some points from regression line for survival times exceeding 200 hr

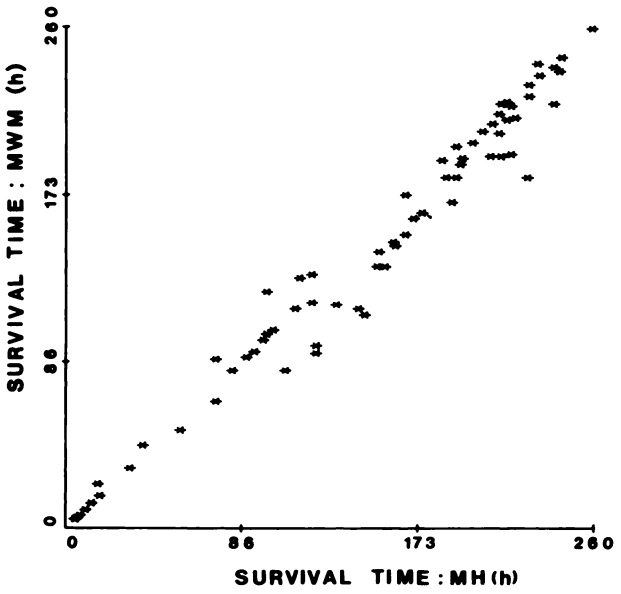

FIGURE 3
Comparison of mean platelet survival time estimated by modified weighted mean (MWM) and multiple hit (MH) models (r = 0.986). Regression curve close to line of identity was obtained

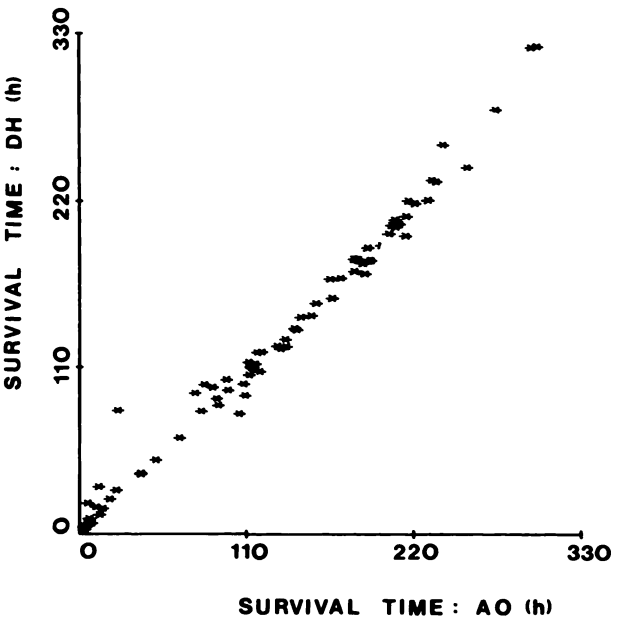

FIGURE 5
Comparison of mean platelet survival time estimated by Dornhorst (DH) and alpha order (AO) models (r = 0.949)

that the weighted mean method applied as recommended by the ICSH, and the variations of the Dornhorst and Meuleman models where the potential age-dependent platelet life span is kept constant at 10 days (the normal platelet survival time), also fit the data fairly poorly. Our modification of the weighted mean model, multiple hit, Dornhorst, Meuleman, alpha order, and polynomial models all performed well and there was no evidence that any one of these is definitely superior.

Although these mathematic models fitted the curves equally well, their estimates of the mean platelet survival times differed. In the whole group, the mean platelet survival time estimated with the multiple hit model differed significantly from the results with the Meuleman, Dornhorst, and alpha order models. Also, in the normal subjects, there were significant differences between results of mean platelet life span estimated with the Dornhorst or Meuleman models compared with the estimate when the curve was fitted with the multiple hit or alpha order models. The mean platelet survival time obtained with the multiple hit and polynomial models also differed significantly. There is no obvious physiologic basis for these differences and it is obvious that the application of, or preference for, any specific model is arbitrary. The major implication of this finding is, however, that it may be hazardous to directly compare results of studies where the platelet life spans were not estimated with the same mathematic model.

The use of more than one mathematic model for the assessment of the data has definite advantages. This is illustrated by our findings. The shape of the platelet survival curve is concave if the basic requirement of a steady state is not satisfied and the rate of platelet production exceeds that of platelet destruction. Although this may be detected by visual inspection of the shape of the platelet survival curve, doing this with the mathematic models is more sensitive and less subjective. A concave survival curve is present when the mean platelet survival time estimated with either the Dornhorst, Meuleman, alpha order, or polynomial models' exceeds that obtained by fitting the data to a linear function. Other indicators of a concave platelet survival curve are that the Dornhorst and Meuleman models' estimates of the platelet life span are longer than the potential age-dependent platelet life span, or, if "alpha" determined in the alpha order model has a negative value. It should be noted, as predicted by theory, that the mean platelet survival time estimated with either the multiple hit or weighted mean methods never exceeded that determined by fitting the data to a linear function.

In patients with immune thrombocytopenic purpura, where the mean platelet survival time was very short, results with the alpha order model differed from those of the other models. In some instances the value of alpha was greater than unity and the estimate of mean platelet life span shorter than that estimated with an exponential function. This indicates that a higher order function is operative. In these cases the other mathematic models, in contrast to the alpha order model, simply fitted the curves to an exponential function. The investigator may, however, be alerted to the presence of such a higher order function by another finding: The estimate of the potential age-dependent platelet survival time will be greater than normal. It is therefore evident that certain patterns of results obtained by fitting the data to several mathematic models may alert the investigator to the possibility that the data on which the estimates were based were invalid, or that the assumptions on which the mathematic model are based are not operative in the particular case.

The usefulness of a mathematic model will also be determined by its sensitivity to "noise." "Pure" noise is introduced by experimental error and is related either to the sampling procedure or to the statistics of counting the radioactivity of the blood sample. "Physiologic" noise could also be introduced by the redistribution of platelets owing to their pooling in organs, and the influence of exercise and posture. Sensitivity to noise will decrease the precision of the measurement of the mean platelet survival time. The sensitivity to noise cannot be directly determined because the "true" mean platelet survival time, represented by the platelet clearance curve under investigation, is not known. We have therefore used the range of the mean platelet survival time observed in normal subjects (where the platelet survival curve is effectively linear) as an approximate indicator of sensitivity to noise. The multiple hit model is relatively insensitive to noise. This is reflected by the coefficient of variation: In normal subjects it was 7.9% for the multiple hit, compared with 6.5% obtained with the linear model. The other models that fitted the data well (modified weighted mean, Dornhorst, alpha order, Meuleman and polynomial function) are somewhat more sensitive to noise: In normal subjects their coefficients of variation varied from 9.9–12.0%. The good correlation of the mean platelet survival times calculated with these models that fitted the data well also indicates that the sensitivity to noise of these models is acceptable.

On the basis of the results of this study, we would make the following recommendations for estimating the mean platelet survival time. The multiple hit model is recommended, but if limited computing facilities are available, our modified weighted mean method is satisfactory. These models are insensitive to noise, but do not indicate the presence of invalid physiologic mechanisms. Therefore, it is always necessary to inspect the platelet survival curve visually. This will provide information on the influence of the collection injury, the

presence of obvious outliers, and the shape of the platelet survival curve. This will alert the investigator to the possibility of an imbalance between platelet production and destruction, to problems with the labeling and harvesting of the platelets, and to the possibility of faulty techniques of blood sampling or counting of radioactivity. It is advisable to use at least one other mathematic model to facilitate the recognition of above mentioned pitfalls. The Dornhorst, Meuleman, alpha order, or polynomial models are equally satisfactory for the detection of a concave shaped platelet survival curve, while the alpha order is recommended for the detection of higher order functions in patients with a very short mean platelet survival time.

APPENDIX

MATHEMATIC FUNCTIONS TO FIT PLATELET SURVIVAL CURVES

The following mathematic functions were fitted to the platelet survival curves.

Linear Function (11,12)

The linear function was calculated by:

$$H_l(t) = H_l(0) - at,$$

where $H_l(t)$ represented the linear regression line, $H_l(0)$ the y-intercept, and "a" the rate of platelet disappearance. The mean platelet survival time, T_l, was given by:

$$T_l = H_l(0)/a.$$

Exponential Function (*10,11*)

The exponential regression curve, $H_e(t)$, was calculated by:

$$H_e(t) = H_e(0)\exp(-kt),$$

where $H_e(0)$ was the y-intercept and "k" the relative rate of platelet disappearance. The mean platelet survival time was given by:

$$T_e = 1/k.$$

Weighted Mean Function (*11, 12*)

The weighted mean regression curve, $H_w(t)$, was calculated by:

$$H_w(t) = [S_e H_l(t) + S_l H_e(t)]/[S_e + S_l],$$

where S_l and S_e were the residual sum of squares associated with linear and exponential regression curves. The residual sum of squares was calculated by:

$$S_l = \sum [(H_l)_i - N_i]^2 \text{ and}$$

$$S_e = \sum [(H_e)_i - N_i]^2,$$

where $(H_l)_i$ and $(H_e)_i$ were linear and exponential regression curve points corresponding to data points N_i.

The weighted mean survival time was calculated by (*10*):

$$T_w = [S_e T_l + S_l T_e]/[S_e + S_l].$$

Alpha Order Function (*14*)

The alpha order function was calculated by:

$$H_a(t) = H_w(0)\,[1 + (1 - a)\,(k_0 + kt)]^{1/(1-a)},$$

where $H_w(0)$ was the y-intercept calculated by the modified weighted mean method, "k" the rate constant, and k_0 an initiation constant. The value of "a" or alpha determined the shape of the function; when a = 0, a linear and when a = 1, an exponential function was obtained. The y-intercept was calculated by:

$$H_a(0) = [H_w(0)][1 + (1 - a)k_0]^{1/(1-a)}.$$

The mean platelet survival time was calculated by:

$$T_a + \frac{1}{k}\,[1 + (1 - a)k_0].$$

Polynomial Function

The polynomial function, $H_p(t)$ was calculated by:

$$H_p(t) = a_0 + a_1 t + a_2 t^2 + \cdots\cdots + a_n t^n,$$

where $a_0, a_1 \cdots a_n$ were constants. The mean platelet survival time was given by:

$$T_p = a_0/a_1.$$

Multiple Hit Function (*11*)

The multiple hit function, $H_h(t)$ was calculated by:

$$H_h(t) = (c/n) \sum_{i=0}^{n-1} \{[(n - 1)/i!] \exp(-at)\,(at)^i\},$$

where "n" was the number of hits, "c" the normalizing constant, and "a" the mean waiting time between hits. The mean platelet survival time was given by:

$$T_h = n/a.$$

The number of hits, "n" reflected the mechanism of platelet destruction; when n = 1 it indicated exponential survival and when "n" became large it reflected linear survival.

Dornhorst Function (*11,15*)

The Dornhorst function, $H_d(t)$, was calculated by:

$$H_d(t) = H_d(0)\,\{[\exp(-kt) - \exp(-kT)]/[1 - \exp(-kT)]\},$$

where $H_d(0)$ was the y-intercept, "k" the relative random rate of platelet destruction, and "T" the potential age dependent platelet survival time. The mean platelet survival time was given by:

$$T_d = [1 - \exp(-kT)]/k.$$

Meuleman Function (*17*)

The Meuleman function, $H_m(t)$, was calculated by:

$$H_m(t) = H_m(0)\,(1 - t/T)\exp(-kt),$$

where $H_m(0)$ was the y-intercept, "k" the random rate of platelet destruction, and "T" the potential age dependent survival time. The mean platelet survival time is given by:

$$T_m = T/(1 + kT).$$

FOOTNOTE

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