Blood platelet behaviour in syphilis

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Blood platelets carry a negative surface charge (Abramson, 1928) and so changes in platelet behaviour may result from changes in this charge. The fact that the migration of platelets in an electric field can be measured by microelectrophoresis was shown by Bangham, Flemans, Heard, and Seaman (1958), using an apparatus which consisted of a capillary tube with platinum electrodes fitted to each end. This capillary tube was filled with plasma rich in platelets.

Using essentially the same technique, Hampton and Mitchell (1966a) showed that small concentrations of adenosine diphosphate (ADP) and noradrenaline increase the electrophoretic mobility of platelets and also that there is a characteristic change in this response pattern in certain pathological conditions. This paper reports typical abnormalities of blood platelet electrophoretic mobility pattern in cases of early syphilis, which are also observable, though to a lesser degree, in late syphilis. After penicillin these abnormal patterns reverted to normal.

Material and methods

The electrophoretic apparatus used for this work was described by Bangham and others (1958), and was manufactured by Rank Bros., Cambridge.

PLATELET-RICH PLASMA (PRP) AND PLATELET-POOR PLASMA (PPP)

If citrated blood is slowly centrifuged at room temperature it is possible to obtain plasma rich in platelets, but containing negligible numbers of red cells or white cells. After removing this supernatant platelet-rich plasma from just above the layer of red and white cells, the remaining blood can be further centrifuged at high speed to obtain plasma poor in platelets.

In the present work PRP was obtained by centrifuging citrated venous blood samples at 150 g. for 10 minutes, and PPP by further centrifugation at 750 g. for 15 minutes. The PRP was then diluted 1 in 10 with PPP and the mixture allowed to stand at room temperature for 1 hour to allow for the decrease in platelet mobility which takes place during the first hour after centrifugation (Hampton and Mitchell, 1966a). The initial electro-

phoretic mobility was then measured. Following this ADP or noradrenaline (both from Sigma Chemical Co.) dissolved in 0.85 per cent. saline solution was added to the PRP/PPP mixture. The mixture was incubated at 25°C. for 10 minutes and the platelet mobility measured again. In each case the effects of ADP and noradrenaline were tested in the two most characteristic concentrations recommended by Hampton and Mitchell (1966b), *i.e.* 0.005 and 0.05 µg./ml. The mobility values after exposure to ADP or noradrenaline were compared with the initial mobility and expressed as a percentage of the initial value.

FIG. 1 Mean platelet electrophoretic mobility changes induced by ADP or noradrenaline in patients with gonorrhoea and in healthy control subjects

TESTS FOR SYPHILIS

Syphilis was diagnosed either by dark ground microscopy in early lesions, or by positive results to serological tests. (In the control patients with gonorrhoea the disease was diagnosed by both microscopical examination of stained urethral smears and by cultures.)

Results

Fig. 1 shows the mean platelet electrophoretic mobility changes induced by ADP or noradrenaline in eight patients with untreated gonorrhoea and in eight age and sex-matched healthy control subjects. These mobility curves show that both groups of subjects had a basically similar pattern of platelet electrophoretic mobility. This type of pattern is characterized by a smaller increase in mobility after the addition of 0.005 $\mu\text{g./ml.}$ of either of the two aggregating agents, ADP or noradrenaline, and a greater rise after a 10-fold increase in the concentration of either of these agents. According to the workers who investigated a large number of people by the same method, this type of biphasic dose-response curve can be found in the healthy section of the population and so can be called 'the normal' (Hampton and Mitchell, 1966c).

FIG. 2 Mean platelet electrophoretic mobility changes induced by ADP or noradrenaline in patients with early syphilis before and after treatment

Fig. 2 shows the mean platelet electrophoretic mobility changes induced by ADP or noradrenaline in seven patients with early syphilis before and 4 weeks after the completion of treatment with procaine penicillin 600,000 units intramuscularly daily for 10 days. The mobilities measured before treatment show a 'shift to the left' in the maximum mobility after the addition of ADP at 0.005 $\mu\text{g./ml.}$ concentration, and also a marked decrease in mobility at 0.05 $\mu\text{g./ml.}$ concentration of this agent. In contrast, the addition of the aggregating agent noradrenaline did not result in such a change.

The dose-response diagrams obtained in eight patients with late syphilis (Fig. 3) show that the curve after ADP administration was still somewhat abnormal, although it had a tendency to return towards the normal biphasic pattern. Treatment with procaine penicillin 600,000 units intramuscularly daily for 10 days brought about complete restoration to normal.

FIG. 3 Mean platelet electrophoretic mobility changes induced by ADP or noradrenaline in patients with late syphilis before and after treatment

Further progress and discussion

Bolton, Hampton, and Mitchell (1967) showed that abnormal platelet behaviour in vascular disease can be transferred to normal platelets by the plasma

from subjects with such disease. Based on these findings, as a next step in the present work, PRP from healthy control subjects was diluted with PPP from patients with early syphilis. The degree of dilution was as follows: 3 ml. abnormal PPP was added to a 1 in 6 mixture of normal PRP-PPP. Altogether three transferring experiments were carried out on three pairs of subjects, and the results are shown in the form of mean values in Fig. 4. It will be seen that the abnormal sensitivity to ADP in syphilis can be transferred to normal platelets by a factor in the plasma.

FIG. 4 Effect of ADP on normal platelets suspended in normal or abnormal plasma

Bolton and others (1967) proposed that the abnormality in the platelet behaviour of patients with atherosclerosis is due to a disorder of plasma phospholipid pattern, or more precisely to an enzyme defect resulting in an abnormal form of plasma lecithin. On the other hand, treponemes contain phospholipids which are related to those in mammalian tissues (Vaczi, Kiraly, and Rethy, 1966). Wright, Doniach, Lessof, Turk, Grimble, and Catterall (1970) proposed that these phospholipids could be responsible for some of the antibodies which cross-react with tissue extracts in the classical serological tests for syphilis, as well as being re-

sponsible for the newly discovered antibody in early syphilis: cardiolipin F. The possibility that the same factor is also responsible for the abnormal platelet behaviour is suggested and that could very well explain the similarity of this abnormal behaviour in such widely different conditions as vascular disease and syphilis. If this is so, then the fact that platelet behaviour abnormalities returned to normal even in cases of late latent syphilis (Fig. 4) after treatment with penicillin makes it doubtful whether the treponeme-like structures found in various tissues in cases of late syphilis (Lancet, 1970) are in fact viable *T. pallidum* organisms still capable of producing disease.

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Summary

The electrophoretic mobility of platelets has been studied in normal subjects, and in cases of early and late syphilis before and after treatment with penicillin. Altered mobility patterns in syphilis were restored to normal after treatment in both early and late cases. The changed mobility patterns were thought to be related to the presence of phospholipids in treponemes.

Comportement des plaquettes sanguines dans la syphilis

SOMMAIRE

On a étudié la mobilité électrophorétique des plaquettes chez des sujets normaux et chez des malades atteints de syphilis récente ou tardive, avant et après traitement par la pénicilline. Dans la syphilis, les troubles de la mobilité revinrent à la normale après traitement, aussi bien dans les cas récents que dans les cas tardifs. Les changements de la mobilité sont considérés comme dus à la présence de phospholipides dans les tréponèmes.