Blood Cell Aggregation and Screen Filtration Pressure

By Roy L. Swank, M.D., and Eli Davis, M.D.

Recent investigations of the conjunctival and nail-bed microcirculation indicate that certain changes in the small blood vessels and the circulating blood are frequently associated with disease. Straight, unkinked arterioles, tortuous veins, and micropools have been observed by Landau and Davis in subjects with arteriosclerosis. Congestion of the venules and capillaries are seen in diabetes mellitus. Intra-arteriolar aggregation of blood elements and capillary ischemia are seen in severe hypertension, in severe diabetes, and in many systemic acute and chronic illnesses. Some investigators consider intravascular aggregation abnormal, but Knisely, Bloch, and Davis and associates find this misleading and accept only diffuse arteriolar aggregation as evidence of a pathological condition.

In most cases, aggregation appears to be accompanied by slowing of the circulation through the capillary bed. Objective measurements of this are lacking. Furthermore, bulk blood viscosity is not changed by the aggregation of blood cells. A detailed study using the capillary-tube viscosity method did not reveal significantly elevated blood viscosity in patients with vascular disease and diabetes mellitus. In both conditions aggregation of circulating blood cells is frequently present. The capillary tube used in these studies had an internal diameter of 300 μ which is sufficiently large to allow free passage of the aggregates, few of which exceed a diameter of 35 μ.

The recently developed screen filtration-pressure method of Swank and co-workers makes it possible to study resistance to flow of blood through pores 20 by 20 μ square. This method is influenced by hematocrit values as is the capillary tube, cone-plate, or G.D.M. low-shear viscometer methods. In addition, it is very sensitive to aggregation, especially of the platelets and leukocytes.

Methods

In the present study, the microcirculation of the conjunctiva and nail bed was observed by one of us (E.D.) and compared with the screen filtration pressure (SFP) of 98 subjects attending the Hadassah Hospital of the Hebrew University Hadassah Medical School.

All subjects were from the Jerusalem area, and all but two were ambulant and came to the clinic by public transportation. Eighty-one patients were examined from among those currently attending the outpatients department, who were known to be in various stages of common illnesses. Seventeen other subjects (medical students, investigators, and technicians) were presumably healthy. Four patients had to be excluded because of poor sampling of blood or for other technical reasons. Blood samples were drawn from an antecubital vein for the screen filtration pressure test between 8:30 and 11:00 a.m. The patients had usually eaten a very light breakfast. All plasma samples were clear and free of hemolysis. Four milliliters of blood were drawn through a disposable needle into each of three, 5-ml metal-tipped syringes. Each syringe contained approximately 0.15 ml of heparin solution (1,000 units/ml). When it was difficult to insert the needle in the vein or the blood did not flow freely, the samples of blood obtained were discarded. In some cases other samples were then drawn from a different vein. A tourniquet was used briefly for inspection of the arm and during insertion of the needle. It was removed during withdrawal of blood. The third, or last, sample drawn was analyzed for screen filtration pressure (SFP) first (first control). To the second 4-ml sample drawn, 10 μg of adenosine diphos-

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phate (ADP) was added 20 to 30 seconds before its SFP was determined. Finally, the SFP of the first blood sample to be drawn was determined (third SFP determination; second control). An example of the three SFP curves as recorded on the storage oscilloscope are shown in figure 1. On several occasions ADP potentiation was repeated on the third blood sample drawn. Less than 5 minutes were required from the onset of blood withdrawal to the completion of the three SFP tests.

The details of the construction and use of the SFP apparatus have been previously described.10,11 The test determines the pressure required to force blood at a constant rate through a screen with multiple pores 20 by 20 μ square and 20 μ deep. The blood passes through this screen, which has a diameter of 1.8 mm, at the speed of 80 mm per second for 10 seconds (2 ml of blood pass the screen in this period). The pressure in millimeters Hg reached at the end of this 10-second period is defined as the screen filtration pressure (SFP). Normally this pressure varies from 30 to 45 mm Hg. The highest normal value for SFP is approximately the same as that one of us (E.D.) found for the upper limits of normal terminal vessel blood pressure.13 After addition of ADP to the blood, the SFP frequently exceeded 225 mm Hg, the pressure that our ink recorder could measure. All such results have been charted and valued as >225 mm Hg.

For the present study several changes in the pressure recording were made. A transistorized differential amplifier, embedded in a bar of aluminum for temperature stability, was substituted for the former stable, but low-frequency magnetic amplifier. Also a Dynisco strain gauge (model PT 25-10) replaced the Statham strain gauge. These changes increased both the sensitivity and accuracy of the apparatus. In addition all SFP measurements were monitored with a Tektronix storage oscilloscope (type 564) with a 2B67 time base unit and 3A3 100 μv/div. differential unit. This proved the reliability of our ink-recorded pressure curves, and, because of the quick response of the oscilloscope, revealed passage of bubbles through the screen in a few instances in which no indication of their presence was shown by the ink writer. Artifactual pressures from bubbles occurred in less than 5% of all tests and were recognized from the ink recording in all but two tests. Comparison of the two control SFP curves indicated that passage of bubbles through the screen artificially increased the SFP usually by no more than 5 mm Hg.

The addition of 1 to 4 μg of adenosine diphosphate (ADP)/ml of blood normally increases the SFP slightly. After trauma ADP potentiates the SFP markedly.14 The potentiation is maximal about 30 seconds after addition of ADP.15 During the present study different concentrations of ADP were tested in samples of blood having both a slight response (normal) or marked response (pathological) to ADP. Concentrations varying from 1 to 4 μg/ml of blood gave essentially the same result. Therefore, the volume of ADP used in all tests was 2.5 μg/ml of blood. The ADP was made up in saline to a concentration of 0.5 mg/ml. This solution (0.025 ml) was introduced into the blood by way of the tip of the syringe 30 seconds before the SFP test.

Two antiserotonin substances,* 1 methyl-D-lysergic acid (−)-butenolamide bimaleate (UML 491) and 9-N-methyl-piperidyliden-4-thioxanthen maleate (BP 400), were tested in blood samples from five subjects with marked ADP potentiation. These substances were added to the blood in quantities of 2 to 5 μg/ml and thoroughly mixed in before the addition of ADP. Previous studies indicated that increases in SFP due to hemorrhage, trauma, and the addition of serotonin and ADP to the blood were prevented or lessened by UML 491.14–16

For analysis, all SFP values were adjusted to a hematocrit of 40. This correction was made between hematocrits of 30 and 50 by adding 0.5 mm Hg to the SFP for each hematocrit unit that was less than 40, or by subtracting 0.5 mm Hg from the SFP for each hematocrit unit that was more than 40. For hematocrits of more

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*Furnished by Sandoz Pharmaceuticals, Hanover, New Jersey.
SFP AND BLOOD CELL AGGREGATION

Figure 2

Relationship of the SFP to different hematocrits for heparinized blood (solid line) and citrated blood (dashed line). The hematocrits were adjusted by adding or removing plasma. Each blood sample was filtered through Dacron wool immediately before the SFP test to remove aggregated platelets and leukocytes. The adjustments of SFP to hematocrit of 40 were done in reference to the straight-line cutting through the SFP hematocrit curve for heparinized blood.

than 50, 1.0-mm Hg pressure was subtracted from the SFP for each hematocrit unit that was more than 50. These corrections are based on the SFP-hematocrit curves shown in figure 2. These curves were obtained by adjusting the hematocrit of each sample with its own citrated plasma or with its own heparinized plasma. Each sample was then filtered through Dacron wool to remove adhesive and aggregated material immediately before the SFP was determined. The curve for heparinized blood was used to adjust the SFP values in the present study.

The microcirculation of the conjunctiva was observed during the morning 1% to 2 hours after the blood was drawn. The results of these observations were recorded independently and without knowledge of the SFP results. The microcirculation of the conjunctiva was observed with a Zeiss slit-lamp at a magnification of 25 to 40. In some cases the nailfold capillaries were examined with a Zeiss WL microscope at a magnification of 100.1,3 These observations supplemented those made of the conjunctiva but have not been included in this analysis.

Microcirculatory Criteria

Arteriolar Red Cell Aggregation5

Significant aggregation (+) is defined as the presence of aggregation in two or more arterioles in each of the whole temporal and nasal sides of the bulbar conjunctiva of both eyes when examined at a magnification of 25. Marked aggregation (++) is defined as the presence of aggregated red cells in two or more arterioles of each microscopic field of each side of the bulbar conjunctiva of both eyes when examined at a magnification of 25.

Ischemia

Ischemia is regarded as present (+) when the capillaries are 3.5 to 5.0μ in diameter. Ischemia is marked (++) when the capillaries are less than 3.5μ in diameter.6 Often the number of capillaries are also markedly reduced when the capillaries are narrowed.

Congestion

The microcirculation is considered congested when the venous limb of the capillary is 12μ or more in diameter (normal variation is 6 to 11μ) and when the smaller venules of the conjunctiva are 33μ or more in diameter (normal diameter is 25μ).3

Results

SFP and the Microcirculation

Alterations from normal in the microcirculation are considered with respect to (1) the SFP, adjusted to a hematocrit of 40, and (2) the increase of the SFP by ADP (fig. 3). The mean values with standard deviations are shown in the table.

The SFP adjusted to a hematocrit of 40 appeared to increase slightly in patients with arteriosclerosis alone and in those with arteriolar aggregation. In patients with ischemia alone, the SFP tended to decrease. However, these trends were not great enough to be statistically significant.

The variation in the adjusted SFP values tended to increase in subjects with arteriolar aggregation ischemia, and congestion (fig. 3). This was confirmed statistically when the three groups of subjects with what we considered normal and questionably pathologically congestion (normal microcirculation, venous aggregation alone, and arteriosclerosis alone) were combined and compared with the other combined groups, all with definitely abnormal microcirculation (table). In the former groups of 25 patients, the variance was 17.5; in the latter groups comprising 69 subjects with definitely abnormal circulation, it was 44.1. The difference was significant, P < 0.01.

When ADP was added to samples of blood from subjects with normal microcirculation,
Figure 3

Relationship of the microcirculation, as observed under the binocular microscope, to the SFP adjusted to a hematocrit of 40, and to the increase in SFP by ADP. Circles indicate females, dots men. The triangles (female, ▲ male) denote the six presumably normal subjects whose microcirculation was abnormal. Only the ADP-potentiated SFP for each of these six subjects is indicated by special characters. The adjusted SFP values were within the normal range and appear as circles or dots.

Table 1

Means and Standard Deviations of Adjusted Screen Filtration Pressures (SFP) and the Increases due to Adenosine Diphosphate (ADP) for the 94 Persons Studied

<table>
<thead>
<tr>
<th>Microcirculation</th>
<th>Cases</th>
<th>SFP, mm Hg*</th>
<th>Increase SFP by ADP, mm Hg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal microcirculation</td>
<td>11</td>
<td>40.9 ± 3.9</td>
<td>49.2 ± 33.2</td>
</tr>
<tr>
<td>Arteriosclerosis alone</td>
<td>8</td>
<td>44.0 ± 3.2</td>
<td>94.9 ± 42.7</td>
</tr>
<tr>
<td>Venous aggregation</td>
<td>6</td>
<td>42.3 ± 5.0</td>
<td>69.5 ± 23.1</td>
</tr>
<tr>
<td>Arteriolar aggregation</td>
<td>23</td>
<td>43.1 ± 6.3</td>
<td>&gt;132.5 ± 64.3</td>
</tr>
<tr>
<td>Arteriolar aggregation (+)</td>
<td>21</td>
<td>42.0 ± 7.2</td>
<td>&gt;149.5 ± 59.0</td>
</tr>
<tr>
<td>with ischemia† (++)</td>
<td>12</td>
<td>42.9 ± 4.2</td>
<td>&gt;168.9 ± 46.5</td>
</tr>
<tr>
<td>Ischemia alone</td>
<td>6</td>
<td>36.3 ± 5.7</td>
<td>&gt;158.3 ± 51.5</td>
</tr>
<tr>
<td>Congestion</td>
<td>7</td>
<td>40.1 ± 7.6</td>
<td>&gt;154.0 ± 52.3</td>
</tr>
</tbody>
</table>

*Adjusted to hematocrit of 40.
†(+) and (++) indicate severity of ischemia.
the SFP increased an average of 49 mm Hg. In subjects with intravenous aggregation, ADP increased the SFP by an average of 70 mm Hg, and in those with arteriosclerosis by 95 mm Hg. These differences from normal appeared to be significant statistically (for venous aggregation $0.01 > P > 0.001$; for arteriosclerosis $0.05 > P > 0.02$). When intrarterial aggregation was present and marked, or when it was combined with ischemia, the ADP potentiation was very marked, and the difference from normal was much greater ($P < 0.001$) than in the aforementioned subjects with venous aggregation or arteriosclerosis (fig. 3). Capillary ischemia alone and congestion were also accompanied by very marked increases in the ADP potentiation of the SFP of a highly significantly degree ($P < 0.001$).

The increased variability of the adjusted SFP in subjects with abnormal circulation was closely related to the degree of ADP potentiation of the SFP. This was shown by charting the adjusted SFP against the increase in SFP by ADP (fig. 4). As the SFP potentiated by ADP increased, the spread of the adjusted SFP values also increased. In 36 subjects with potentiated SFP from 0 to 100, the variance was 7.1. In the remaining subjects the ADP potentiation of the SFP was greater than 100, and the variance was 32.7. The variance was significantly greater in the latter than in the former group ($P < 0.001$). The mean adjusted SFP for the subjects with potentiated SFP up to 100 was 40.2 and for those with potentiated SFP above 100 was 43.2. Even though these differences were not great, they were significant statistically ($P < 0.01$).

A total of 17 subjects presumed normal were studied. In 11 of these the microcirculation was judged normal. As stated before, the ADP potentiation of the SFP in these cases averaged 49 mm Hg. In the other six subjects the microcirculation was found to be abnormal, and in these the SFP potentiation by ADP was greater (△ female; and △ males; fig. 3). The adjusted SFP measurements were not altered significantly from normal in these 17 subjects.

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**Figure 4**

SEP adjusted to a hematocrit of 40 in relationship to the potentiation of SFP by ADP. Note that as ADP potentiation increased, spread of SFP values also increased.
Relationship of SFP to Age and Other Factors

The patient’s age did not influence the SFP, nor the SFP potentiation by ADP. The average SFP in 27 subjects less than 40 years of age was 40, and in 64 persons 40 to 84 years old, was 41 mm Hg. There was, however, a tendency for the spread of SFP values to be greater in older patients, but these older patients were usually more ill.

The cases were divided along ethnic lines into three groups, European-American, Mediterranean, and Oriental, and the average SFP and spread of values in each group was studied. No significant differences were detected in these values nor in the SFP potentiated by ADP.

The erythrocyte sedimentation rate did not influence the SFP, nor the potentiation effect of ADP.

The main diseases from which the patients suffered, included 19 cases of diabetes mellitus, 14 cases of arteriosclerosis with clinical complications other than congestive cardiac failure, seven of chronic pulmonary disease, six of severe vasomotor rhinitis, five of chronic renal disease, four of hypertension, four of acrocyanosis and Raynaud’s disease, three of osteoporosis, three of bilateral nerve deafness, and three of hypercholesteremia. The other diseases were a fair sample of the usual cases attending a general medical clinic. In chronic pulmonary disease and arteriosclerosis, there was a tendency for the SFP to be somewhat elevated (by nearly 10%), but the SFP elevation by ADP was not significantly different in most disease categories. Exceptions, however, were the three cases of bilateral nerve deafness and the three cases of hypercholesteremia. In all six, the ADP produced a marked increase in SFP. Unfortunately the number of these cases tested was too small to warrant conclusions.

Further division on the basis of whether the patients were moderately ill or very ill revealed that both of the ill groups had a greater variation in the SFP values, and a tendency of the SFP to be higher than was observable in apparently healthy persons. Also, the SFP potentiated by ADP was higher in the moderately ill and severely ill patients than in those presumed well. No statistically significant difference between the two ill groups was apparent.

Effect of Antiserotonin Agents

UML 491 and BP 400 did not alter the SFP values nor the ADP effect.

Discussion

It was stated earlier that intra-arteriolar aggregation of blood cells is associated with disease, and by an apparent slowing of the circulation. Yet the SFP of venous blood in subjects with intra-arterial aggregation was not significantly higher than in patients with completely normal circulation. Two possible explanations of this will be presented and discussed: (1) The forces holding the aggregates together were weak; the aggregates were readily broken apart upon entering the capillary beds; and the circulation was not altered or slowed. (2) The forces holding the cells together were considerable and did cause interference with the circulation; the capillary beds filtered or removed the cohesive forces or substances from the blood; consequently the SFP of the venous blood became essentially normal.

The latter explanation is supported by the following evidence: (1) The SFP of blood has been shown to be reduced by passage through the vascular bed of the lungs.14, 16, 17 It seems reasonable to assume that this would also occur in other vascular beds of the body. (2) The SFP values of blood samples from subjects with arteriolar blood cell aggregation or with a high ADP potentiation of the SFP varied significantly more than they did in subjects with a normal microcirculation or in those with a low ADP potentiation of the SFP. This could be due to a variable efficiency of filtration or removal by the capillary bed of the cohesive factors which caused aggregation of blood cells in the arterial blood. In consequence the forces tending to cause aggregation of blood cells in the veins would vary in strength, as would also the SFP. These forces would likely vary in the same individual from time to time as well as between individuals. The solution to these
questions may be answered by serial SFP studies on the same individual, and by concurrent sampling for SFP of arterial and venous blood in patients with intra-arterial aggregation.

In contrast to the slight changes observed in the adjusted SFP values, dramatic changes in the potentiation of SFP by ADP were observed in patients with definitely abnormal microcirculation (in those with intra-arterial aggregation, capillary ischemia, and congestion of the small blood vessels). In each of these situations some degree of hypoxia or ischemia of tissues was suspected. In the presence of arteriosclerosis without arteriolar aggregation, and in venous aggregation alone, much less potentiation of the SFP by ADP was observed, and statistical analysis indicated that these values probably differed significantly from normal. This may indicate, contrary to prevailing opinion,5, 7, 8 that venous aggregation and arteriosclerosis are indeed an evidence of abnormal circulation though of a less severe magnitude than present in arteriolar aggregation, ischemia, and congestion.

Six of the 17 medical students and technicians, who were presumed healthy, showed abnormalities in the microcirculation and demonstrated marked SFP potentiation by ADP. When blood was drawn, four of these six were exceptionally nervous and tense. Two were found to be suffering from acrocyanosis, and one medical student, the son of a hypertensive father and a diabetic mother, had several micropools in his conjunctiva. In a previous study,5 one of us (E.D.) investigated 40 persons presumed healthy, aged 17 to 50 years, who came for routine medical examinations for staff appointments. Clinical examination and all routine laboratory tests gave normal results in 26 of the 40 subjects. In 14 a raised blood sedimentation rate was found which persisted for 6 months, and in four of these the sedimentation rate was more than 40 in the first hour by the Westergren method. Arteriolar aggregation was present in four of these 14 subjects.

It is clear that not all presumed well persons are free from evidence of disturbed circ-

ulation. The presence of abnormal responses of the SFP to ADP in patients with Raynaud’s phenomenon and in acrocyanosis, and in presumed healthy, but very tense young individuals, indicates the marked degree of change in the circulation probably occurring in these instances. Nervous tension may have been a factor causing arteriolar aggregation in some of our presumed normal subjects, many of whom had never experienced having their blood taken. This must have been a much less important factor in the ill patients, all of whom had had their blood taken many times, and all of whom appeared to be undisturbed by the procedure. Davis and associates18 have shown that 60% of persons informed that they are about to get an injection react to the announcement with narrowing of, and red cell aggregation in, the small blood vessels.

It is known that ADP causes platelet adhesiveness.15, 19 It is also known that ADP increases the SFP slightly in normal dogs, and markedly in severely traumatized dogs.14 If, however, the buffy coat is removed from the blood, or the blood is filtered through Pyrex glass or Dacron wool, the potentiation of the SFP by ADP is abolished. The first of these procedures removes most of the platelets and leukocytes, the second removes those which are adhesive.18 It seems, therefore, that the potentiation of the SFP is due to adhesive and aggregated platelets (and probably also leukocytes) caused by the ADP. These blood elements may become preconditioned to the action of ADP in their passage through tissues suffering from varying degrees of ischemia or hypoxia. Marked potentiation only occurred when the microcirculation was abnormal and tissue ischemia or hypoxia was likely, whereas slight or no potentiation occurred in subjects with normal microcirculation.

Earlier studies showed that the ADP potentiation after trauma was abolished by the serotonin antagonist, UML 491.14 For this and other reasons, it was felt that the potentiation of the SFP by ADP in trauma probably involved serotonin. This substance in the pres-
ence of ADP has caused platelets and leukocytes to be adhesive and to aggregate and the SFP to increase. In the present studies UML 491 and BP 400, two serotonin antagonists, did not influence the ADP potentiation of the SFP in blood from patients with abnormal microcirculation. It appears, therefore, that potentiation of the SFP by ADP in our patients with abnormal microcirculation differs from that previously observed in traumatized dogs.

Summary
Changes in the microcirculation of the conjunctiva were compared with the screen filtration pressure (SFP) of venous blood in presumed normal subjects and in others with known systemic disease. The presence of arteriolar aggregation and of other circulatory abnormalities was not attended by significantly different mean SFP values. When, however, 2.5 μg of adenosine diphosphate (ADP) per milliliter were added to blood in vitro 20 to 30 seconds before the SFP determination, the SFP increased. In subjects with normal microcirculation, the increase was slight; in those with abnormal circulation, it was marked. When abnormal changes in the circulation were present, or ADP potentiation was high, there was an increase in the spread of control SFP values. Also when the ADP potentiation was high, the control SFP values were slightly higher than when the SFP potentiation was low. Two serotonin antagonists did not prevent or decrease the increase in SFP by ADP.

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References
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