The Relation of Blood Platelet Survival and Distribution to $^{14}$C-Serotonin Distribution and Excretion

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A VALID REPRESENTATION of the lifespan and destruction of a specific cell type is provided by studies of labeled cells if a number of requirements are met. These include that the label should not be injurious to the cell, that there is no elution of the label, that there is no reutilization of label at time of cell death, that the age distribution of the labeled cell population should be known, and that there is no labeling of the precursors of the cell. The use of $^{14}$C-5-Hydroxytryptamine ($^{14}$C-5-HT) as a blood platelet label probably does not meet all of these specifications. However, $^{14}$C-5-HT infused bound to platelets is retained in the body to a far greater extent than when infused directly or when massive platelet destruction occurs in vivo, suggesting a unique role of platelets in determining the in vivo distribution and subsequent fate of 5-HT. In order to further explore this relationship, the in vivo distribution and the excretion of $^{14}$C-5-HT introduced as a platelet label was compared to the survival and distribution of platelets themselves measured independently by enumeration.

MATERIALS AND METHODS

Carbon-14 labeled serotonin ($^{14}$C-5-Hydroxytryptamine; $^{14}$C-5-HT) was obtained and prepared for use as described previously. Male Sprague-Dawley rats weighing approximately 350 Gm. were given two microcuries of $^{14}$C-5-HT intravenously. Twenty-four hours following infusion of $^{14}$C-5-HT, 20 per cent of the activity was located in blood platelets. Aortic blood of ether-anesthetized rats was withdrawn using a 20-gauge needle and siliconized syringes. Seventy to 80 per cent of the rat's blood volume was obtained in this manner, expelled into siliconized conical centrifuge tubes containing 0.1M sodium citrate or modified ACD solution as anticoagulants and centrifuged to obtain platelet rich plasma (PRP). The PRP was withdrawn using siliconized pipettes and the red cells were discarded. The PRP was spun in a refrigerated centrifuge to obtain a platelet button. After decanting all but 1 to 2 ml. of the supernatant plasma, the platelets were resuspended in repeated aspiration and expulsion of the button using a series of siliconized pipettes with fire.
smoothed ends ranging from 0.5 mm. up to 2.0 mm. in diameter. The platelet count of PRP was determined using phase contrast microscopy prior to centrifugation, the volume of PRP was measured, and the total number of platelets in the final concentrate was calculated from these figures. The labeled platelets were then infused into male Sprague-Dawley rats weighing approximately 150 Gm. via a tail vein or through an isolated saphenous vein using plastic syringes.

At various time intervals following infusion of labeled platelets, the abdominal cavity was opened and blood was withdrawn. The abdominal and thoracic organs were rapidly removed, weighed, and multiple samples were taken for assay of radioactivity. The gut was divided into three approximately equal parts and samples were taken from each portion. The tissue samples averaged 25 to 50 mg. and were analyzed for radioactivity following combustion in a modified Schoniger oxygen flask. Twenty-five ml. of a 1:5 mixture of hyamine 10X and methanol were employed throughout the studies as a scrubbing solution to trap the \(^{14}CO_2\) generated during combustion. Two ml. aliquots of the hyamine-methanol mix were transferred to counting vials containing a solvent-scintillator solution (toluene, PPO, POPOP) for determination of \(^{14}C\) activity in the sample using a liquid scintillation counter. Recovery of \(^{14}C\) activity from tissues was approximately 100 per cent as estimated by adding known amounts of \(^{14}C\) activity to comparable samples of tissue taken from control animals.

In each experiment an aliquot of the injected labeled platelet concentrate was combusted to determine the total amount of activity injected. Total organ radioactivity was estimated on the basis of the ratios of the weights of samples burned to the total organ weight, and this measurement was expressed as per cent of injected dose. Analysis of multiple samples from the same organ did not show significant variation in distribution of \(^{14}C\) activity within the organs. In some experiments urine and stool were collected for analysis of \(^{14}C\) activity. Animals were sacrificed in triplicate or quadruplicate for all studies of tissue distribution of \(^{14}C\) serotonin.

The identity of \(^{14}C\) activity was 5-HT in tissues was determined using paper chromatography. Tissues were homogenized in distilled H\(_2\)O using a semimicro jar centrifuged, and an appropriate amount of nonradioactive 5-HT was added to the supernate. Radioactivity was determined with a four-pi gas flow paper strip counter.

The disappearance of \(^{14}C\) from blood platelets in intact animals was determined using whole blood samples obtained from the tail vein. It can be demonstrated that within 1 hour following injection, virtually 100 per cent of blood \(^{14}C\)-5-HT is located in platelets. Tails were clipped and blood was allowed to drip on a glass slide which contained dried heparin. One-tenth ml. of blood was immediately pipetted onto duplicate planchets containing an equal volume of distilled water with Tween 80 (Polyox 80). Planchet radioactivity was determined using a gas flow proportional counter. Samples for platelet enumeration were taken directly from tail vein blood. Total blood \(^{14}C\) activity and recovery of platelets after infusion was determined by the ratios of \(^{14}C\) activity or number of platelets infused to the amount calculated in the total blood volume. The blood volume of 12 randomly selected 150 Gm. recipient rats was determined with \(^{51}Cr\) labeled erythrocytes and approximated 7 per cent of body weight. This value is slightly higher than reported by others using different technics. In some animals, organ distribution of the \(^{51}Cr\) activity was determined on samples removed after anesthesia and bleeding through the abdominal aorta as described previously.

Splenectomized rats were allowed to recover for 3 weeks prior to use as platelet recipients.

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in the designated experiments. In 15 animals the mean platelet count 3 weeks postsplenectomy was 1,320,000 as opposed to 980,000 presplenectomy, an increase of 35 per cent. Thrombocytopenic animals were prepared by 250 kv. whole body irradiation. A total of 800 r was administered in five consecutive daily doses, resulting in an average platelet count of 50,000 per mm.³ eight days after completion of irradiation. Control animals of the same age and weight, radiated at the same time, were routinely observed during experiments, using thrombocytopenic animals to be certain that recovery of marrow function did not ensue during the lifespan studies.

**Results**

As a frame of reference for the studies in rats, Figure 1 depicts the detailed kinetics of ¹⁴C-5-HT in a human subject following infusion of autologous in vitro labeled platelets. Circulating platelet ¹⁴C activity decreased exponentially to half the initial value by 5.3 days. After 28 days less than 3 per cent of infused activity remained in the body. During the first 10 days only 5 per cent of the activity was excreted in the feces. The major route of excretion was via the kidneys with 10 per cent of ¹⁴C activity in the urine at the end of the first day.

If the total amount of activity recovered is calculated for each day by summing the per cent present in urine, blood, and stool, and this value is subtracted from 100 per cent, a value representing activity residual in the body and not present in the total blood volume ("missing activity") is determined. The "missing activity" represents a second pool of ¹¹C-5-HT which is apparently extravascular. The disappearance of the "missing activity" (Fig. 1) parallels almost exactly the rate of decline of blood activity, suggesting that the two pools are in equilibrium with each other.

In contrast, direct injection of ¹¹C-5-HT, not bound to platelets, results in
rapid urinary excretion, 60 per cent to 75 per cent of injected radioactivity appearing in the urine within 24 hours, most of it within the first 4 hours (two subjects). After 24 hours, however, the curve of radioactive decay resembles that observed when $^{14}$C-5-HT is injected bound to platelets.

**Distribution of $^{14}$C-5-HT in Blood and Tissues of Rats Following Infusion of $^{14}$C-5-HT Labeled Platelets**

Figure 2 depicts the distribution of $^{14}$C-5-HT in blood and various organs of rats at intervals of time following infusion of labeled platelets. The $^{14}$C activity in tissues is present as $^{14}$C-5-HT since greater than 90 per cent of the radioactivity moved in a manner identical (Rf. 47) to added carrier 5-HT by paper chromatography. The recovery of $^{14}$C-5-HT in blood at 1 hour and 1 day is similar to that seen in human experiments. Twenty-five to 30 per cent of the injected $^{14}$C activity appeared in spleen, liver and lungs, with the greatest proportion (18 per cent) being in spleen. Under identical experimental circumstances only 3 per cent of injected $^{51}$Cr labeled red blood cells were found in the spleen at any time following infusion. Virtually no $^{14}$C activity was recovered from brain, and notably the gut never contained more than 3 per cent of the injected dose. The distribution of $^{14}$C-5-HT injected free in saline is not qualitatively different but by contrast the total amount recovered is only 20 per cent of the total injected dose.

Figure 3 demonstrates that the disappearance curves of $^{14}$C-5-HT activity from blood and from liver, spleen, and lung taken together are essentially
parallel after 2 days. Prior to that time there appears to be a buildup in activity in the combined organs, suggesting that an equilibrium between platelet and tissue \( ^{14} \text{C}-5\text{-HT} \) or between labeled and nonlabeled platelets is reached in these organs over this period of time.

Comparison of the Disappearance of Platelets and \( ^{14} \text{C}-5\text{-HT} \) from the Circulation

Labeled platelets were infused in numbers calculated to raise the platelet count by at least 50 per cent in 14 normal rats. The mean platelet count in the rats averaged 980,000 per mm\(^3\) prior to infusion of labeled platelets and 1,430,000 platelets per mm\(^3\) after infusion. At 1 hour, the per cent \( ^{14} \text{C} \) recovered was greater than the number of platelets recovered (Fig. 4). As will be seen subsequently, the increased recovery of \( ^{14} \text{C}-5\text{-HT} \) as opposed to platelets is a usual finding. At 1 day and thereafter, the per cent \( ^{14} \text{C}-5\text{-HT} \) platelets remaining were similar. It is uncertain from this experiment that the \( ^{14} \text{C}-5\text{-HT} \) is located only in the population of infused platelets. Eight thrombocytopenic animals were given platelet infusion. A rise in platelet count from a mean of 50,000 per mm\(^3\) to between 600,000 and 1,000,000 platelets per mm\(^3\) was achieved. The recovery of platelets and \( ^{14} \text{C} \) serotonin was similar to that seen in the intact animals (Fig. 4). The disappearance of label and platelets in thrombocytopenic animals is similar. In animals with normal platelet counts the correlation is not as good.
Fig. 4.—Comparative disappearance of platelets and $^{14}$C-5-HT from the blood in normal and thrombocytopenic rats. The disappearance of $^{14}$C serotonin and platelets are plotted on rectilinear coordinates in Figure 4. The asterisks indicate platelet counts which are not significantly different from the base line platelet count in the animals.

**Organ $^{14}$C-5-HT as an Index of Platelet Sequestration**

The previous data suggest that organ $^{14}$C activity might be related to pools of platelets. If so, removal of an organ such as the spleen might result in an increase in the proportion of radioactivity recoverable in blood as well as platelets.

In splenectomized, nonthrombocytopenic animals the recovery of $^{14}$C-5-HT in the blood increased from 61 per cent to 84 per cent while recovery of platelets rose from 46 per cent to 68 per cent at 1 hour (Fig. 5). There was no increase in $^{14}$C activity of lungs or liver when compared to nonsplenectomized animals. Similar results were obtained in splenectomized, thrombocytopenic animals.

Since the enhancement of recovery of $^{14}$C and platelets by splenectomy could possibly be due to lack of sequestration and destruction of damaged platelets in the spleen, a shortened lifespan associated with increased recovery might occur. Such is not the case (Fig. 6). There was no early component of loss of platelets from the circulation and the survival curve was apparently linear for the first four days (cf. normal platelets in Figure 4).
Fig. 5.—The recovery of $^{14}$C-5-HT and platelets in intact and splenectomized rats 1 hour following infusion. The differences between the mean recoveries in intact and splenectomized animals is significant ($p < 0.01$). A minimum of six animals was used for each group.

The Variability of Platelet Survival in the Rat

When the disappearance of infused platelets in intact animals is compared with the radiated thrombocytopenic animals (Fig. 4), the former appeared to show a more nearly linear disappearance of platelets than the latter. Figure 7 shows the survival curves of infused platelets in 16 thrombocytopenic animals. Three separate experiments are depicted in which variable numbers of platelets were injected to raise the platelet count to nearly normal (600–900,000/mm³) and to supranormal levels (1,200,000–2,000,000/mm³). In all but one of the six rats with initial platelet counts less than 900,000/mm³, the survival curves are more curvilinear and shorter (3 days) than for the ten rats with initial platelet counts above 1.2 million/mm³. As the platelet count declines below normal levels (900,000–1,000,000) in the latter, the curves tend to be-
Fig. 6.—The disappearance of platelets in splenectomized, thrombocytopenic rats.
Asterisks denote platelet counts not significantly different from base line counts. The
values are expressed as a per cent of the 1 hour postinfusion count.

come nonlinear. These data are summarized in Figure 8. The effect of shortening of survival to 3 days is probably not an artifact produced by variable degrees of injury of infused platelets, since as shown in Figure 9 the absolute recovery of platelets in the circulation is related directly to the number infused.

DISCUSSION

Several observations indicate that platelets labeled with $^{14}$C-5-HT do not accurately reflect platelet survival, especially situations in which synthetic rates of 5-HT are high, as in the carcinoid syndrome. However, the pattern of $^{14}$C-5-HT distribution in the rat following intravenous infusion, either free in saline or bound to platelets, does not coincide with the known distribution of 5-HT. While appreciable amounts of endogenous 5-HT are present in spleen, liver, and lung, the largest single pool is in the gut. In contrast, 40–50 per cent of $^{14}$C-5-HT remains in blood platelets and 30–40 per cent is in various organs, mainly the spleen, with lesser amounts in liver and lung. Little is found in the gut. Further, the disappearance of $^{14}$C-5-HT from these organs parallels the disappearance of $^{14}$C-5-HT from circulating blood platelets.

Additional evidence that the distribution of $^{14}$C-5-HT is determined by the distribution of platelets is provided by comparing the recovery of infused labeled platelets and of $^{14}$C-5-HT in intact and splenectomized animals. The
increased recovery of platelets in splenectomized animals when compared to controls is reflected by increased recovery of $^{14}$C-5-HT in blood without an increase in $^{14}$C activity of other organs. Therefore, shortly after infusion of $^{14}$C-5-HT labeled platelets, $^{14}$C-5-HT present in the spleen is most likely located in viable platelets. Whether the $^{14}$C-5-HT present in liver and lungs is similarly present in platelets is not known, but the analogy seems reasonable. These observations are consistent with studies by Synder, Wurtman, and Axelrod\textsuperscript{14} which indicate that $^{14}$C-5-HT present in the spleen of Sprague-Dawley rats is located primarily in blood platelets.

Since the platelet survival curves, based on enumeration, provide no evidence of immediate sequestration and subsequent release of platelets into the circulation, the distribution of $^{14}$C-5-HT and of viable platelets 1 hour after infusion may parallel the normal distribution of platelets. If so, significant numbers of platelets would be present in spleen and perhaps liver and lungs at all times. The total size of the splenic pool of platelets can be estimated to approximate 40 per cent of the pool of circulating platelets based on the ratios of $^{14}$C activity of spleen (18 per cent) to blood (47 per cent) 1 hour following infusion of labeled platelets. The increased recovery (69 per cent) of platelets in blood in all splenectomized animals as opposed to the recovery (49 per
Fig. 8.—The mean per cent recovery of platelets on succeeding days in the three experiments depicted in Figure 7. The platelet count one hour after infusion taken as 100 per cent. In addition to the change in shape of the curves, the infused platelets have disappeared by 3 days in the animals receiving the smallest number of platelets while final disappearance in the other groups is prolonged to 4 or 5 days.

Fig. 9.—The relation of the platelet count in the recipient animals to the number of platelets infused. Four separate experiments totaling 32 infusions of platelets.
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cent) in all intact animals indicate that 20 per cent of newly infused platelets take residence in the spleen. If 20 per cent of platelets are in spleen and 49 per cent are recovered in the blood of intact animals post-infusion, then the proportion of splenic platelets to blood platelets again is close to 40 per cent, a value which compares well with the $^{14}$C estimates of the size of the splenic pool. There is added significance to the data if the average increase in platelet count 3 weeks post-splenectomy (35 per cent) in these animals is compared to the estimates of splenic pool size. Post-splenectomy thrombocytosis may be due to reduction in the space of distribution of platelets without alteration in the rate of platelet production. Post-splenectomy thrombocytosis is not due to increased platelet lifespan. The lifespan of platelets determined by enumeration as done here or by Di-isopropyl fluorophosphonate $^{32}$P labeling$^{15}$ is not prolonged post-splenectomy. Removal of a splenic inhibitor to production$^{10}$ is a possible explanation of post-splenectomy thrombocytosis, but would not explain the increased recovery of infused platelets. There are other observations which are consistent with the existence of a pool of platelets in the spleen in man and animals,$^{17}$ although the existence of such a pool in rats has been questioned by other workers.$^{19}$

The correlation between the distribution and disappearance of $^{14}$C-5-HT and platelets is good but not absolute. The recovery of $^{14}$C-5-HT in blood is usually higher than the concomitant recovery of platelets. Comparison of $^{14}$C and platelet recovery and disappearance suggests that significant elution of $^{14}$C-5-HT occurs during the first day post-infusion. Since there is a small residual of $^{14}$C-5-HT circulating after platelets have disappeared, it is possible that reutilization of label occurs also.

The shape of the disappearance curve of the platelets themselves is variable and dependent on the total number of platelets present. In animals with normal or supranormal numbers of platelets, survival curves determined by enumeration are nearly linear functions for the first 3 days and all platelets have disappeared between the fourth and fifth days. In amegakaryocytic, thrombocytopenic, animals transfused to less than normal platelet levels, platelet survival is more curvilinear and platelets have virtually disappeared by 3 days. The data concerning survival of platelets derived by enumeration of platelets are compatible with the idea that platelet death in a randomly aged platelet population is primarily a function of age-related processes, although utilization of platelets,$^{20,21}$ irrespective of age, may also occur. Normally the influence of random utilization on platelet loss from the circulation is small since the curves approach linearity. In thrombocytopenic animals, however, the fraction of platelets entering into such processes is proportionately greater and the generation of survival curves of increasing curvilinearity and moderate shortening results. The data suggest that moderate shortening of platelet survival in thrombocytopenic recipients as well as changes in the shape of platelet survival curves may well indicate physiologic loss of platelets from the circulation and are not necessarily related to pathologic destruction of platelets. The findings are similar to those obtained in rabbits by Ebbe, Baldini, and Dono-
van. In thrombocytopenic man, study of the survival of platelets by the technic of enumeration has usually resulted in a curvilinear survival pattern.

**Summary**

1. The distribution of $^{14}$C-5-HT following infusion is different from that of endogenous serotonin. One hour after infusion it is, in major part, a function of the distribution of blood platelets.

2. The spleen in rats is the site of a pool of platelets. Based on both $^{14}$C and platelet recovery data in normal and splenectomized animals, this pool approximates 40 per cent of the total circulating platelets.

3. Postsplenectomy thrombocytosis may relate to removal of a platelet reservoir with shift of the platelets normally contained therein to the peripheral circulation.

4. The shape of platelet survival curves in rats is neither strictly linear nor curvilinear but normally is determined primarily by age-related processes in the platelet. Platelet survival curves tend to become curvilinear in thrombocytopenic animals, indicating that there is probably an additional small, fixed random loss of platelets from the circulation.

5. The disappearance of $^{14}$C-5-HT from blood platelets approximates but is not completely representative of the disappearance of the platelets themselves. It is probable that elution from platelets and possibly reutilization of platelet label occurs.

**Summario in Interlingua**

1. Le distribution de $^{14}$C-5-hydroxytryptamina post-infusional differe ab illo de serotonina endogene. Un hora post l'infusion illo es, in grande parte, un function del distribution del plachettas sanguinee.

2. Le splen de rattos es le sito de un pool de plachettas. A base de datos de reobtention de $^{14}$C e de plachettas in animales normal e splenectomisate, il pare justificare concluder que iste pool contine aproximativamente 40 pro cento del circulante plachettas total.

3. Thrombocytosis post splenectomia es possihilemente relationate al eliminacion de un reservior de plachettas con le transferimento, ad in le circulation peripheric, del plachettas normalmente continite in illo.

4. Le conformation del curvas del superviventia plachettal in rattos es ni strictemente lineari ni curvilineari sed es normalmente determinate super toto per processos intraplachettal que es relationate con le etate. Le curvas de superviventia plachettal tende a devinir curvilineari in animales thrombocytopenia, lo que indica que il occurre probabilemente un micre per dita additional fixemente alcatorisate de plachettas ab le circulation.

5. Le disparition de $^{14}$C-5-hydroxytryptamina ab le plachettas sanguinee es approximativemente sed non completemente un representation del disparition del plachettas mesme. Es probable que elution ab plachettas e possihilemente reutilisation del marca del plachettas occurre.
REFERENCES


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