

PRURITUS IN POLYCYTHEMIA VERA: TREATMENT WITH ASPIRIN AND POSSIBILITY OF PLATELET INVOLVEMENT

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Abstract. The characteristic temperature-dependent pruritus in polycythemia vera (PV) is described. The triggering factor seems to be a sudden decrease in skin temperature, e.g. after a hot bath or shower. The sudden onset and limited duration of the pruritus might suggest an activation or release of some humoral factor(s). In a controlled study we showed that aspirin alleviates this particular pruritus. Therefore, the possibility of prostaglandin and platelet involvement was considered. It was found that substances such as PGE₂ and serotonin, produced and released by platelets, could elicit pruritus in healthy volunteers when injected intradermally and that PGE₂ enhanced the cutaneous responses to serotonin. Studies of platelet aggregation did not reveal any abnormalities in the PV patients but ADP was shown to sensitize platelets to adrenaline-induced aggregation *in vitro*. Although not proven the following hypothesis is suggested: a combination of ADP, emerging from erythrocytes, and catecholamines released from adrenergic vasoconstrictor nerves when the skin is cooled down, might stimulate platelets to aggregation in skin vessels and to production and release of pruritogenic factors.

Key words: Aspirin; Platelet aggregation; Polycythemia vera; Prostaglandins; Pruritus; Serotonin

Pruritus is common in patients with polycythemia vera (PV). It has a rather unique clinical picture. The Polycythemia Vera Study Group (2) observed itching in 43% of their 325 patients. Typically, the pruritus has a pricking character, is induced or aggravated by hot baths or showers and can be so extremely severe and distressing that some patients are unable to expose their bodies to water (see e.g. 4).

As the blood picture normalizes following treatment with phlebotomy and/or chemotherapy the pruritus frequently subsides, but returns on hematologic relapse (26). A correlation between elevated blood and/or urine histamine levels and pruritus in myeloproliferative diseases was observed by Gilbert et al. (4) who also showed that the pruritus was partially or completely suppressed

by cyproheptadine, a histamine and serotonin antagonist.

A clue to further understanding was a clinical impression that aspirin seemed to improve this particular pruritus (Franzén, personal communication). Aspirin is known to inhibit platelet release of dense granules (10) as well as prostaglandin (PG) synthesis in both platelets (22) and various tissues (24). Dense granules contain constituents such as serotonin (27) which might be pruritogenic. Prostaglandins E and PG endoperoxides produced during platelet aggregation (6, 21) can induce and/or potentiate itching (5, 12, 13, 16). Thus, it was conceived that platelets and/or prostaglandins might be involved in the common and often intolerable pruritus occurring in PV.

The present paper first gives a description of the clinical picture of pruritus in PV. Secondly, the therapeutic effect of aspirin was studied according to a double-blind cross-over scheme. Thirdly, in an attempt to explain the pathogenesis of pruritus in PV we studied cutaneous responses (i) to platelet factors such as PGE₂ and serotonin in order to estimate their pruritogenic effect and (ii) to histamine in order to estimate skin sensitivity. We also studied platelet function and various laboratory data.

MATERIAL AND METHODS

Patients

A questionnaire was mailed to 86 patients with PV, registered in 1963-75, and treated and regularly followed up at the Oncologic Department (Radiumhemmet), Karolinska Hospital. The diagnostic criteria were based upon laboratory data and bone marrow aspirations. Patients older than 85 years were excluded. Seventy-two patients, 29 men and 43 women, mean age 68 (43-85) years, responded to the questionnaire.

Eight of the patients as well as 10 more patients referred to us from other clinics with severe, temperature-pro-

Table 1. Characteristics of subjects studied

M=males; F=females

	Cutaneous responses to				Platelet function			
	Aspirin and pruritus Patients	PGE ₂ and 5-HT Healthy volunteers	Histamine		Aggregation		Circulating and spontaneous aggregates	
			Patients ^a	Controls ^b	Patients ^a	Controls ^c	Patients ^a	Controls ^b
No. of subjects	17	12	14	13	14	10	14	13
M	8	2	6	4	6	4	6	4
F	9	10	8	9	8	6	8	9
Mean age (years)	67 (58-78)	28 (19-42)	68 (58-78)	65 (50-75)	68 (58-78)	69 (49-81)	68 (58-78)	65 (50-75)

^a PV patients with pruritus.^b Patients with benign circumscribed non-pruritic dermatoses.^c PV patients without pruritus.

voked pruritus in progress, i.e. a total of 18 patients, were followed up more closely, including personal interviews. Eight of these patients were men, 10 women, with a mean age of 67 (58-78) years. These patients had not obtained treatment for their PV during the past 2 months. Seventeen of the 18 patients took part in a double-blind cross-over study to investigate the possible therapeutic effect of aspirin. Platelet function and cutaneous responses to histamine were examined in 14 of the patients. Routine laboratory studies of blood were performed in all 18 patients, serotonin was analysed in 14 patients and in 9 patients a more extended screening of various laboratory data was carried out. Cutaneous responses to PGE₂ and serotonin were studied in 12 healthy volunteers.

Controls

The control population consisted of 1) a group of 13 patients with benign circumscribed non-pruritic dermatoses (such as leg ulcers) hospitalized at our department, 2) 10 patients with PV without present or past history of pruritus, followed up at the Oncologic Department. For further information, see Table 1.

Questionnaire

In the questionnaire the patients were asked whether they had experienced itching after discovery of their blood disease. They were further questioned about the frequency of the pruritus (daily, weekly, or seldom?), its intensity (moderate, disturbing, intolerable?) temperature dependency (in connection with hot or cold baths/showers?) and relationship to baths or showers (during or after the baths/showers?). The questionnaire also included queries about episodes of thrombosis and hemorrhages.

Aspirin and Pruritus

In the 17 patients selected for the controlled study, aspirin, 500 mg tablets (Magnecyl, ACO, Solna, Sweden) was compared with identically appearing placebo tablets. The

patients received three tablets a day for one week according to a random-order, double-blind, cross-over scheme. The two treatment periods were separated by a drug-free week. The patients were instructed not to take any analgetics or other drugs known to interfere with platelet function for at least one week before the study. These periods were chosen since aspirin inhibits platelet aggregation/release irreversibly and the clinical effect lasts for several days up to one week after discontinuing the therapy (18). The patients were asked to take a hot bath at least three times a week, to keep a daily record of their pruritus and to report which of the two drugs they preferred. The patients were interviewed after each treatment period.

Studies on Experimental Pruritus

Experimental pruritus was provoked by using the same technique as previously described (11, 12, 13). Small volumes, 0.02 ml of the solutions studied, were injected intradermally on the lateral aspect of the upper arms. The duration of the pruritus was recorded and the area of the flare response measured planimetrically after 5 min. PGE₂ (Upjohn Co., Kalamazoo, Michigan, USA) was stored in ethanol (1 mg/ml) at -20°C until use. A sample of the stock solution was taken on the day of each experiment and further diluted in sterile, pyrogen-free physiological saline containing 10% (v/v) Sørensen phosphate buffer (Na₂HPO₄+KH₂PO₄, 67 mM), pH 7.4. Serotonin creatinine sulphate (Sigma, St. Louis, Mo, USA) and histamine (ACO, Solna, Sweden) were dissolved and diluted in buffered saline and passed through a Millipore filter (Millex TM, 0.22 µm) before use. Cutaneous responses were observed for each of the solutions and for a combination of PGE₂ and serotonin.

Studies on Platelet Function

Platelet aggregation studies

Preparation of platelet rich plasma (PRP). Blood was collected from the antecubital vein with a needle directly

Table II. Thrombotic and hemorrhagic episodes correlated to pruritus in 72 PV patients

	Thrombosis	Bleeding
Patients with temperature dependent pruritus (n=38)	10 (26%)	5 (13%)
Patients without temperature dependent pruritus (n=34)	10 (29%)	3 (9%)

into 0.1 vol of 3.8% trisodium citrate. Plastic containers and equipment were used. PRP was prepared by centrifugation at 140 g for 15 min at room temperature. Platelet counts were adjusted to approximately $250 \times 10^9/l$ by dilution with platelet-poor plasma (PPP) obtained by centrifuging the blood, after the PRP had been removed, at 1 900 g for 15 min. Platelets were counted in a counting chamber.

Technique. Platelet aggregation in PRP was performed by recording light transmission in a Payton Dual Channel Aggregometer (Payton Associated Ltd, Scarborough, Ontario, Canada) at 37°C and stirring speed 900 rpm. Agents used to induce aggregation were ADP (Sigma) and adrenalin (ACO) at final concentrations of 0.03, 0.1, 0.33, 1.0, 3.3 and 8.3 μM . In addition, platelets were preincubated with ADP at final concentrations of 0.03, 0.1 and 0.33 μM for 1.5 min prior to addition of adrenalin at the concentrations mentioned above. The preincubation time of 1.5 min was chosen since any ADP-induced primary aggregation had by then been terminated.

Circulating aggregated and spontaneously aggregated platelets

Preparation of platelet rich plasma. Platelets in circulating aggregates formed in vivo and platelet tendency to aggregate (spontaneous aggregation) were measured by modifications of methods described by Holdrinet 1969 (9) and Wu and Hoak 1974 (28). Blood was collected from the antecubital vein and drawn into three plastic tubes containing: 1) 0.1 vol of 3.8% trisodium citrate in 0.4 vol Veronal acetate buffer, pH 7.35; 2) 0.1 vol of 0.1 M EDTA in 0.4 vol of the same buffer; and 3) 0.1 vol of 3.8% trisodium citrate and 0.1 vol of 20% formalin in 0.3 vol of the buffered solution mentioned. The contents were mixed thoroughly. After incubation at room temperature for 15 min the samples were centrifuged at 140 g for 10 min at room temperature in order to prepare PRP. The platelets were counted in a counting chamber.

Principle. Aggregates which are present in vivo at the time of venipuncture, disperse in the presence of EDTA and further aggregation is inhibited. In the presence of citrate+formalin such aggregates are preserved and will sediment during centrifugation. Consequently, the difference in PRP platelet counts in EDTA and in citrate+formalin solutions represents circulating aggregated platelets. The percentage of circulating aggregated platelets can be expressed as follows:

% circulating aggregated platelets =

$$\frac{\text{platelet-count (EDTA)} - \text{platelet-count (citrate+formalin)}}{\text{platelet-count (EDTA)}} \times 100$$

In the presence of citrate some platelets will aggregate during a mild centrifugation and sediment. Therefore, the difference in PRP platelet count in EDTA and in citrate solutions represents platelet tendency to aggregate in vitro ("spontaneous aggregation"). This can be expressed in per cent as follows:

% spontaneously aggregated platelets =

$$\frac{\text{platelet count (EDTA)} - \text{platelet-count (citrate)}}{\text{platelet-count (EDTA)}} \times 100$$

Laboratory Screening

Laboratory data included hemoglobin, hematocrit, white cell count with differential analysis and platelet count. The thorough laboratory screening included in addition: serum bilirubin, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, lactate dehydrogenase, electrolytes, cholesterol and triglycerides, protein electrophoresis, serum complement levels (C₃ and C₄), blood urea nitrogen, serum creatinine, uric acid, cryoglobulins, cryofibrinogen, red cell fragility test, Coomb's test, tests for circulating immune complexes (platelet aggregation (25) and C_{1q}-binding capacity) and prothrombin complex determinations and investigations. In urine, routine analyses and 5-HIAA determinations were performed. In addition, serotonin concentration in whole blood was analysed in 14 PV patients and 9 of their controls, i.e. PV patients without pruritus.

RESULTS

Data from the questionnaire

Fifty-one of the 72 PV patients (71%) reported periods with pruritus. In 13 of these patients the pruritus was not associated with hot baths or showers. Pruritus appearing in connection with hot baths or showers was described by 38 patients (53%), 14 men and 24 women. In 5 of these patients even contact with cold water could elicit pruritus. The pruritus began immediately upon immersion in hot water in 2 patients and started promptly after a hot bath or shower in 28, while 8 patients did not answer this question. Sixteen of the patients (22%) complained of pruritus at the time of the present study and in 12 (17%) the pruritus was severe. Twenty of the 72 patients had a history of thrombosis and 8 of hemorrhages. There was no significant correlation to pruritus (Table II).

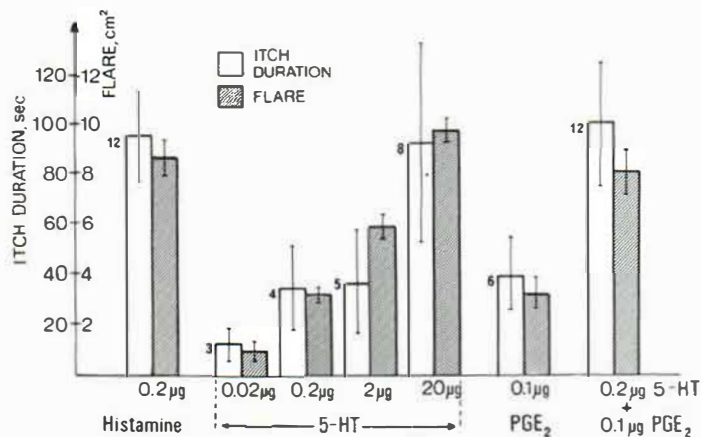


Fig. 1. Itch and flare responses in 12 healthy volunteers after intradermal injections of serotonin (5-HT), PGE₂ and a mixture of serotonin and PGE₂ (mean ± S.E.). Histamine was included as a control. The flare area was measured 5 min after the injections. Figures at the itch-duration columns denote numbers of subjects perceiving itch.

Data from patient interviews

Eighteen patients with severe pruritus elicited by hot baths or showers were examined. Some of the patients could avoid or alleviate pruritus by using colder water. Many patients perceived only insignificant pruritus after a hot bath or shower if they could avoid cooling down abruptly afterwards. One patient was even able to take a sauna without problems—unless he took a cold shower afterwards. The temperature dependency was also emphasized by the fact that some patients noted pruritus when undressing, when getting under the chilly bed clothes at night, or when they felt warm and perspired. Some patients also complained of pruritus on body areas exposed during cold weather. The pruritus seemed to become worse the more abruptly the skin was cooled down.

The pruritus started promptly after hot baths or showers, had a pricking character and lasted for a period of 15–60 min. Two patients reported a longer duration (about 2 hours). The severity of the pruritus varied and in 7 patients it was almost intolerable.

Eight of the patients had experienced the typical temperature-provoked pruritus for 1–10 years before the diagnosis of PV was made. In 6 patients the pruritus started about the same time as the diagnosis of PV was made and in 2 patients it started later. No definite information could be obtained from 2 patients. Seven of the 18 patients had noticed good relief of pruritus when they were treated with phlebotomy and/or chemotherapy. One patient reported no effect of this treatment and in 10 patients it was impossible to draw any retrospective conclusions.

Aspirin and pruritus

Seventeen patients took part in a double-blind study. Fifteen of the 17 experienced a greater alleviation of pruritus when treated with aspirin than with placebo. Two patients preferred the placebo tablets. The difference was statistically significant ($p < 0.01$) according to the sign test (23). Aspirin relieved pruritus partially in 14 patients and completely in one.

Cutaneous responses to prostaglandin E₂ and serotonin

PGE₂, 0.1 µg, when injected intradermally in healthy volunteers produced a bright red flare which gradually subsided and was replaced by a dusky red erythema. Six of the 12 volunteers investigated noticed pruritus at the injection site. Serotonin (0.02–20 µg) produced an erythematous response of the flare type, which disappeared within one hour. Pruritus was experienced by 8 subjects but in 4 subjects pruritus could not be provoked. However, when PGE₂, 0.1 µg, was injected in a mixture with serotonin, 0.2 µg, all subjects felt a marked itching sensation at the injection site. The flare and itch responses were more pronounced when induced by the mixture than by any of the agents given alone (Fig. 1). By interpolation in the dose-response curve for serotonin the flare reaction induced by the mixture of serotonin and PGE₂ was calculated to be equivalent to that induced by 8 µg serotonin and the itch duration corresponded to that induced by more than 20 µg of serotonin. Thus, PGE₂ seemed to enhance the cutaneous responses to serotonin.

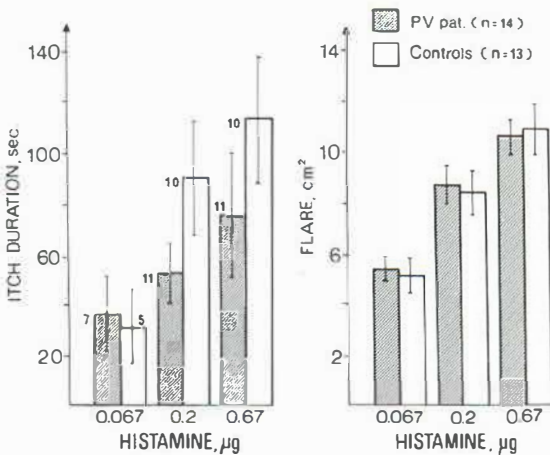


Fig. 2. Dose-response relations for histamine-induced itch and flare in 14 PV patients with pruritus and in 13 control subjects (mean \pm S.E.). Figures above the itch-duration columns denote numbers of subjects perceiving itch.

Cutaneous responses to histamine

In order to establish the proneness to itching in the pruritic PV patients, experimental itch was induced by i.d. injections of increasing concentrations of histamine (Fig. 2). The number of individuals perceiving pruritus increased with increasing concentrations of histamine, but 3 of the 14 patients and 3 of the 13 controls did not feel pruritus after any of the concentrations used. The itch response showed wide interindividual variations without statistically significant differences between patients and controls. An erythematous response of the flare type appeared in all subjects without significant difference between the two groups.

Platelet aggregation

Platelet aggregation in PRP from PV patients with and without pruritus did not differ significantly when ADP and adrenalin were used as aggregating agents (Fig. 3a, b). Moreover, no differences were observed between the two groups when platelets were preincubated with ADP, 0.03, 0.1 and 0.33 μ M for 1.5 min prior to addition of adrenalin (exemplified with ADP 0.33 μ M in Fig. 3c). The areas below the curves were compared and statistically analysed according to the Mann-Whitney U-test (23).

On the other hand, platelets from PV patients with pruritus were found to be significantly sen-

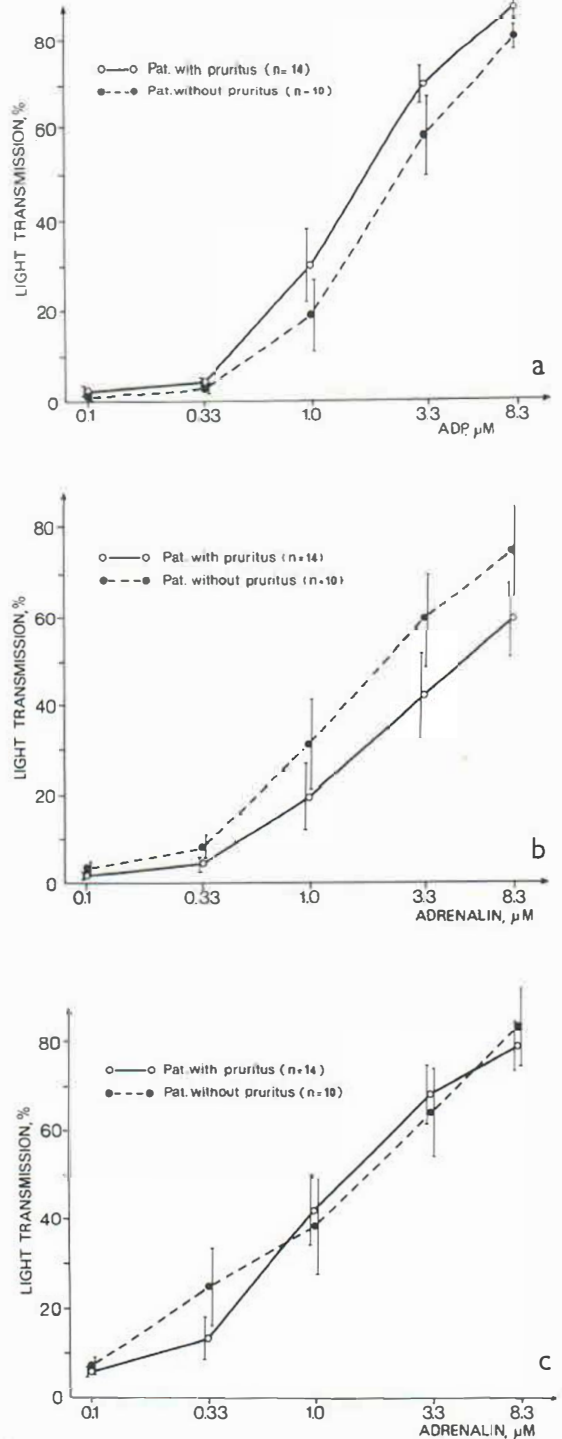


Fig. 3a-c. Platelet aggregation in 14 PV patients with pruritus and in 10 PV patients without pruritus (mean \pm S.E.). Aggregating agents were (a) ADP, (b) adrenalin, (c) adrenalin after preincubation of platelets with ADP, 0.33 μ M for 1.5 min.

Table III. Numbers of patients with an ADP-induced increased/decreased sensitivity to adrenalin-induced aggregation

Statistical analysis was based on changes in the ED₅₀ values

	ADP 0.03 μM	ADP 0.1 μM	ADP 0.33 μM
PV patients with pruritus (n=14)			
Increase	9 ^a	9 ^b	12 ^b
Decrease	2	2	1
No change	3	3	1
PV patients without pruritus (n=10)			
Increase	4	6	7 ^a
Decrease	2	1	1
No change	4	3	2

^a p<0.05.

^b p<0.01.

sitized to adrenalin-induced aggregation after preincubation with ADP, 0.03 μM (p<0.05), 0.1 μM (p<0.01) and 0.33 μM (p<0.01) and platelets from non-pruritic PV patients (controls) were sensitized after preincubation with ADP 0.33 μM (p<0.05). This sensitization can be visualized for ADP 0.33 μM by comparing Fig. 3b with 3c. The statistical evaluations are based on the ED₅₀ values for adrenalin, i.e. the doses of adrenalin inducing 50% platelet aggregation were calculated by interpolation in the dose-response curves. An ADP-induced sensitization will cause a shift of the dose-response curve to the left and thus a decrease in the ED₅₀ value. The ADP-induced changes of ED₅₀ values were analysed with Wilcoxon's matched-pairs signed-ranks test (23) (Table III).

Circulating and spontaneously aggregated platelets

No significant differences were observed between the PV and the control patients (Fig. 4).

Table IV. Laboratory data in 18 PV patients

The blood samples were taken at the first visit to the Department of Dermatology before evaluation of the aspirin effect. M=male; F=female

	Hemoglobin (g/l)		Hematocrit (%)		WBC (×10 ⁹ /l)	Platelets (×10 ⁹ /l)	Basophils (×10 ⁹ /l)
Mean (±S.E.)	M 162±10	F 152±6	M 50±3	F 48±2	10.2±0.8	314±36	0.16±0.04
Reference range	M 140-170	F 120-150	M 42-50	F 37-43	4.0-9.0	150-400	0.04-0.09

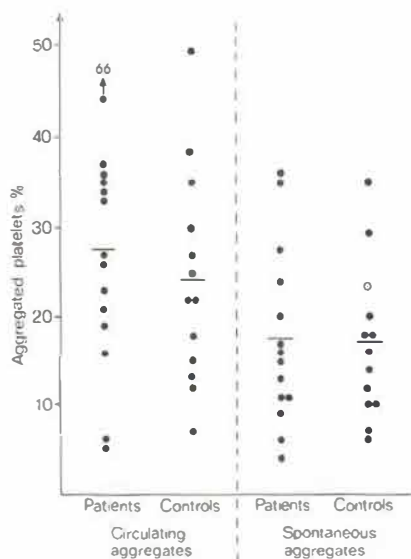


Fig. 4. Circulating and spontaneously aggregated platelets in 14 pruritic PV patients and 13 control subjects. The number of platelets in aggregates is expressed as a percentage of the total number of circulating platelets. For details, see text. The crossbars indicate the geometrical mean of each group.

Laboratory screening

Hemoglobin and hematocrit concentrations around the upper limits of normal were observed as well as a slight leukocytosis and an increased basophil count (Table IV). The thorough laboratory screening gave normal results. No significant difference in serotonin concentration was observed between PV patients with and without pruritus.

DISCUSSION

The pruritus in PV is characteristically temperature dependent. In our study, 71% of the patients answering the questionnaire had experienced periods

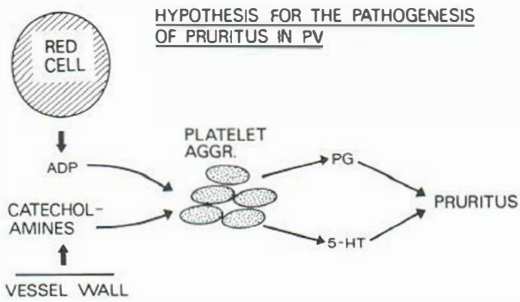


Fig. 5

of intermittent pruritus, which was temperature dependent in 53%. What has not been stressed in earlier reports is that the pruritus in PV starts promptly *after* a hot bath or shower and that it is of fairly short duration, lasting for 15–60 min. The triggering factor seems to be a sudden decrease in skin temperature. We noticed that the pruritus seemed to become worse the more abruptly the skin was cooled down. The clinical features described here are suggestive of a role of some humoral factor(s) released or activated in the skin when it is cooled down.

The humoral factor(s) postulated is probably not histamine as suggested by Gilbert et al. (1966) (4). It has recently been noted that intradermally injected histamine in low concentrations produces erythema without itching (Hägermark, unpublished) and no skin lesions of the urticarial type were observed in our PV patients. Furthermore, antihistamines, with the exceptions of cyproheptadine (4), have not been reported to relieve this type of pruritus. However, histamine cannot be totally excluded due to the known correlation between pruritus and the elevated basophil count (4). An elevated basophil count was also observed in our PV patients.

In a controlled study, aspirin, 500 mg three times a day, was shown to alleviate the pruritus in this particular disease. In our experience, most other pruritic conditions fail to respond favourably to aspirin. This effect of aspirin in PV might mean that it inhibits release or activation of the humoral factor(s) postulated. Since PV is a blood disease and aspirin is known to inhibit platelet aggregation, the involvement of platelets was considered. Aspirin also inhibits prostaglandin synthesis in various tissues (24) and in platelets (22). Prostaglandins E and prostaglandin endoperoxides induce and/or

potentiate itching (5, 12, 13, 16). Of the platelet dense granule constituents, which are released in connection with aggregation, serotonin might be conceived to be pruritogenic. According to Shelley & Arthur (1957) (19) serotonin does not elicit pruritus, but 8 of our 12 healthy volunteers experienced pruritus when serotonin was injected intradermally. Moreover, when serotonin was combined with PGE₂, pruritus was elicited in all subjects studied and the response was more pronounced than that induced by PGE₂ or serotonin alone. The pruritogenic factors in PV might thus be prostaglandins and serotonin produced/released from platelets. It is interesting to recall that cyproheptadine, the only histamine antagonist reported to relieve pruritus in PV (4) also acts as a serotonin antagonist and inhibits platelet function *in vitro* (14).

In vitro, no functional platelet abnormalities were detected with respect to aggregation. This may reflect a discrepancy between platelet function *in vitro* and *in vivo* (7). The platelet count in peripheral blood was normal, which does not exclude the possibility of an increased total number of platelets (15). The platelets responded normally to the aggregating agents ADP and adrenalin. The number of circulating and spontaneously aggregated platelets seemed to be normal. Moreover, the concentration of serotonin (mainly located in platelets) in blood did not differ between PV patients with and without pruritus and 5-HIAA determinations in urine were normal. A suspicion of a decreased itch threshold in the skin could also be eliminated, since skin sensitivity studied by histamine injected intradermally in small volumes was normal.

However, we found that ADP sensitized the aggregation provoked by adrenalin in PV patients with pruritus. In controls, i.e. PV patients without pruritus, this was observed only for the highest ADP concentration. It has previously been demonstrated that preincubation with catecholamines potentiates ADP-induced aggregation (1, 17). ADP in blood is located mostly in red cells and there is a possibility that ADP can be released from red cells without hemolysis (3). A significant role of ADP released from erythrocytes is indicated by the fact that platelet adhesiveness is proportional to the hematocrit value of the blood (8, 20). Furthermore, since pruritus in PV typically appears when the skin is suddenly cooled down, vasoconstriction might be involved in some way.

Although still not proven, we suggest that the following sequence of events might lead to pruritus in PV (Fig. 5). When the skin is cooled down the adrenergic vasoconstrictor nerves release catecholamines from the nerve endings. Some of the transmitter substances might diffuse into the vascular lumen and, in combination with ADP from red cells, stimulate platelets to produce and release pruritogenic factors such as PGE₂ and serotonin.

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