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. and Alvarez and Summerskill (1958) in 50 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. Enteric-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 enteric-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-stickiness (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 thromb-elastogram. 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 occured 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 $(P = 2 \times 10^{-8})$.

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)

Haemostasis	P			Mean Value	
	Healthy Adults	Rheumatoid Arthritics	Combined	Before ASA	During ASA
Prothrombin Time (sec.)	0·11	0·11	0·026	13·7	14·1
Haematocrit (vol. per cent.)	0·031	0·44	0·049	43	41·6
Recalcification Time (sec.)	0·062	0·047	0·002	135	120
Platelet-stickiness (inverse relation)	0·11	0·06	0·02	235	275
Bleeding Time (sec.)	0·016	0·002	$3 \cdot 10^{-5}$	161	302

TABLE II

BLEEDING TIME IN 31 PATIENTS FOR WHOM THE ASA MEDICATION WAS KNOWN TO THE AUTHOR

ASA	No. of Patients	Sex	Bleeding Time (sec.) for Each Patient							
Not given	8	Male ..	140	142	143	107	149	138	148	127
	14	Female ..	242 110	159 91	139 146	133 216	176 156	209 177	185	200
Given	3	Male ..	229	235	248					
	6	Female ..	375	258	208	293	193	359		

P = 0·00007 (Wilcoxon two-sample test)

TABLE III

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

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

P = 0·0008 (Wilcoxon two-sample test)

TABLE IV

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

ASA	No. of Patients	Sex	Bleeding Time (sec.) for Each Patient							
Not given	4	Male ..	81	78	115	115				
	19	Female ..	50 64 122	35 41 82	103 126 58	45 75	108 140	103 101	60 118	110 67
Given	2	Male ..	108	302						
	8	Female ..	257	200	239	97	227	238	55	78

P = 0·006 (Wilcoxon two-sample test)

TABLE V

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

Estimation	Bleeding Time (sec.) for Each Patient						
Before ASA ..	125	92	85	84	100	68	96
During ASA ..	180	139	194	177	322	162	182

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, Huntingdon, and Montgomery, 1945).

There is a weak indication (P = 0·049) that the packed cell volume decreases during ASA 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

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 technique, 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 hypocoagulability 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 haemostasis 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 initially, 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 = $3 \cdot 10^{-5}$), the very nature of the bleeding time determination calls for further discussion. According to Ivy, Shapiro, and Melnick (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 clotted 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 (Tables 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 significant prolongation (Table V). The statistical analysis of the data was performed with the Wilcoxon two-sample test, rather than with means and standard deviations. For clinical purposes the normal bleeding 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 demonstrate 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 susceptibility 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 $0 \cdot 1$ to $8 \cdot 7$ 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 $0 \cdot 5$ to $1 \cdot 1$ 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 gastroscopic 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-stickiness 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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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 échelle et dans une situation qui ressemblait un essai par la méthode de *double-blind*. On en discute les implications.

La influencia de la aspirina sobre los parametros hemostáticos

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 fué 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.