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Platelet aggregation and multiple sclerosis

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Measurements of blood platelet aggregation were carried out in 30 patients suffering from multiple sclerosis (MS) and in 15 healthy individuals. Compared with the control group, the MS patients showed an increase in both spontaneous and induced (ADP and serotonin) platelet aggregation. The possible pathogenetic significance of these results is discussed.

Key words: Increased platelet aggregation – frequency and possible pathogenetic value in multiple sclerosis.

The perivenous distribution of MS plaques has been known since the pioneering study of *Rindfleisch* in 1863. Since then, various authors have described thromboses of the cerebral veins situated at the centre of MS plaques. These findings, as well as the results of his own investigations, led *Putnam* (1937) to formulate his vascular hypothesis for multiple sclerosis. In this, the venous thromboses – which *Putnam* had shown to contain platelets – were interpreted as a consequence of abnormal blood clotting in the vascular system of MS patients (*Putnam* 1937).

In the intervening period, many studies have been carried out on the platelets of MS patients, in particular measurements of platelet adhesiveness. In contrast, platelet aggregation has only rarely been investigated.

The following is a report on our own measurements of platelet aggregation in MS patients and control subjects.

MATERIAL AND METHODS

MS patients and control subjects

Measurements of platelet aggregation were performed in 30 MS patients (21 women and 9 men), aged between 16 and 61 years (average age 40.2 years).

In all cases, the patients were known to have a "definite MS" according to the criteria of *Rose et al.* (1976). In order to preclude the possibility of interference with platelet behaviour, the following criteria were applied to exclude patients from the study: migraine, current infection, arteriosclerosis, pregnancy, major surgery or myocardial or cerebral infarction within the last

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year, treatment with inhibitors of platelet aggregation, treatment with corticosteroids and/or immunosuppressive agents within the last 6 months.

The control group consisted of 15 healthy subjects (6 women and 9 men), aged between 22 and 63 years (average age 38.6 years). The same exclusion criteria applied to this group as to the MS patients.

Measurements of platelet aggregation

Measurements were made of both spontaneous platelet aggregation (aggregation without the addition of aggregation-inducing agents) and induced platelet aggregation (aggregation after the addition of aggregation-inducing agents).

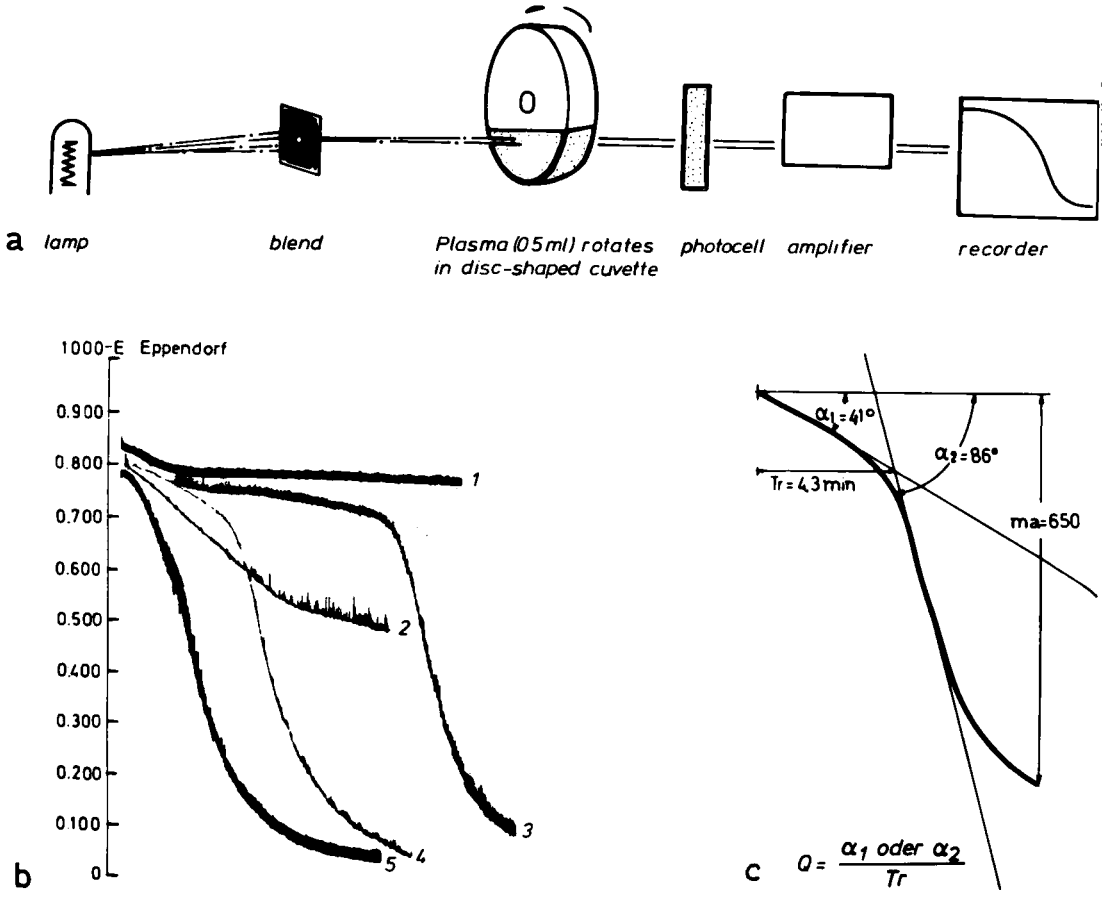

Fig. 1. (a) Principle of photometric PAT III; (b) examples of PAT III curves; (c) evaluation of PAT III curves (Breddin et al. *1977*).

Spontaneous platelet aggregation was measured using the photometric platelet aggregation test (PAT III) of *Breddin et al.* (1977).

Principle: a small quantity of platelet-rich plasma (PRP) is rotated in a disc-shaped plastic cuvette. The light beam of a photometer is directed through the plasma sample, so that changes in optical density caused by the formation of platelet aggregates can be continuously registered using suitable recording equipment (Fig. 1).

For evaluation of PAT III curves the following parameters can be measured:

(1) angle α_1, between the horizontal line and the tangent of the transmission curve from the start of rotation;

(2) angle α_2, between the horizontal and the tangent of the steepest part of the aggregation curve;

(3) reaction time T_r from the start of rotation to the onset of maximal aggregation;

(4) angle α_2/T_r relation (quotient Q); if there was no maximal aggregation (α_2 missing) α_1 values were arbitrarily divided by 10, since registration was stopped after 10 min.

The following 3 gradations were used:

no spontaneous aggregation (normal finding): α_1 and α_2 less than 40° and quotient Q less than 10;

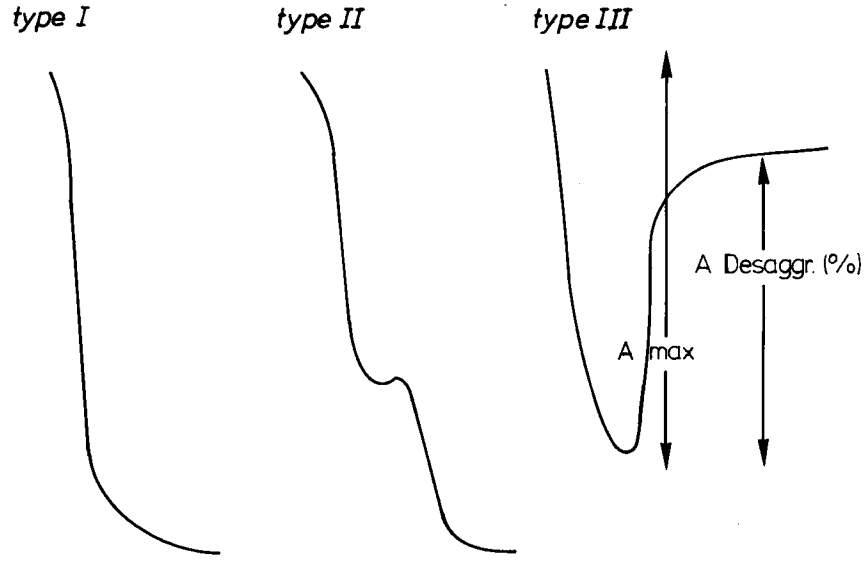

Fig. 2. ADP- and serotonin-induced aggregation curves: type 1 curve = irreversible aggregation without disaggregation; type II curve = irreversible aggregation after disaggregation; type III curve = reversible aggregation.

moderate tendency to spontaneous aggregation: α_1 or α_2 greater than 40° and/or quotient Q greater than 10;

strong tendency to spontaneous aggregation: α_1 or α_2 greater than 60° and/or quotient Q greater than 20.

Induced platelet aggregation was measured using the photometric method of *Born & O'Brien,* as modified by *Breddin et al.* (1977).

Normally the addition of ADP to PRP leads to aggregation with subsequent disaggregation, the degree of disaggregation exceeding 40 % (type III curve, Fig. 2).

On the suggestion of *Krzywanek et al.* (1977), the following were taken as indicative of increased ADP-induced aggregation: appearance of a 2nd aggregation after previous disaggregation, with the degree of disaggregation being less than 40 % (type II curve, Fig. 2) or irreversible aggregation without previous disaggregation (type I curve, Fig. 2).

In man, the addition of serotonin to PRP normally leads to minimal aggregation with more or less complete disaggregation (*Gordon et al.* 1978). The following was regarded as indicative of increased serotonin-induced aggregation: appearance of a 2nd aggregation wave, with no regard to the

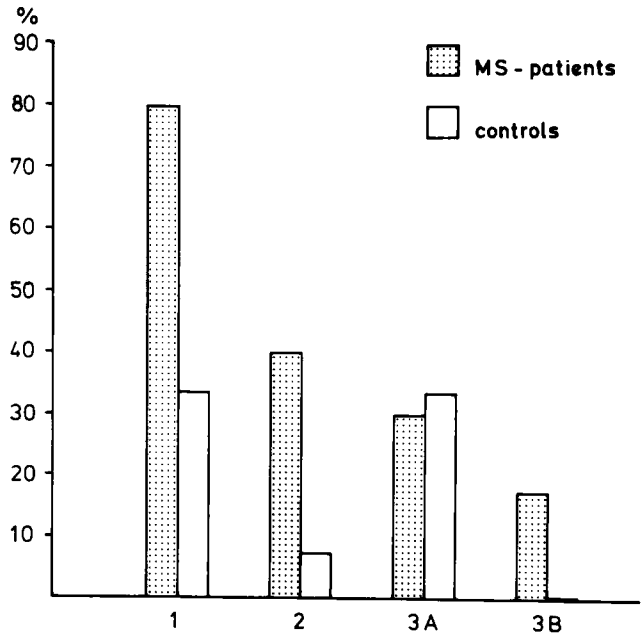

Fig. 3. Results of the platelet aggregation measurements in patients and control subjects. 1 = increased ADP-induced aggregation; 2 = increased serotonin-induced aggregation; 3A = moderate, 3B = strong tendency to spontaneous aggregation.

degree of disaggregation, or reversible aggregation without previous disaggregation.

All measurements of platelet aggregation were carried out at an identical time after blood sampling, both in the patient group and in the control group. The final concentration of ADP in PRP was 10^{-6} M; the final concentration of serotonin in PRP was 5×10^{-6} M. The aggregation curves were recorded for periods of not less than 10 min. Details of the experimental conditions and possible sources of error can be found in the study of *Breddin et al.* (1977).

RESULTS

ADP-induced platelet aggregation was increased in 80 % of the MS patients compared to about 30 % of the control group; this difference was statistically significant ($P < 0.01$).

Serotonin-induced platelet aggregation was increased in 40 % of the MS patients, and in less than 10 % of the control group; this difference was highly significant ($P < 0.001$).

There was a moderate tendency to spontaneous aggregation in about 30 % of both the MS patients and the control group. A strong tendency to spontaneous aggregation was seen in about 15 % of the MS patients, but in none of the control subjects. The difference as regards spontaneous aggregation was only significant at the level $P < 0.2$.

Out of the 30 MS patients under investigation, 6 were suffering from an acute exacerbation of the disease at the time of measurement. ADP- and serotonin-induced platelet aggregation was increased in all 6 of these patients; a strong tendency to spontaneous aggregation was seen in 4 out of the 6 patients, while the remaining 2 showed no tendency to spontaneous aggregation.

The results of the platelet aggregation measurements in the patient and control groups are graphically compared in Fig. 3.

Statistical analysis was performed using the 2×2 χ^2 test.

DISCUSSION

These results indicate that compared with clinically healthy subjects, the MS patients showed a (significantly) increased ADP- and serotonin-induced platelet aggregation. An increased tendency to spontaneous aggregation of the blood platelets of MS patients could also be demonstrated. It is worth noting that ADP- and serotonin-induced platelet aggregation was increased in all patients suffering from an acute exacerbation of the disease. However,

since so few patients experienced an exacerbation, it is not possible to put a statistically definitive interpretation on the above point.

Our findings are in agreement with the results of previous studies on platelet adhesion in MS patients. All authors found an increased adhesiveness of the blood platelets (*Nathanson & Savitsky* 1952, *Caspary et al.* 1965, *Wright et al.* 1965, *Dohnal et al.* 1970, 1971), together with a regular correlation between the extent of increased adhesiveness and the activity of the disease process. In no case was a correlation with the duration of the disease reported. *Millac* (1967) described a correlation to measurable disability. Through the addition of "encephalitogenic factor" to the plasma of MS patients during acute exacerbations, *Field & Caspary* (*Field & Caspary* 1964, *Caspary & Field* 1967) succeeded in producing an increase in platelet adhesiveness (this effect was absent in patients with no exacerbation) and in inducing the release of serotonin from the platelets.

In studies in 36 MS patients, *Couch & Hassanein* (1977) found a significantly increased ADP-induced platelet aggregation, as compared to a control group. In this case the degree of increase in aggregation was particularly pronounced during or shortly after an exacerbation.

The results of adhesion and aggregation studies in MS patients may be interpreted differently. *Nathanson & Savitsky* (1952) for instance, regard the increased adhesiveness of the blood platelets of MS patients as a secondary phenomenon, since they were able to demonstrate similar increases in patients with Guillain-Barré syndrome, head trauma or cerebral tumours. Concerning this question, *Millar et al.* (1966) suggest that the increased adhesiveness of platelets from MS patients may be the outcome of the breakdown of neural tissue. The breakdown may give rise to a liberation of basic protein in the blood stream (where it may complex with lipoproteins) and/or to the liberation of proteinases, which may degradate the surface membrane of the platelets. In contrast, *Field & Caspary* (1964) and also *Sharp* (1965), discuss the possibility that increased platelet adhesion in MS patients could lead to thromboses of small cerebral veins, and thus to accelerated plaque formation. According to *Couch & Hassanein* (1977), it is possible that substances released during the "platelet release reaction" – such as serotonin, peptides and lysosomal enzymes – could exert an influence on the formation and spread of plaques in the brain. Other causes under discussion for the platelet alterations found in MS patients include: changes in the lipid composition of the serum (*Baker et al.* 1964) and/or of the platelet membrane (*Gul et al.* 1970), as well as antigen-antibody reactions affecting the aggregation behaviour of the blood platelets (*Caspary & Field* 1967).

A completely new hypothesis was formulated by *Srivastava et al.* (1975). These investigators found a reduced synthesis of the prostaglandins PGE_1 and PGE_2 in the platelets of MS patients. Platelet aggregation is inhibited

by PGE_1 and stimulated by PGE_2. Since the inhibitory effect of PGE_1 is roughly 10 times greater than the stimulating effect of PGE_2, the reduced synthesis of the aggregation-inhibiting PGE_1 is of greater consequences, with the net result being an increased platelet aggregation. These authors do not exclude the possibility that viruses contained in the platelets might be responsible for the disturbed prostaglandin synthesis. Several viruses are known to possess the ability to invade thrombocytes (*Danon et al.* 1959, *Schulz & Landgräber* 1966).

In summary, the following statements can be made:

(1) Neither the results of our own investigations nor of any previous studies provide a conclusive answer to the question of whether the platelet alterations in MS patients are epiphenomena of multiple sclerosis, or whether they are independent, pathogenetically relevant phenomena.

(2) It is possible that the increased platelet aggregation found in MS patients could – even if not the primary cause of the disease – exert a contributory influence, via cerebrovascular pathologic mechanisms, on the formation and spread of MS plaques, and thus on the disease process. This would, for example, provide a possible explanation for the ability of "stress situations" to bring on exacerbations of the disease.

(3) If these results were to be confirmed in a greater number of MS patients, then, in our opinion, it would be justified to use inhibitors of platelet aggregation in an attempt to treat patients with multiple sclerosis.

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