Relationship of Platelet Serotonin to Disturbances of Clotting and Hemostasis

By Murray Weiner, M.D. and Sidney Udenfriend, Ph.D.

The presence of serotonin (5-hydroxytryptamine) in high concentration in platelets and its absence from normal platelet-free plasma suggest the possibility that this vasoconstrictor substance may play a role in hemostatic mechanisms. Platelet serotonin content measured by a fluorometric method in 94 patients showed an average content of 0.22 μg./ml. blood. In none of the disease groups studied, including hypertension, was a significant alteration found in platelet serotonin. However, the administration of reserpine resulted in a marked and prolonged depletion of platelet serotonin that was not accompanied by any significant change in any of the clotting factors. The in vitro addition of serotonin in amounts up to 50 μg./ml. also failed to alter any of the clotting factors studied. Platelet serotonin did not correlate with diagnosis, age, weight, blood pressure, cephalin-fluocel- lation, or capillary fragility. However, markedly anemic patients (below 10 Gm. per cent hemoglobin) and patients whose blood urea nitrogen was above 30 mg. per cent, tended to have a low platelet serotonin content.

In 1912 O'Connor demonstrated that serum had a vasoconstrictor activity that was distinctly greater than that of plasma and was not due to epinephrine. Janeway and co-workers found that platelets were essential to the vasoconstrictor activity of serum. This observation was later confirmed by several other investigators. In 1948, a crystalline vasoconstrictor substance was isolated from serum by Rapport and associates and named "serotonin." This substance, subsequently identified as 5-hydroxytryptamine, is derived biologically from tryptophan.

The presence of serotonin in high concentration within platelets and its absence from normal platelet-free plasma suggest the possibility that this compound may play a role in hemostatic mechanisms that in turn may influence cardiovascular function. The huge amounts of serotonin associated with metastatic carcinoid are presumed to be related to at least some of the cardiovascular disturbances associated with this disease.

This paper presents the results of experiments designed to determine whether serotonin is involved in clotting or hemostasis, and whether the concentration of circulating serotonin is altered in a variety of disease states.

Methods

Platelet suspensions were prepared from blood collected and handled with siliconized glassware. Disodium ethylenediamine tetracetate (EDTA) was used as anticoagulant (0.3 ml. of 5.0 per cent Na₂ EDTA to 9.7 ml. of blood). Platelet counts were done by direct chamber count with a diluent containing 1.5 per cent Na₂ EDTA and 0.7 per cent NaCl. After determination of the platelet count of the whole blood specimen, the blood was centrifuged for 20 min. at 500 r.p.m. The plasma was separated from the red cells and recentrifuged at 2000 r.p.m. for 40 min. The supernatant was then decanted and the platelet button resuspended in 3.3 ml. of isotonic saline. The platelet count of this suspension was determined and 3.0-ml. aliquots were used to determine the serotonin content spectrophotometrically. This procedure can measure as little as 0.1 μg. of serotonin.

In vitro coagulation studies were performed on plasma specimens obtained from a mixture of 1 part 3.8 per cent sodium citrate to 9 parts of blood. The recalcification time was done by adding 0.1 ml. of 0.025 M. CaCl₂ to a mixture of 0.1 ml. plasma and 0.1 ml. water or appropriate serotonin solution. Prothrombin time was performed by a 1-stage technic with whole plasma and 12.5 per cent saline-diluted plasma. Serum prothrombin time (prothrombin consumption) was determined by a method previously reported. Residual thrombin activity ("antithrombin" test) was determined by a new simplified technic.

This test is based on the observation that the ability of fresh serum to clot fibrinogen disappears rapidly, on incubation, apparently due to the de-
TABLE 1.—Effect of Adding Serotonin in Vitro to Plasma

<table>
<thead>
<tr>
<th>Prothrombin time (sec.)</th>
<th>Recalculated thrombin consumption (sec.)</th>
<th>Platelet-poor plasma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prothrombin time (sec.)</td>
<td>Recalculated thrombin consumption (sec.)</td>
<td>Platelet-poor plasma</td>
</tr>
<tr>
<td>Total dose—mg. seratonin/100 platelets</td>
<td>.02 00 00 00 05 01 18 26 09 .17 .40 .12</td>
<td></td>
</tr>
<tr>
<td>C. 5 treated patients after discontinuing oral reserpine therapy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Days since last dose</td>
<td>Total dose—mg. seratonin/100 platelets</td>
<td>Average</td>
</tr>
<tr>
<td>Patient I</td>
<td>Patient II</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>3 hours</td>
<td>1 day</td>
</tr>
<tr>
<td>0</td>
<td>1.02</td>
<td>02</td>
</tr>
<tr>
<td>3 hours</td>
<td>.38</td>
<td>02</td>
</tr>
<tr>
<td>1 day</td>
<td>.11</td>
<td>02</td>
</tr>
<tr>
<td>3 days</td>
<td>.10</td>
<td>02</td>
</tr>
<tr>
<td>5 days</td>
<td>.00</td>
<td>02</td>
</tr>
</tbody>
</table>

*By the method used, serum prothrombin time (prothrombin consumption) above 25 sec. is normal. The values noted are not considered significantly different.

**Table 2.—Relationship of Reserpine Therapy to Platelet Serotonin Content**

<table>
<thead>
<tr>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>mg. seratonin/100 platelets</td>
</tr>
<tr>
<td>.74 .96 .47 .36 .48 .30 1.10 1.00 .99</td>
</tr>
</tbody>
</table>

The capacity of the clotting region is a function of both the initial amount of thrombin formed and the antithrombin activity. The test is performed with serum from the system used to measure prothrombin by the 1-stage technic. If the initial prothrombin time is normal, it is assumed that a normal amount of thrombin was formed and that residual thrombin after a specific time interval is a function of antithrombin activity.

Prothrombin time is done in the usual way with the aid of a nichrome wire loop, which is left in the tube as the clot forms. Exactly 30 seconds after clotting, the clot is lifted with the wire loop and gently compressed against the glass wall to obtain serum. The clot is then removed and exactly 1 minute after clotting, 0.1 ml. of the residual serum is added to 0.2 ml. of standard fibrinogen solution (Chilcott). Normally the fibrinogen will clot in 12 to 18 seconds. Higher clotting times indicate increased antithrombin activity if initial prothrombin time is normal.

Fibrinolysis was studied by the method of von Kaulla. Observations were also made with the coagulograph ("Thromboelastograph" of Hartshorne), an instrument that records continuously the dissensibility of a clot. Capillary fragility was determined in patients by counting the petechiae produced by negative pressure of 20 mm. Hg applied for 2 minutes to the volar surface of the forearm 2 inches below the antecubital fossa.

**Results**

Influence of Serotonin on the Clotting Tests. The recalification time, prothrombin time, serum prothrombin time, residual thrombin time ("antithrombin" activity), clot retraction, and lysis activity of normal and platelet-poor plasma were compared with and without the addition of serotonin in concentrations up to 50 µg. per ml. In none of the tests was there a significant difference as illustrated by the typical experiments in table 1. The pattern of the coagulograph also remained unaltered by the addition of serotonin in concentrations up to 45 µg. per ml. The abnormal coagulograph patterns of platelet-poor plasma could not be corrected by the addition of serotonin.

Body depots of serotonin, including platelets, can be markedly depleted by large doses of reserpine in animals. Haverback and associates have demonstrated similar results with repeated small doses of reserpine. This finding has been confirmed for platelet serotonin after repeated oral doses of therapeutic magnitude or single intravenous doses (3 mg.) in man (table 2). In rabbit, dog, and man such deple-
tion has failed to influence the above clotting tests. Bleeding time and capillary fragility were also not detectably altered. These results are in agreement with those of Shore and associates\textsuperscript{23} and of Haverback.\textsuperscript{22}

**Correlation of the Serotonin Content of Platelets with a Variety of Disease States.** The platelet serotonin content of 94 patients at a chronic disease hospital were studied and compared with their diagnosis, age, weight, blood pressure, blood urea nitrogen, hemoglobin, platelet count, cephalin flocculation, and capillary fragility. The average serotonin content was 0.87 μg. per 10\(^9\) platelets, and 0.22 μg. per ml. blood with a range as illustrated in figure 1. Scattergrams of serotonin concentrations vs. age, weight, blood pressure (systolic and diastolic), cephalin flocculation, and capillary fragility failed to demonstrate any correlation.

Patients with a hemoglobin concentration over 10 Gm. per cent also failed to show any correlation of hemoglobin with platelet serotonin. However, of 10 patients with a hemoglobin value below 10 Gm. per cent, 9 had less than the average amount of serotonin per platelet.*

* The 10 patients with hemoglobin values below 10 Gm. per cent had an average platelet serotonin of 0.57 μg. per 10\(^9\) platelets, compared to 0.92 μg. per 10\(^9\) for 84 patients with hemoglobin concentrations above 10 Gm. per cent. Statistical analysis gave a t value of 3.1, indicating a 2 per cent probability that the difference between the means is due to chance.

Patients with platelet counts between 100,000 and 500,000 did not show any correlation between count and serotonin content per 10\(^9\) platelets. However, in 4 instances with counts above 500,000, the serotonin content per platelet was below average and in 2 instances with counts less than 100,000 the serotonin was above average.

Patients whose blood urea nitrogen was less than 30 mg. per 100 ml. showed no correlation of this factor with platelet serotonin content. However, of 8 patients with urea nitrogen values above 30, 7 had less than average amounts of serotonin per platelet.†

Patients were classified into diagnostic groups as in figure 2. Apparently none of these groups demonstrated any distinct abnormality of platelet serotonin. Platelet serotonin did not correlate with hypertensive cardiovascular disease or with blood pressure.

**DISCUSSION**

Serotonin has been found to occur primarily in gastrointestinal tissue, platelets, and brain. Although the presence of serotonin in platelets suggests that it may be a factor in hemostasis, these studies, as well as those of Sjoerdsma, Weissbach, and Udenfriend,\textsuperscript{21} Shore, and co-workers\textsuperscript{23} and Haverback and co-workers\textsuperscript{22} indi-

† The 8 patients with urea nitrogen values above 30 mg. per cent had an average platelet serotonin of 0.61 μg. per 10\(^9\) platelets, compared to 0.91 μg. per 10\(^9\) platelets for 86 patients with urea nitrogen values below 30 mg. per cent. Statistical analysis gave a t value of 2.5 indicating a 5 per cent probability that the difference between the means is due to chance.
icate that marked changes in the amount of platelet serotonin are not accompanied by any disturbance in hemostasis. Zucker and Borelli and Schullman and associates have found no deficiency of serotonin in patients with hemorrhagic phenomena related to coagulation and platelet deficiencies. Although Fenichel and Seegers have found serotonin to influence one specific reaction (clot retraction) involving a platelet factor, our studies and those reported by Zucker fail to reveal any influence by either depletion or addition of serotonin on clotting, clot retraction, or clot lysis.

The present findings that patients with high urea nitrogen values and high platelet counts tend toward lower platelet serotonin is in agreement with the findings of Zucker that were obtained by bioassay. The tendency toward lower platelet serotonin with severe anemia and high blood urea suggests that reduced platelet serotonin may occur with non-specific debility or nutritional deficiency, since no specific disease group was found to have a low platelet serotonin content.

Earlier studies of serotonin as a possible etiologic factor for hypertension failed to prove such a relationship. This study shows that hypertensive patients do not have abnormal amounts of circulating platelet serotonin. However, subjects taking therapeutic doses of reserpine develop a marked depletion of platelet serotonin, which persists for many days after discontinuing the drug. The depletion of platelet serotonin that accompanies the hypertensive effect of reserpine apparently does not influence any of the known factors related to hemostatic mechanisms.

**Summary**

Platelet serotonin content was measured in normal subjects and in a variety of disease states by a spectrophotofluorimetric method. It was not disturbed in any of the clinical groups studied, including hypertensive patients. Capillary fragility, as measured by a negative pressure method, was not correlated with platelet serotonin. Platelet serotonin content tended to be reduced in patients with markedly elevated urea nitrogen or severe anemia. With abnormally high platelet counts the concentration per platelet was also low.

In man, reserpine in doses commonly used clinically caused a marked and prolonged depletion of platelet serotonin without influencing the clotting mechanism or hemostasis. Serotonin added in vitro was found to be without effect on coagulation, clot retraction, or fibrinolysis.

**Summario in Interlingua**

Le contento de serotonina in le plachettas esseva mesurare in subjectos normal e in patientes con un varietate de statos pathologic. Le metodo usate esseva le spectrophotofluorimetrica. Le serotonina plachettal non esseva disturbate in ule del gruppos clinic studiate. Isto valeva etiam pro patientes hypertensive. Le fragilitate capillar, mesurate per un methodo a pression negative, non se monstrava correlationate con le serotonina plachettal. Le contento de serotonina in le plachettas tendeva a monstrare se reducute in patientes con marcate elevationes del nitrogeno ureal o con grados sever de anemia. In casos de anormalmente alte numerationes plachettal, le concentration de serotonina in le plachetta individual esseva etiam basse.

Reserpina, administrate in le doses que es de uso commun in le practica clinic, causava un marcate e prolongate depletion del serotonina plachettal sin influenza le mechanismo coagulatori o le hemostase. In observationes in vitro, le addition de serotonina monstrava nulle effecto super le coagulation, le retraction del coagulo, o le fibrinolysve.

**REFERENCES**

After being frequently urged to write upon this subject, and as often declining to do it, from apprehension of my own inability, I am at length compelled to take up the pen, however unqualified I may still feel myself for the task.

The use of the Foxglove is getting abroad, and it is better the world should derive some instruction, however imperfect, from my experience, than that the lives of men should be hazarded by its unguarded exhibition, or that a medicine of so much efficacy should be condemned and rejected as dangerous and unmanageable.—William Withering. *An Account of the Foxglove, and Some of Its Medical Uses.* Birmingham, 1785.
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