The Relationship of Human Platelet Density to Platelet Age: Platelet Population Labeling by Monoamine Oxidase Inhibition

By Kevin G. Chamberlain, Michael Tong, Edmond Chiu, and David G. Penington

The relationship between platelet density and platelet age appears to vary between species with relatively few labeling studies in humans reported. In this study, irreversible monoamine oxidase (MAO) inhibitors were used to biochemically label the circulating platelet population in 18 humans. Platelet samples were then isolated during the 15 days after drug ingestion. The platelets were separated by density on continuous linear Percoll gradients and the density distributions were divided into five fractions containing approximately equal numbers of platelets. Baseline MAO activity was strongly correlated with platelet density. Twenty-four hours after a single dose of tranylcypromine, platelet MAO activities in the density subpopulations were reduced to 14% to 17% of the baseline values. During the first five days after inhibition, the rates of recovery of MAO activity (percentage per day) were inversely proportional to platelet density. The recovery rates in the two most dense fractions were initially slow but increased after five days. Percentage recovery of MAO activity in the least dense fraction was significantly greater than the percentage recovery in the most dense fraction on days 2, 3, 5, and 8 (P < .01, sign test). These results support the hypothesis that normal human platelets show a small increase in density with age, but they do not exclude the additional possibility that human platelet lifespan is positively correlated with platelet density.

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MATERIALS AND METHODS

Materials. All chemicals were of analytical grade. Prostaglandin E1 (PGE1) and bovine serum albumin were obtained from Sigma Chemical Company. 14C-tyramine was supplied by Amersham (Melbourne). Percoll (colloidal silica coated with polyvinyl-pyrolidone) and density marker beads were supplied by Pharmacia (North Melbourne, Australia).

Subjects. A pilot study was performed with the cooperation of five women 26 to 44 years of age who had been taking MAO inhibitors daily for 1 to 6 months under the care of a psychiatrist (EC). Subjects no. 1 to 4 were taking Parnate (Smith, Kline & French; tranylcypromine sulphate) and subject no. 5 took Nardil (William R. Warner; phenelzine sulphate). All subjects were being treated for depression but they had recovered and treatment was no longer considered necessary. All subjects gave informed consent to blood sampling on days 1, 3, 5, 8, and 15 after drug withdrawal. The second group of subjects consisted of ten healthy volunteers (six women) whose mean age was 25 ± 5 years (range, 21 to 35 years). These subjects were fully informed in writing of the dietary and other restrictions necessary during the 24 hours preceding and the seven days after the ingestion of the MAO inhibitor, and they gave written consent to the trial. The study protocol was approved by the research ethics committees of both St. Vincent's Hospital and Melbourne University. Systolic and diastolic blood pressures were measured in both recumbent and upright positions after the subjects had rested quietly for a few minutes. Subjects were excluded from the study if their baseline supine blood pressure exceeded 130/90.

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Supported by a grant from the National Health and Medical Research Council of Australia.

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0006-4971/89/7305-0029$3.00/0

This group of subjects took a single oral dose of Parnate (2 × 10 mg) at 9 AM on day 0, after the baseline blood sample had been taken. Further blood samples were taken at 9 AM on days 1, 2, 3, 5, 8, and 15. Single blood samples were also taken from another group of 14 normal laboratory personnel (seven men; mean age, 33 ± 8 years) and platelet density subpopulations were prepared for biochemical measurements, including β-thromboglobulin (βTG) content.

Preparation of human platelet total populations. The methods for the preparation of human platelet density subpopulations have been described in detail elsewhere and so a brief description is given here. Blood (21 mL) taken from the antecubital vein was rapidly mixed with 3.75 mL of an anticoagulant made of acid citrate dextrose containing 2.0 μg/mL PGE, and 1.44 mg/mL theophylline. In many cases this twice amount of blood was taken and processed identically. A total platelet population was separated following the method of Rand et al with minor changes. Modified Tyrode’s buffer (MT) consisted of calcium-free Tyrode’s solution with the addition of 0.375% trisodium citrate dihydrate, 0.225% citric acid monohydrate, 0.02% EGTA, 0.3% glucose, and 0.35% bovine serum albumin (BSA). The pH was finally adjusted to 6.7 and the osmolality to 291 mOsm/kg. This buffer was used to dilute the blood before each of three low-speed centrifugations (200 g × 10 min) to prepare diluted platelet-rich plasma (PRP). After the addition of PGE, (0.4 μg/mL), the platelets were pelleted from the combined PRP pool (2,000 g × 15 min) and resuspended in 6.0 mL of MT buffer. This platelet suspension was subjected to one or two very brief centrifugations (600 g × 30 seconds) in order to remove any residual erythrocytes or leukocytes. The mean platelet recovery after this step was 86 ± 11% (n = 20) and the combined erythrocyte and leukocyte contamination was <0.2%. One milliliter of the total platelet population was set aside and the other 5 mL was applied to a swing-out rotor of a Sorvall RC-5B centrifuge (with the rate gradient tube was then centrifuged at 3,500 g × 1 h) and the platelets were pelleted (2,000 g × 15 min) and resuspended in PBS-EGTA to give a final concentration of approximately 2 × 10^7/mL. The platelets were counted and aliquots were frozen at −70°C.

The platelet density subpopulations were labeled 1 to 5 in order of increasing density. In the following sections the extreme density fractions will be referred to as the low density fraction (LD, fraction 1) and the high density fraction (HD, fraction 5) but it should be realized that these conventional labels have been used by other investigators to refer to density fractions containing different percentages of the total platelet population.

Platelet counting and sizing. Platelets were counted in quadruplicate after dilution in Isoton II (Coulter Electronics), using a Coulter Model ZF Cell Counter equipped with a 50 × 60 μm orifice tube. The output from the Coulter counter was sorted using a Canberra Series 30 Multichannel Analyser to provide a frequency distribution of platelet volume. Platelet modal and median volumes were recorded and the mean platelet volume was estimated using the relation: mean = [(median^3/mode)^1/3]. Erythrocyte and leukocyte counts were performed manually using a counting chamber and phase contrast optics.

Biochemical assays. MAO was assayed using 14C-tyramine as substrate following the procedure of Mann and Thomas apart from doubling the specific radioactivity of the substrate and raising the incubation temperature to 37°C. All the platelet samples from each subject were accumulated and then analyzed in the same assay. Control samples stored at −70°C showed negligible loss of MAO activity over a period exceeding 6 months. Percoll levels as high as 10% (vol/vol) had no effect on platelet MAO activity (final Percoll levels in the study samples were <1%).

Platelet βTG content was estimated by specific radioimmunoassay as previously described. Platelet samples for βTG assay were counted and then lysed in 1% Triton X-100 before freezing. Before assay the samples were diluted extensively. Neither residual Percoll nor Triton X-100 interfered with the assay at the low final concentrations used. Platelets in PRP were treated similarly; in this case the βTG content of the corresponding platelet-poor plasma was subtracted before calculation of the platelet βTG content.

Statistics. All statistical comparisons were performed using the nonparametric sign test. Results are expressed as mean ± SD unless otherwise indicated.

RESULTS

The ten subjects receiving the single 20-mg dose of Parnate showed a significant increase in mean supine systolic blood pressure of 9 ± 7 mm Hg (P < .05) 24 hours after the dose, but the blood pressure declined thereafter and was not significantly higher than baseline on the second day. The day 0 values for mean platelet count (254 ± 38 × 10^9/L), mean platelet volume (6.7 ± 0.8 FL) and mean modal platelet density (1.063 ± 0.003 g/mL) did not change significantly during the subsequent 15 days of the trial.

Table 1 summarizes measurements made on the platelet density fractions prepared from the ten single-dose subjects on day 0. The first column shows that, on average, each of the five density fractions contained close to 20% of the platelets recovered from the gradient. Both the mean platelet volume and the platelet MAO activity increased in a monotonic fashion with platelet density but the MAO activity showed a steeper gradient with platelet density.

Samples from another 14 normal subjects were processed in identical fashion and the β-thromboglobulin (βTG) content was assayed in both the total population and the five density fractions. Table 1 reveals that the βTG content also showed a steep dependence on platelet density. For each of the last three variables in Table 1 the mean values estimated...
for the HD fraction were significantly greater than the mean values obtained for the LD fraction \( (P < .01) \). On the other hand, the mean values estimated for the total platelet population did not differ significantly from the mean values obtained for the modal fraction (fraction 3), suggesting that passage through the gradient had not altered the properties of the platelets significantly. In particular, there was no measurable loss of \( \beta TG \) from the alpha-granule compartment as a result of passage through the Percoll gradient.

In the present protocol, the platelets were pelleted once before the gradient to increase the number of platelets that could be applied to it. Although previous workers from our laboratory had reported that losses of lactate dehydrogenase and \( \beta TG \) were <2.5% after a single pelleting, it was decided to test again for \( \beta TG \) release using the present buffer and conditions. Platelet \( \beta TG \) content measured in platelet-rich plasma from three subjects \( (4.9 \pm 3.7 \mu g/10^8) \) was not significantly greater than the \( \beta TG \) content measured after pelleting and resuspension of the platelets in MT buffer \( (mean, 5.5 \pm 3.4 \mu g/10^8) \), or even after a second pelleting and resuspension in PBS-EGTA \( (5.8 \pm 2.3 \mu g/10^8) \). In another 13 subjects, mean platelet volumes were measured in samples taken before and after pelleting in MT buffer. The mean volume after pelleting \( (7.11 \pm 0.69 \mu L) \) was virtually identical to that before pelleting \( (7.13 \pm 0.66 \mu L) \) showing that platelet swelling had not occurred.

Twenty-four hours after the single 20-mg dose of Parnate, the MAO activities in the platelet density fractions were reduced to less than 20% of their baseline values. Recovery of activity proceeded over the next two weeks, so that by day 15 the MAO activities were not significantly different from the values obtained on day 0. In order to compare the patterns of recovery in the density subpopulations, each activity was expressed as a percentage of the appropriate baseline activity (defined as the mean of the day 0 and day 15 activities).

Figure 1 shows the MAO activities of fractions 1, 3, and 5 (calculated as percentages of their individual baseline activities) for the period following Parnate ingestion. Fractions 2 and 4 have been omitted from Fig 1 for the sake of clarity, but at each time point the activity of fraction 2 fell between that of fractions 1 and 3 while the activity of fraction 4 decreased to between that of fractions 3 and 5. Twenty-four hours after Parnate ingestion there were no significant differences in the degree of MAO inhibition in any of the density fractions. Between days 1 and 5 the LD platelets regained MAO activity at a faster rate than the platelets in the denser fractions. As a result, the extent of recovery of MAO activity in the LD subpopulation was significantly greater than in the HD population on days 2, 3, 5, and 8 \( (P < .01) \).

The recovery period was further analyzed by dividing it into three time segments: the lag phase (days 1 to 2), the early recovery phase during which none of the platelet MAO activities reached baseline (days 2 to 5), and the late recovery phase (days 5 to 8). Table 2 shows the average recovery rates (percentage per day) for all the density subpopulations during these three segments of the recovery period. Each recovery rate was calculated using the formula \( R = 100(a_2 - a_1)/bA(t_2 - t_1) \), where \( A \) = baseline MAO activity,
Table 2. Average Recovery Rates (% per day) of Platelet MAO Activity in Density Fractions After a Single Dose of Parnate

<table>
<thead>
<tr>
<th>Density Fraction</th>
<th>Segment of the Recovery Period</th>
<th>Days 1-2</th>
<th>Days 2-5</th>
<th>Days 5-8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>1.8 ± 3.8</td>
<td>11.7 ± 1.7</td>
<td>10.2 ± 2.2</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>8.0 ± 4.1*</td>
<td>17.1 ± 2.8</td>
<td>8.6 ± 2.9*</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>5.5 ± 4.7</td>
<td>14.5 ± 2.6</td>
<td>10.0 ± 4.0</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>4.9 ± 4.7</td>
<td>14.4 ± 3.2</td>
<td>10.6 ± 3.4</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>3.2 ± 6.2</td>
<td>10.5 ± 2.4</td>
<td>12.9 ± 3.8</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>2.3 ± 4.4</td>
<td>8.7 ± 2.3</td>
<td>12.0 ± 3.2</td>
<td></td>
</tr>
</tbody>
</table>

NOTE. Recovery rates were calculated by dividing the average increase in MAO activity per day in each segment by the maximum decrease in MAO activity after inhibition and multiplying by 100. Results are shown as mean ± SD (N = 10). Percentages 1-5 increase in density from 1 (LD) to 5 (HD). Statistical comparisons of LD and HD means were performed using sign tests.

*P < .05.
†P < .01.

b = maximum degree of inhibition (0 ≤ b ≤ 1.0), while a1 and a2 are MAO activities measured at times t1 and t2. The factor b is included because the recovery rate calculated as a percentage of the initially inhibited MAO activity is the variable most directly related to platelet turnover.

The maximum degree of platelet MAO inhibition occurred during the first 24 hours after Parnate ingestion, and so could only be estimated by graphic extrapolation in the present study. If the intracellular concentration of Parnate reached similar levels in all platelets, and if the inhibition reaction follows simple first-order kinetics with respect to the enzyme, the degree of inhibition should be the same in all subpopulations, independent of the enzyme concentration in the platelets. Examination of Fig. 1 shows that the single-dose data seems to conform quite well with this hypothesis. Extrapolation of the plots for the three density subpopulations towards zero time shows that the plots intersect with each other at times between 8 and 16 hours and a residual activity between 12 and 14% of baseline. Given that the percentage activities do not differ significantly even at 24 hours, it was decided to assign the same maximum degree of inhibition to each subpopulation (mean residual activity, 13%; mean degree of inhibition [b], 0.87). The results would not be significantly altered if the individual degrees of inhibition measured at 24 hours were used instead; in fact the differences between the density subpopulations would be slightly amplified.

Table 2 shows that between days 1 and 2 there was a lag phase in which the recovery of MAO activity was retarded. During this lag phase there was a clear negative correlation between recovery rate and platelet density. The increase in activity in the LD platelets (8.0 ± 4.1% per day) was more than three times that of the HD platelets (2.3 ± 4.4% per day), and this difference was statistically significant (P < .05). The mean recovery rate in the total population was unexpectedly low in this period due to some high values for the total samples on day 1 which were probably unreliable measurements.

During the second segment (days 2 to 5), the mean recovery rates were more than double the corresponding rates in the lag phase, but the inverse relationship between recovery rate and platelet density was maintained. The mean daily recovery rate in the LD platelets (17.1 ± 2.8% per day) was almost twice the rate in the HD platelets (8.7 ± 2.3% per day) (P < .01). In the next segment (days 5 to 8), the recovery rate in the total population decreased only slightly, but there were major changes in the relative recovery rates in the density fractions.

In the third segment, the mean recovery rate in the LD fraction fell to half the value of the preceding segment. The main reason for this was that the MAO activity of the LD fraction reached baseline during this segment for four out of the ten subjects. In contrast to this, the MAO activities in the HD fractions were still far from baseline in all cases and the recovery rates in fractions 4 and 5 actually increased between the second and third segments. This increase in recovery rate failed to reach significance for each fraction alone, but when these two fractions were pooled by taking means, the increase in recovery rate from a mean value of 9.7 ± 1.7% per day to 12.4 ± 2.9% per day was statistically significant (P < .05).

The results from the five patients who were treated chronically with MAO inhibitors were analyzed separately from the single-dose results because of differences in drug regimens and in the timing of blood samples. Table 3 shows the mean MAO activities obtained in this group as a percentage of the day 15 values. Unlike the single-dose group, the chronic group showed significantly higher percentage activity in the LD fraction compared to the HD fraction even on day 1 (P < .05). Otherwise the pattern of recovery was similar to that shown in Fig. 1 for the single-dose group. Data were only available for four of the subjects on day 5. Nevertheless, the LD platelets recovered their MAO activity much more quickly than the HD platelets and the extent of recovery was significantly higher on days 1 and 3 (P < .05).

DISCUSSION

It is important that the methods used to prepare density subpopulations of platelets do not cause artifactual changes in the platelets. In the present study, the absence of significant βTG release and the consistent patterns obtained in platelet volume, βTG content, and MAO activity suggest that the methods used were reliable and generated minimal artifact. In addition, density subpopulations of platelets obtained by these methods have now been studied by electron microscopy and show normal ultrastructure with little evidence of platelet activation or release.21

Steep gradients in enzyme activity with platelet density have also been found with three other mitochondrial enzymes in addition to MAO.4 Although Corash et al.22 were unable to demonstrate any relationship between mitochondrial content and platelet density in electron micrographs of their density subpopulations, a significant increase in mitochondrial concentration with platelet density has been reported in more recent studies,21.23 and this increase is likely to account for the steep gradients in enzyme activity.

The half-life of tranylcypromine in plasma is short (1.5 to 3.5 hours),12,24 and so the maximum inhibition of platelet
MAO activity is likely to occur within the first 24 hours after Parnate ingestion. The renewal of MAO activity in all the density fractions was somewhat retarded beyond this period, in a similar fashion to the lag in the renewal of cyclooxygenase activity after aspirin inhibition.25,26 The likely explanation for this lag phase is partial inhibition of preformed enzyme in the megakaryocyte compartment.27,28 In the total platelet population the renewal of MAO activity after the lag phase showed a near-linear pattern in control subjects and could be used to estimate megakaryocyte-platelet regeneration times.16 The patterns of MAO renewal in the density subpopulations were significantly different and these differences provided clues to the relationship between platelet density and platelet age. Table 2 shows that the rates of recovery of MAO activity varied inversely with platelet density from day 1 through day 5. Two models will be considered in attempting to explain these results. In the first model of platelet turnover, we assume that platelets may only be lost from a density subpopulation by destruction and these platelets may only be replaced by new platelets from the megakaryocyte compartmt. In this model, the rate of renewal of MAO activity in the second segment (days 2 to 5) should simply equal the platelet turnover rate in the subpopulation. Since the platelet turnover rate (percentage replacement per day) is inversely related to platelet lifespan, this model suggests that mean platelet lifespan is positively correlated with platelet density. Such a correlation has also been proposed by other investigators,7,11,29,30 and is made more plausible by reports that platelet lifespan is significantly related to mean platelet volume31,32 and by the finding that human mean platelet volume is correlated with platelet density (Table 1).

The second model postulates that human platelets increase slightly in density as they age in the circulation.7,10 The differences in platelet ultrastructure and composition that occur during thrombopoiesis are probably the major determinants of platelet density.5,31 Nevertheless, a small shift of the whole platelet density distribution towards higher density could occur during the platelet lifespan. This second model would result in an apparently higher rate of platelet turnover in the LD subpopulation even if all platelets had the same lifespan. Unlike the situation in the first model, platelets could enter or leave density subpopulations by increasing their density as well as by entering or leaving the circulation. While all the platelets lost from the LD fraction would only be replaced by new platelets from the megakaryocyte compartment, at least some of the platelets lost from the HD fraction would be replaced by circulating platelets that had undergone the necessary increase in density. Immediately after Parnate ingestion these platelets would contribute very little MAO activity to the HD fraction. The renewal of activity in the HD fraction would therefore begin more slowly than the renewal in the LD fraction but it would accelerate with time as the average MAO activity in the adjacent fraction itself began to increase.

The pattern is made more complex by the fact that during the initial lag period even the newly formed platelets contain partially inhibited MAO. Despite this complexity, the data fit the second model quite well. During the lag phase, the ratio of recovery rate in the LD fraction to the recovery rate in the HD fraction was at its maximum (3.5). In the second segment (days 2 to 5) this ratio decreased but the HD recovery rate tripled and then increased again during the third segment (days 5 to 8). These features of the data cannot be fully explained by the first model, which predicts that the rates of renewal would remain relatively constant in each density fraction. The data are quite compatible with a combination of both these models. It is worth noting that both the suggested explanations, an increase in platelet density with platelet age and a correlation between platelet density and platelet lifespan, generally predict that LD platelets would be younger on average than HD platelets, although both fractions could still contain platelets with a wide range of ages.

The present data are not compatible with the hypothesis that normal human platelets might decrease in density as they aged in the circulation. This model predicts that the rate of renewal of MAO activity in the HD platelets would initially exceed the corresponding rate in the LD platelets. It also predicts that the recovery of MAO activity in the LD platelets would begin slowly but then accelerate with time. Neither of these trends is apparent in the present data.

These results are consistent with the results from other studies in which the labeling of normal human platelet populations was achieved by other methods. Boneu et al17 used aspirin to irreversibly inhibit platelet cyclo-oxygenase activity and then studied the recovery of malondialdehyde synthesis in platelet density subpopulations from three subjects. The LD platelets regained full activity more rapidly than the HD platelets, which showed a slow recovery rate.
HUMAN PLATELET DENSITY AND PLATELET AGE

initially, followed by a more rapid rate after six days. Mezzano et al10 and McDonald and Ali11 measured the recovery of thromboxane synthesis after aspirin inhibition and found that the LD platelets showed significantly greater recovery than the HD platelets by day 3.

Mezzano et al10 and Boneu et al13 radiolabeled platelets with 51Cr and obtained results consistent with their aspirin studies in that the LD platelets lost radioactivity rapidly while the activity of the HD population remained on a plateau or even increased for several days before declining. Realizing that these results might indicate a selective uptake of the HD platelets by the spleen followed by a slow release of these platelets, Mezzano et al14 repeated their study with three splenectomized subjects and obtained very similar results to those found with control subjects. Watson and Ludlam3 labeled human platelets with 111In and also found a rapid loss of activity in the LD fraction and a transient increase in activity in the HD fraction in five normal subjects. These investigators also studied five splenectomized subjects and suggested that the less pronounced increase in the specific activity of the HD fraction in these subjects might indicate temporary splenic sequestration of HD platelets in normal subjects.

Boneu et al13 used continuous density gradients to show that the approximately gaussian distribution of 51Cr-labeled platelets was initially indistinguishable from the distribution of unlabeled platelets but shifted to slightly higher density during the first three days after reinjection. The investigators pointed out that there did not appear to be any narrowing of the distribution that would be expected if the LD platelets had a shorter lifespan than the HD platelets. Watson and Ludlam35 reported a similar shift in the platelet density profiles from two subjects both one and two days after reinjection of 111In-labeled platelets but chose to interpret this in terms of initial sequestration and delayed release of the HD platelets.

One study of human platelets presenting evidence in direct conflict with these results is the cohort study of Amorosi et al36 in which three human subjects were injected with 75Se-selenomethionine. The ratio of the specific activities of HD to LD platelets was found to be 2.5 to 2.9 initially but it decreased to near unity within a few days suggesting that young platelets are relatively dense but decrease in density as they age. The investigators used discontinuous gradients of water-immiscible oils to separate platelets of different density and these methods may cause more artificial changes in platelets than the methods used in more recent investigations.1 In addition, the small number of subjects studied (three) and the lack of statistical analysis reduce the value of this study. With this one exception, the labeling studies in humans generally fail to support the hypothesis that human platelets decrease in density with age.

Much of the controversy over the relationship between platelet density and age has arisen from attempts to extrapolate from one species to another. In particular, studies of rabbit platelets have consistently produced results opposing those found in human studies. Cohort-labeling studies in rabbits by Charmatz and Karpatkin7 using 75Se-selenomethionine, Rand et al18 using 35S-sulphate, and Corash and Shafer4 using 3H-amino acids showed that the label appeared more rapidly in the HD platelets but after about two days this was followed by a relative increase in the labeling of the LD platelets. Rand et al38 labeled circulating rabbit platelet populations with 51Cr and found a relatively rapid loss of label from the HD platelets while the LD platelets did not begin to lose radioactivity for 24 hours. In further experiments using both 111In and 51Cr, these investigators found that the mean survival time of LD platelets was 47 ± 19 hours compared to 76 ± 22 hours for HD platelets. Packham et al9 labeled rabbit platelet populations using aspirin and found that the recovery of thromboxane synthesis was significantly greater in the HD platelets compared to the LD platelets two and three days after aspirin injection.

All these results are quite consistent and give strong support to the hypothesis that young rabbit platelets are relatively dense and become less dense as they age in the circulation. The divergence between these results and those reported in human studies suggests that rabbits may be unsuitable laboratory models for the study of human platelet kinetics. The rabbit platelet mean lifespan is very short, 60 ± 29 hours (gamma model)39 compared to the human platelet lifespan of 230 ± 14 hours (gamma model).39 While Hanson and Slichter29 estimated that 18% or less of normal human platelets are destroyed randomly, Trowbridge and Martin40 estimated that the ratio of random to constant destruction was three times higher in rabbits than in humans. It is possible that rabbit platelets become less dense as they age due to events that cause degranulation without platelet consumption. It has been reported that thrombin-degranulated rabbit platelets are able to circulate with normal lifespan in rabbits.41 Although degranulated platelets have been detected in humans with various disorders, it is important to distinguish the normal process of human platelet senescence from the depletion of granule contents and associated loss of platelet density that have been reported to occur in some disease states42,43 or after cardiopulmonary bypass.44

Results obtained in other species seem to vary considerably. In the rat, no variation in platelet density with platelet age was detected.45 Studies in baboons46 and Japanese crabeating monkeys (Macaca fascicularis) have shown a small increase in platelet density with age similar to that proposed in humans. On the other hand, Corash et al30 studied a different species of monkey (rhesus) and obtained data supporting a decrease in platelet density with age. The importance of species differences in this field has been underlined by the work of Mezzano et al47 who reported that platelets in splenectomized dogs decreased in density with aging. These investigators used the same methods that they had used to gain evidence of the opposite change with platelet aging in normal and splenectomized humans.30,34 Furthermore, these investigators48 recently reported that dog platelets show an inverse relationship between platelet density and platelet mean volume, in contrast to both humans and rabbits.1,18

The present results are in accord with most of the labeling studies in humans and support the hypothesis that normal human platelets show some increase in density as they age in
the circulation. The present results do not exclude the additional possibility that human platelet lifespan may also show a positive correlation with platelet density. Some possible mechanisms for the postulated increase in platelet density with normal platelet aging are outlined below.

The radiolabelling experiments of Boneu et al. and Watson and Ludlam in humans, Martin and Penington in monkeys, and Savage et al. in baboons have shown density increases of about 0.001 to 0.003 g/mL when comparing a relatively aged platelet population with a normal, mixed platelet population. If we assume that the increase in density occurs at a uniform rate, the total shift in density would be about twice this over the lifespan of the platelet, ie, an average of about 0.004 g/mL (0.4%). One possible mechanism for such a shift might be a slight shrinkage involving a loss of water and electrolytes only. Using the formula derived by Holme and Murphy for such transitions, a density change of 0.004 g/mL would require a 7% decrease in volume. Such shrinkage might be caused by alterations in the activity of ion pumps or leakage pathways in the plasma membrane.

Another possible mechanism for an increase in platelet density is an increase in platelet mass without proportional changes in platelet volume or intracellular osmotic pressure. Assuming a mean platelet volume of 6.6 fL and a mean platelet density of 1.064 g/mL, the mean platelet mass would be 7.0 mg/10^12 and an increase in platelet density of 0.4% would require an increase in platelet mass of about 30 μg/10^6. Serotonin accumulates in a bound form in human platelet-dense granules during the platelet lifespan but the maximum accumulation would be insufficient (<2 μg/10^9) to bring about the desired change in density. Platelet glycogen, on the other hand, is very dense, osmotically inactive, present in moderately large amounts (90 to 100 μg/10^9) and able to be rapidly synthesized. Murphy and Gardner showed that stored platelets containing only 12% of the normal glycogen content were able to resynthesize normal levels of glycogen within 24 hours after transfusion into aplastic patients. It is possible that the glycogen content of normal platelets continues to increase slowly during their entire lifespan. After allowing for the appropriate increase in platelet volume to accommodate the newly synthesized glycogen, an increase in platelet glycogen content of 10 to 15 μg/10^9/day during the platelet's lifespan is estimated to cause an increase in platelet density of the required magnitude. Platelet glycogen content has already been found to show a steep correlation with platelet density. Further studies will be needed to investigate such possible mechanisms for the small increase in human platelet density with age that has been proposed by previous workers and is further supported by the present study.

ACKNOWLEDGMENT

We wish to thank Jenny Cooke and Janet Macpherson for their skilful preparation of the platelets. The biochemical assays were expertly performed by Ian Trounce, Ian Andrew, Margaret French, and Sophie lossifidis. Maria Froebel gave valuable assistance in the preparation of the figure. We thank Professor Brian Davies for providing facilities for the performance of some of the MAO assays.
HUMAN PLATELET DENSITY AND PLATELET AGE

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The relationship of human platelet density to platelet age: platelet population labeling by monoamine oxidase inhibition

KG Chamberlain, M Tong, E Chiu and DG Penington