INFLUENCE OF ASPIRIN ON HAEMOSTATIC PARAMETERS

BY

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In recent years faecal blood loss due to ingestion of acetyl salicylic acid (ASA) has been demonstrated by various authors, both by chemical methods such as the benzidine reaction, and by physical methods with erythrocytes labelled with radioactive substances. Stubbé (1958) reported occult faecal blood loss in 70 per cent. of patients and healthy volunteers taking ASA.

Scott, Porter, Lewis, and Dixon (1961), reporting a 70 per cent. incidence of faecal blood loss with ASA, found no indication as to the actual site of bleeding and no correlation between dyspepsia and blood loss. Stubbé (1958) time and again found normal values for the bleeding time and assumed a local irritant action of ASA on the gastric mucosa to be responsible for the faecal blood loss. Enterico-coated ASA tablets, at least those commercially available, provide "no protection of the gastric mucosa" as faecal blood loss was reported in about 32 per cent. of cases by Scott and others (1961) and in 65 per cent. of cases by Stubbé (1958) and also by Lange (1957). Also Pierson, Holt, Watson, and Keating (1961) found that enterico-coated ASA induced the same rate of bleeding as non-coated ASA; according to these authors the intestinal mucosa is also susceptible to ASA irritation. With a special experimental coating of ASA tablets, faecal blood loss was reduced to a minimum (Stubbé, Pietersen, and van Heulen, 1962).

In addition to the 50 to 70 per cent. incidence of faecal blood loss presumably due to gastric irritation, there are incidental reports of manifest bleeding during medication with ASA after tooth-extraction (Smith and MacKinnon, 1951), post-biopsy bleeding and epistaxis (Frick, 1956), haematuria, bleeding gums, menorrhagia (Wising, 1952), and after tonsillectomy (Neivert, 1945). Here, factors other than gastric irritation must be present.

On account of the unknown pathogenesis of ASA-induced occult faecal blood, and of the reports of frank bleeding following the ingestion of ASA, a study was made of the influence of ASA on haemostasis.

Methods and Material

In this study ASA was given in a daily dosage of 3 g., two tablets of 0.5 g. dissolved in water three times a day after meals. Six healthy adults (three males and three females) and eight adult female patients with definite rheumatoid arthritis (A.R.A. criteria) were examined. Patients taking corticosteroids, Butazolidin, anticoagulants, or recent salicylate medication were excluded. With other medications such as gold salts or antimalarials, care was taken that the dosage had not been changed in the 2 months before the experiment. The following haemostatic parameters were studied before ASA ingestion and during the second week of ASA ingestion.

Vascular Factors and Platelets

- Capillary fragility (suction cup)
- Bleeding time (Ivy, modified)
- Direct total thrombocyte count (Feissly)
- Platelet-stickness (Wright)
- Clot retraction, correlated with haematocrit (Tocantins)
- Factor III in a platelet suspension for thromboplastin generation

Plasmatic Factors

- Recalcification clotting time (Howell)
- Heparin tolerance test (Marbet and Winterstein)
- Thrombelastogram (Hartert)
- Thrombin generation (Pitney and Dacie)
- Thromboplastin generation (Biggs and Douglas)
- Prothrombin consumption (Quick)
- Prothrombin time (Quick)
- Fibrinogen concentration (Clauss)
- Factor II, V, and VII activity (Koller)
- Factor X (Bachmann)
- Antithrombin immediate (thrombin time)
The urine was tested for haematuria by an orthotolidine tablet, sensitive to 25 red cells per c.mm. (Watson-Williams, 1955), and for the presence or absence of salicylate by the ferri-chloride test.

Inquiry was made for manifest bleeding from nose, gums, skin, etc.

For bleeding time determination the technique of Ivy, Nelson, and Bucher (1941) was used with the following modifications:

1. To prevent hyperaemia by 95 per cent. alcohol, the skin area tested was not cleaned at all.
2. Ivy and his colleagues recommended ample time between the three readings. Here three puncture wounds were made within four seconds, 2 to 3 cm. apart, assuming traumatic hyperaemia will influence all three wounds alike.
3. Instead of the Sharpe and Smith lancet with a blade length of 2-5 mm., a Becton and Dickinson disposable blade was used, which had a sharp cutting point 2-4 mm. deep with a base of 1-5 mm. and a thickness of 0-07 mm.
4. Blood droplets at each puncture site were blotted away every 10 to 15 seconds until bleeding ceased. The average time in seconds of the three readings was taken to be the bleeding time. If one site did not bleed, the average of the two other sites represented the bleeding time. If only one site bled, the test was repeated.

The results of these tests, when analysed statistically by the Wilcoxon test for symmetry, pointed to a marked change in the bleeding time, and this alone was then studied on a larger sample.

Instead of investigating a patient before and during ASA ingestion, a group of patients not taking ASA was compared with a group taking 3 g. ASA daily. All the cases were in-patients, the majority of them for rheumatoid arthritis. No restriction was made on the duration of ASA medication, some patients having taken ASA for several months without interruption. With the first group of 31 patients, 22 without and nine with ASA, the author knew who was taking ASA and who not (Table II, below).

For reasons explained later, another two groups were compared: 22 patients in 1958 of which twelve were off and ten on ASA, and 33 patients in 1961 of which 23 were off and ten on ASA (Tables III and IV, below), in which the author was unaware of the medication given.

All the tests were performed by the author. These data were analysed by the Wilcoxon two-sample test. Finally, the bleeding time tests were performed by a technician, in a double-blind situation, on seven patients, with definite rheumatoid arthritis, again before and during acetyl salicylic acid ingestion (Table V, below).

**Results**

The haemostatic mechanism in the eight patients with rheumatoid arthritis was essentially the same as in the six healthy adults. Of the eight patients with rheumatoid arthritis, five showed an increased plasma-fibrinogen concentration, and subsequently an increased maximal amplitude on the thromboelastogram. The other haemostatic parameters, however, were normal.

Hence it was justifiable to analyse the changes induced by ASA in these two groups together. Only those haemostatic parameters which showed a statistically significant difference at a 0-05 level with the Wilcoxon test for symmetry, are listed in Table I, with their P values, and their mean value before and during ASA ingestion. Thus no significant changes occurred in the platelet count, the capillary fragility, clot retraction, thromboplastin generation, or Factor II, V, VII, or X activity. Haematuria remained absent.

The difference in bleeding time between the patients that were off and those that were on ASA is highly significant by the Wilcoxon two-sample test: P = 0.00007 for the first group of 31 patients (Table II). In the blind situation the P value remains significant, P = 0.0008 in 1958 (Table III) and P = 0.006 in 1961 (Table IV). These three groups together contain 86 patients, 57 off ASA and 29 taking ASA 3 g. daily. The difference in bleeding time between these patients is highly significant (P = 2 × 10⁻⁹).

The bleeding times estimated by a technician in a double-blind situation on seven patients before and during ASA show prolongation in all cases (P = 0.008) (Table V).

### Table I

**Changes in Haemostatic Parameters, During 2nd Week of ASA, Significant at the 0.05 Level (Wilcoxon Test for Symmetry)**

<table>
<thead>
<tr>
<th>Haemostasis</th>
<th>P</th>
<th>Mean Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Healthy Adults</td>
<td>Rheumatoid Arthritis</td>
</tr>
<tr>
<td>Prothrombin Time (sec.)</td>
<td>0.11</td>
<td>0.11</td>
</tr>
<tr>
<td>Haematocrit (vol. per cent.)</td>
<td>0.031</td>
<td>0.044</td>
</tr>
<tr>
<td>Recalcification Time (sec.)</td>
<td>0.062</td>
<td>0.047</td>
</tr>
<tr>
<td>Platelet-stickiness (inverse relation)</td>
<td>0.11</td>
<td>0.06</td>
</tr>
<tr>
<td>Bleeding Time (sec.)</td>
<td>0.016</td>
<td>0.002</td>
</tr>
</tbody>
</table>
TABLE II
BLEEDING TIME IN 31 PATIENTS FOR WHOM THE ASA MEDICATION WAS KNOWN TO THE AUTHOR

<table>
<thead>
<tr>
<th>ASA</th>
<th>No. of Patients</th>
<th>Sex</th>
<th>Bleeding Time (sec.) for Each Patient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Not given</td>
<td>8</td>
<td>Male</td>
<td>140 142 143 107 149 138 148 127</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>Female</td>
<td>242 110 159 91 146 133 176 209 185 200</td>
</tr>
<tr>
<td>Given</td>
<td>3</td>
<td>Male</td>
<td>229 235 248</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>Female</td>
<td>375 258 208 293 193 359</td>
</tr>
</tbody>
</table>

\( P = 0.0007 \) (Wilcoxon two-sample test)

TABLE III
BLEEDING TIME IN 22 PATIENTS FOR WHOM THE ASA MEDICATION WAS NOT KNOWN (1958)

<table>
<thead>
<tr>
<th>ASA</th>
<th>No. of Patients</th>
<th>Sex</th>
<th>Bleeding Time (sec.) for Each Patient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Not given</td>
<td>4</td>
<td>Male</td>
<td>115 167 129 67</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>Female</td>
<td>140 215 159 136 144 160 199 133</td>
</tr>
<tr>
<td>Given</td>
<td>5</td>
<td>Male</td>
<td>251 214 363 343 281</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>Female</td>
<td>213 297 126 217 264</td>
</tr>
</tbody>
</table>

\( P = 0.0008 \) (Wilcoxon two-sample test)

TABLE IV
BLEEDING TIME IN 33 PATIENTS FOR WHOM THE ASA MEDICATION WAS NOT KNOWN (1961)

<table>
<thead>
<tr>
<th>ASA</th>
<th>No. of Patients</th>
<th>Sex</th>
<th>Bleeding Time (sec.) for Each Patient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Not given</td>
<td>4</td>
<td>Male</td>
<td>81 78 115 115</td>
</tr>
<tr>
<td></td>
<td>19</td>
<td>Female</td>
<td>50 35 103 45 108 103 60 110</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>Male</td>
<td>108 302</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>Female</td>
<td>257 200 239 97 227 238 55 78</td>
</tr>
</tbody>
</table>

\( P = 0.006 \) (Wilcoxon two-sample test)

TABLE V
ASA-INDUCED PROLONGATION OF BLEEDING TIME IN A DOUBLE-BLIND TRIAL ON SEVEN PATIENTS

<table>
<thead>
<tr>
<th>Estimation</th>
<th>Bleeding Time (sec.) for Each Patient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before ASA</td>
<td>125 92 85 84 100 68 96</td>
</tr>
<tr>
<td>During ASA</td>
<td>180 139 194 177 322 162 182</td>
</tr>
</tbody>
</table>

\( P = 0.008 \) (Wilcoxon test for symmetry)

Signs of manifest bleeding in the patients taking ASA did not occur during this study.

Discussion
ASA prolonged the prothrombin time from 13·7 to 14·1 sec., which proved to be statistically significant. Clinically, however, this small prolongation is of no importance whatsoever. Link, Overman, Sullivan, Huebner, and Scheel (1943), Shapiro, Redish, and Campbell (1943), Meyer and Howard (1943), and Quick and Clesceri (1960) have all described a slight to moderate fall in prothrombin concentration, hence a prolongation of prothrombin time on a relatively large dose of 5·3 to 8 g. ASA daily. A marked fall in prothrombin concentration is seen only when massive doses of ASA are given, and even then the level is seldom low enough to cause haemorrhage (Butt, Leake, Solley, Griffith, Huntington, and Montgomery, 1945).

There is a weak indication \( P = 0.049 \) that the packed cell volume decreases duringASA ingestion, which agrees with citations in Goodman and Gilman (1955). The mean value before aspirin is somewhat low (43 vol. per cent.) due to the subnormal values, as expected, in the patients with rheumatoid arthritis. It is stressed that this slight decrease from 43 to 41·6 vol. per cent. occurred during a 2-week experiment, and that is not necessarily related to the recent reports of aspirin-induced...
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anaemia (Summerskill and Alvarez, 1958).

The whole blood recalcification clotting time decreased from 135 to 120 sec. (P = 0.002). It is
known that the clotting time is shorter in plasma than in whole blood. The decrease in blood-clotting
time during ASA ingestion is probably directly
related to the lower haematocrit value or increased
plasma volume. Clotting time measured by the
heparin tolerance test was not influenced by ASA.

Platelet-stickiness, measured with Wright's tech-
nique, decreased in all but one case (P = 0.02). With the same technique Bounameaux, and van
Cauwenberge (1954) studied the effect of sodium salicylates in rats. Low dosage had no effect, but
very high doses (30 mg./100 g. bodyweight) reduced
platelet-stickiness, inducing hypoagulability of
blood independent of prothrombin concentration.
The exact role of platelet-stickiness in haemostasis is
not yet clarified. The platelets and their stickiness
play a major role in the very first stages of haem-
ostasis by adhering to endothelial cells, and in the
formation of the platelet plug. A relation between
bleeding time and platelet-stickiness seems plausible

The bleeding time in the six healthy adults and
eight patients with rheumatoid arthritis studied ini-
tially, increased in all cases, from a mean of 161 sec.
before ASA to 302 sec. in the 2nd week of ASA
ingestion. Although this change proved to be
strongly significant (P = 3·10^{-5}), the very nature of the
bleeding time determination calls for further
discussion. According to Ivy, Shapiro, and Mel-
nick (1935) and Ivy and others (1941), the upper
limit of normal bleeding is 240 sec., but in their 115
healthy volunteers, the bleeding time rarely exceeded
180 sec. In setting a "clinically normal" maximum
bleeding time at 240 sec., patients with mild bleeding
tendency may be diagnosed as normal. Biggs and
MacFarlane (1962), using Ivy's technique, found a
normal bleeding time to be from 2½ to 7 minutes.
Without ASA medication, the author found a mean
bleeding time in 81 tests in adults of 137 sec.,
standard deviation of 45·5. Normal bleeding time
estimated by a technician in the same laboratory
using an identical technique is 92 sec. (range 68 to
141). The difference in "normal values" for the
Ivy bleeding time found by various investigators
using identical techniques is due to differences in the
pressure applied on the blade and in determining
the end-point of bleeding. It is difficult to determine
when bleeding has stopped and when a small amount
of red-tinted serum starts being expressed from the
cotted blood. It is at this end-point of bleeding,
that unconscious bias can easily occur. Hence it
was imperative to perform the bleeding time test in
a "double-blind situation". In the two groups
examined by the author in a double-blind situation
(Table III and IV), the differences with and without
ASA remained statistically significant, and the
bleeding time estimated by a technician in a double-
blind situation on seven patients before and during
ASA medication also showed a statistically signifi-
cant prolongation (Table V). The statistical anal-
ysis of the data was performed with the Wilcoxon
two-sample test, rather than with means and standard
deviations. For clinical purposes the normal bleed-
ing time was 137·3 sec. ± S.D. 45·5 in 81 tests.
During the ingestion of 3 g. ASA daily, the bleeding
time measured 255·4 sec. ± S.D. 76·1 in 64 tests.

Beaumont and Willie (1955) and Beaumont,
Willie, and Lenègre (1955) were the first to dem-
strate a prolongation of the bleeding time due to
ASA in adult patients with cardiac disease. With
30 to 40 mg. ASA/kg. body weight, bleeding time
increased from 3·67 to 5·8 minutes in about 80
per cent. of cases (Duke's technique). Capillary
fragility was not influenced by ASA. In twelve
healthy children (Beaumont, Caen, and Bernard,
1956), the bleeding time increased from 2·75 to 4·06
minutes on the third day of ASA ingestion. In
children with haematological disorders such as
leukaemia and haemophilia, ASA caused further
prolongation in the already prolonged bleeding time.
Very interesting is the observation of Beaumont and
others (1956) that, in three children with platelet
disorders and prolonged bleeding time aspirin did
not induce further prolongation. This supports the
conclusions of Bounameaux and van Cauwenberge
(1954), who demonstrated that sodium salicylate
decreased platelet stickiness in rats. In Beaumont's
three cases poor platelet function presumably could
not be further reduced by aspirin. Hofmann (1956)
induced progressive prolongation of bleeding time
in mice, correlating closely with increasing doses of
ASA or sodium salicylate. Blatrix (1963) also
demonstrated a prolongation of the bleeding time
and an increase in the volume of blood loss with
ASA doses in excess of 40 mg./kg. bodyweight daily.
Quick and Clesceri (1960) found no prolongation of
the bleeding time (Duke's technique) in ten normal
adults on 6 g. ASA daily, but the results on the one
subject, reported as representative for his group of
ten, do show an increase in bleeding time from 1½
to 3 minutes.

In general the bleeding time in patients on ASA
is in the range encountered in mild haemorrhagic
diathesis. The changes in haemostasis induced by
ASA are definitely not sufficient to cause major
haemorrhage, and if this occurs individual sus-
ceptibility to ASA must be assumed (Frick, 1956).
On the other hand, haemostasis is impaired and this
should be reckoned with, as menorrhagia or bleeding following tooth-extraction or tonsillectomy may occur. Performing a closed hepatic biopsy during ASA ingestion is thus contraindicated. ASA increased the incidence of late haemorrhage following tonsillectomy from 0·1 to 8·7 per cent. (Fox and West, 1947).

This study does not clarify the pathogenesis of ASA-induced faecal blood loss. However, from incidental observation, we are inclined to conclude that a mild haemorrhagic diathesis may cause faecal blood loss, e.g. in low-grade haemophilia, low-grade thrombocytopenia, and von Willebrand’s disease. No reports on the incidence of faecal blood loss in these diseases are available. During anticoagulant therapy (also a mild haemorrhagic diathesis) an increase from the “normal faecal blood loss of 0·5 to 1·1 ml. daily” was demonstrated by Watson and Pierson (1961). The possibility remains that the ASA-induced faecal blood loss is caused not only by gastric or intestinal mucosal irritation, but also by the concomitant mild haemorrhagic state. This would help to explain the 70 per cent. incidence of faecal blood loss while gastroscopical studies revealed gastric mucosal bleeding due to a direct irritation by ASA in only 20 per cent. of cases (Weiss, Pitman, and Graham, 1961). Enteric-coated aspirin tablets, which presumably do not irritate the gastric mucosa, may still induce a mild haemorrhagic diathesis with subsequent increased faecal blood loss.

Summary

Aspirin in a dose of 3 g. daily by mouth induced definite prolongation of the bleeding time and a decrease in platelet-stickness in six healthy adults and eight patients with rheumatoid arthritis. The prolongation of the bleeding time was confirmed in a larger sample in a double-blind situation. The implications are discussed.

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REFERENCES


L’influence de l’aspirine sur les paramètres hémostatiques

Résumé

L’aspirine à la dose de 3 g. par jour par voie buccale induisait une prolongation définie du temps de saignement et une diminution de la viscosité thrombocytaire chez six adultes sains et huit malades atteints d’arthrite rhumatismale. La prolongation du temps de saignement fut confirmée sur une plus grande étude et dans une situation qui ressemblait à un essai par la méthode de double-blind. On en discute les implications.

La influencia de la aspirina sobre los parametros hemostátiicos

Sumario

La aspirina a la dosis de 3 g. diarios por vía oral producía una prolongación definida del tiempo de sangría y una diminución de la viscosidad trombocitaria en seis adultos sanos y ocho enfermos con artritis reumatoide. La prolongación del tiempo de sangría fue confirmada en un mayor número de casos y en una situación de ensayo por el método de double-blind. Se discuten las implicaciones de esta investigación.