Effects of warfarin on blood rheology in navicular disease

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A preliminary investigation has been undertaken of blood rheology in horses and ponies, its variation in navicular disease and the changes following treatment with warfarin. Erythrocyte flexibility, measured by a centrifuge packing technique, is higher in horses (30 per cent min⁻¹) than in ponies (23.8 per cent min⁻¹). There are corresponding differences in blood viscosity. The high erythrocyte flexibility in horses is caused by an unknown factor present in plasma. The erythrocyte flexibility in horses with navicular disease is even higher, at 38.5 per cent min⁻¹. Treatment with warfarin reduces the flexibility to just below the normal value. A significant fall in plasma viscosity and erythrocyte flexibility was found after treatment of four ponies with 6 mg warfarin daily for one week.

BLOOD rheology is the study of the flow properties of whole blood and its various constituents, including the shape and deformability of red blood cells. Although this discipline has become established in human medicine over the last 30 years, little reference has been made to it in veterinary medicine and only passing reference has been made to animal rheology in haematological journals. Rheological changes in blood are well recognised as being complicating factors in human diseases, such as sickle cell anaemia and diabetes mellitus.

This paper represents the first comparable report of significant rheological changes, and their correction, occurring in an equine disease. As this field is unfamiliar to many veterinary readers, a brief description of the techniques used, and a definition of the units, is given below.

The forelimb lameness in horses, known as navicular disease, was probably first described by Jeremiah Bridges (1752). It was at one time given the descriptive name ‘navicular joint disease' by Turner (Youatt 1836). Early in the development of the disease it becomes clinically evident when trotting as a shortening of the forelimb stride, the feet being placed on the ground toe first. The condition is nearly always present in both forelimbs and is slowly progressive. About one third of all cases of chronic forelimb lameness in horses in England has been attributed to navicular disease (Colles 1982a). From descriptions of the disease by Bridges (1752), Moorcroft (1819) and Turner, it was generally accepted that the lameness involved ulcerations and erosions of the fibrocartilage of the navicular bone and lesions of the deep digital flexor tendon. However, over the last decade Colles (1979a) has shown that cartilage erosion is seldom the cause of lameness and tendon lesions are not the decisive factor.

As long ago as 1885, Walley suggested that the disease might be caused by a circulatory disturbance, as more recently have Hickman (1964) and Nemeth (1972). In 1977, Colles and Hickman showed that arterial thrombosis is a common pathological change in navicular disease and suggested it led to ischaemic necrosis. Recent investigations have established (Colles 1983) that the clearance time of a contrast agent in blood flowing through the feet is extended from about 12 seconds in normal animals, to one to two minutes in cases of navicular disease, and Svalostoga and Smith (1983) have shown a raised subcortical bone blood pressure in diseased cases. This suggested that navicular disease may in part be due to venous congestion, producing a rise in blood pressure, which in turn initiates secondary arterial disease.

Current treatment is aimed at improving blood flow by correct shoeing, regular exercise and anti-coagulant therapy with warfarin. Following the introduction of warfarin treatment by Colles (1979b), 82 per cent of treated horses were able to return to work and 61 per cent have remained sound for four years (Colles 1983). A number of treated horses have been free of the disease and working for five to eight years.

Little is known of the pharmacokinetics of warfarin in the horse. As a number of cases have responded satisfactorily with low doses, in which the one-stage prothrombin time would not have been considered significantly lengthened in man, it was decided to investigate the possibility that the improvement in blood flow was due to a modification of the rheological properties of blood. Colles (1982b) had noted a small, but significant, drop in plasma viscosity following treatment with warfarin. Because of the sparsity of rheological data on horses a preliminary
Materials and methods

Horses

The following groups of horses were used in this study: (1) nine mature healthy horses, comprising three thoroughbreds, three crossbred horses and three crossbred ponies; (2) two crossbred horses with chronic navicular disease; (3) six crossbred horses under treatment with warfarin for navicular disease; (4) four healthy crossbred ponies which were each given 6 mg warfarin by mouth daily for five days to assess the short term changes in blood rheology following warfarin administration; (5) seventy-seven horses of mixed breeds, which were used to assess plasma viscosity before and after warfarin treatment.

Blood sampling

Blood was obtained by jugular venepuncture and anticoagulated with lithium heparin at a concentration of 12.5 iu ml$^{-1}$ of blood. All rheological measurements were undertaken within six hours of blood collection.

Rheological techniques

Viscosity can be regarded as the internal friction within a fluid that restrains laminar flow and its effect is present whenever relative motion exists between adjacent layers of a liquid. The coefficient of viscosity, η, is the constant of proportionality between the force applied to generate flow and the ratio of the resultant difference in velocity of two adjacent fluid layers, of unit area, to their distance apart. The ratio of the difference in velocity to distance apart is called the ‘shear rate’. If the force is measured in dynes cm$^{-2}$, the velocity in cm sec$^{-1}$, and the distance apart in cm, the coefficient of viscosity is given in units of ‘poise’ dyne sec cm$^{-2}$. In SI units, with the force per unit area in Pascals (kg m$^{-1}$ sec$^{-2}$) and the velocity in m sec$^{-1}$, the coefficient of viscosity is given in units of Pascals.sec (Pa.sec). One poise is equal to 0.1 Pa.sec. The coefficient of viscosity of water at 20°C is 0.01 poise, or one centipoise (cp), which is the most common unit in international use, and adopted in this paper.

With simple fluids, such as water, η does not vary with the flow rate, more accurately shear rate, and the flow behaviour is termed ‘Newtonian’. Complex fluids such as blood exhibit non-Newtonian behaviour, the viscosity, η, increasing as the flow rate (shear rate) falls. This is believed to be mainly due to additional Rouleaux formation and aggregation between red cells at slow flow rates.

There are two current methods of measuring blood viscosity; by monitoring the flow rate of blood through a capillary tube when subject to a constant driving pressure or by placing a small volume of blood between two surfaces rotating relative to each other and assessing the associated drag. A fluid flowing through a tube has zero velocity at the vessel wall and maximum velocity at its centre. If the fluid is Newtonian, the variation of velocity with radial distance is parabolic and the flow obeys Poiseuille’s law. The difference of velocity between two adjacent fluid layers thus varies across the tube, i.e., the shear rate varies. Hence capillary viscometers can only be used to obtain accurate measurements of viscosity with Newtonian fluids, which only pertains with blood at high shear rates.

The majority of the following results were obtained using a Coulter-Harkness capillary viscometer at 37·5°C. As designed, the pressure of the mercury column in the Harkness capillary viscometer could not be sufficiently reduced to give flow rates with a mean shear rate of 200 to 250 sec$^{-1}$, comparable to measurements with the Wells-Brookfield rotational visometer discussed later. This difficulty was simply overcome by applying a small back pressure to the mercury column outlet to the air through pressure tubing connected to a sealed plastic bottle which could be squeezed by a clamp. The clamp was tightened just sufficiently to reduce the blood flow to a mean shear rate of about 230 sec$^{-1}$. At this flow rate it was established that the blood of horses is effectively Newtonian and hence its viscosity, relative to plasma, could be accurately assessed.

In principle rotational viscometers have a geometric construction that permits the fluid to be subject to a uniform shear rate. The variation of the coefficient of viscosity with shear rate can then be ascertained by using different speeds of rotation. This is important in situations of slow flow and high η, such as occurs during flow through large veins in some disease states. A Wells-Brookfield cone-on-plate rotational viscometer was used in an attempt to obtain this information for horses’ blood. The geometric construction of this viscometer is basically that of an inverted cone, point downwards, which can be rotated just above a flat plate.

While this method gives values with normal human blood, at high shear rates, about 5 per cent higher than the capillary technique, with horses’ blood and plasma the results were erratic. On occasions, measurements of plasma viscosity on the Wells-Brookfield were more than twice the value obtained...
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with the Coulter-Harkness viscometer. Preliminary investigations suggested that there is an artefact when using rotational viscometers without a guard ring, owing to the formation in horses' blood of a relatively viscous film at the air-plasma interface. No simple method of avoiding this effect in the Wells-Brookfield viscometer was discovered. The viscosity of whole blood varies with haematocrit in a semi-logarithmic manner and the values of viscosity have been standardised to a fixed haematocrit, by the procedure given in the results section.

In addition to the influence of haematocrit and plasma viscosity, the viscosity of blood depends on the shape and flexibility of red blood cells. The latter factor is a measure of the viscoelastic properties of the cells and is ascertained by measuring the rate at which the cells are deformed when subjected to stress. In the present case this factor was measured using the stroboscopic recording method of Amin et al (1983). This technique has the advantages that no additional preparation or washing of the cells is involved and only 0.05 ml of blood is required. In principle it relies on the fact that at a haematocrit of 35 to 42 per cent randomly oriented red blood cells must be in contact with each other. During centrifugation of blood, at a haematocrit of greater than 35 per cent, the separation of cells and plasma is brought about by the weight of cells, one on top of the other, deforming and squashing the cells at the bottom of the centrifuge tube. The less flexible are the cells, the slower is the rate of packing and rigid cells cannot be made to pack at all. The rate cells pack in whole blood, under a constant acceleration of 200 g, is recorded photographically, using stroboscopic illumination. The rate of packing, in per cent min \(^{-1}\), is ascertained from the recorded change of the length of the red cell column with time. To allow for variations of the individual haematocrits, measured at 13,000 g, a calibration curve of the packing rate with haematocrit was obtained for each breed and the quoted packing rates have been corrected using these curves to a standard haematocrit of 45 per cent. The stress applied to pack the cells by centrifugation also depends on the difference of cell to plasma specific gravities. The small variations of this factor between different horses, and man and horse, had no significant effect on the packing rate.

**Plasma fibrinogen, haematocrit and mean corpuscular volume**

The fibrinogen concentration was estimated using the thrombin clot technique of Rampling and Gaffney (1976). The haematocrit was obtained by centrifugation on a Hawksley microhaematocrit centrifuge, at 13,000 g for three minutes. The red cell count was measured using a Coulter counter. Values of the mean corpuscular volume (MCV) were calculated by dividing the haematocrit by the cell count.

**Results**

The observed rheological parameters for horses and ponies are shown in Table 1. The erythrocyte flexibility was very high, the mean packing rate for horses being 30 per cent min \(^{-1}\) and for ponies 23.8 per cent min \(^{-1}\), compared with about 7 per cent min \(^{-1}\) for man. The increased flexibility in horses is associated with an unknown factor in horse plasma (Amin and Sirs 1982). If human red cells are suspended in horse plasma their packing rate is increased to 31.5 per cent min \(^{-1}\). The factor can be removed from red cells by repeated washing and resuspension in Ringer Locke solution. The packing rate for horse erythrocytes is then 4.0 per cent min \(^{-1}\) and for human cells 1.2 per cent min \(^{-1}\). If horse plasma is defibrinated, by incubation at 56°C, and washed horse cells resuspended in serum, there is no effective change of the packing rate. This suggests that the factor in plasma is not fibrinogen. An increase of erythrocyte flexibility should in principle lower the whole blood viscosity at high shear rates (Chien et al 1967). If allowance is made for the different haematocrits, by using the relationship:

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(\eta_r)^H = (\eta_r)^H_s
\]

where \(\eta_r\) is the relative viscosity (ie, blood viscosity/
due to the different shape of horse and human erythrocytes. By progressively increasing the back-pressure in the capillary viscometer, and decreasing the flow, the viscosity of blood at shear rates down to 20 seconds could be estimated. At this slower flow it was observed that the viscosity of horses' blood was nearly twice that of human blood, at an haematocrit of 45 per cent and 37 °C.

In two untreated horses with chronic navicular disease, the erythrocyte flexibility was significantly elevated to the same level of 38·5 per cent min⁻¹. The flexibility values for six horses with navicular disease, treated with warfarin, were 23·1, 30·7, 28·8, 26·0, 35·2 and 24·1 per cent min⁻¹. The values were significantly higher than normal in untreated horses and lower after treatment. A similar significant reduction of plasma viscosity has been observed following treatment with warfarin. Before treatment the mean plasma viscosity of 77 horses was 1·62 cp, with a standard deviation of 0·10. Following four weeks treatment with warfarin the mean plasma viscosity was 1·56 ± 0·08. The difference is statistically significant at a probability level of less than 0·1 per cent.

To obtain some indication of how quickly and to what extent rheological changes occur, and to establish if the effect of warfarin only occurred in animals with navicular disease, a preliminary trial was undertaken with four control ponies. Measurements of blood and plasma viscosities, erythrocyte flexibility, plasma fibrinogen level, haematocrit and MCV were made just before treatment. Each animal was then given a daily dose of 6 mg warfarin by mouth. Measurements of the rheological parameters were made 48 hours later and then on each of the following five days. The values for each parameter for the pony with the most systematic variation is shown in Table 2. The other three ponies had similar variations but with wider fluctuations during the first three days of treatment. In particular, the flexibility rose transiently in two ponies before falling below its initial level. By the sixth day of the trial all the ponies were showing comparable changes in their blood rheology. The mean percentage changes of the haematocrit, plasma viscosity, blood viscosity, relative viscosity (ln [ηr] H⁻¹), fibrinogen concentration, erythrocyte flexibility and MCV were +0·5, -11, -8, +6, +4·5, -15 and -1 per cent respectively. The change of plasma viscosity was statistically significant with P<0·05 and the decrease of flexibility at P<0·01.

There was an associated increase of (ln [ηr]) H⁻¹, with decrease of flexibility, but with the small number of ponies it was not possible to show that it was statistically significant. The differences between the other factors were not significant. In another experiment horse and human cells were incubated with warfarin at 37·3 °C for several hours with no significant rheological changes relative to controls without warfarin.

### Discussion

It has been known for some time that the blood rheology of horses is unusual in several respects (de Haan 1918, Fahraeus 1921). There are significant differences in erythrocyte aggregation, Rouleaux formation and plasma protein composition. Higher aggregation increases blood viscosity at low shear rates. Rouleaux formation requires a change of cell shape, the biconcave surfaces of the cells being flattened in the region of contact (Chien et al 1971, Rowlands and Skibo 1972). The high flexibility of horse cells facilitates this shape change and promotes aggregation, with an associated increase of low shear rate viscosity, as observed in this study. Other species, such as sheep and cattle, are known to have relatively inflexible cells and have negligible Rouleaux formation. An increase of erythrocyte flexibility may occur in man in a number of pathological conditions with high fibrinogen levels (eg, during the post-operative period, in bronchitis and some forms of hypertension). There is an associated increase of venous thrombosis in man in these circumstances. It would appear that in the horse this risk is offset by its ability to reduce its haematocrit at rest to 30 to 35 per cent, by sequestration of cells in the spleen. The lower haematocrit has a pronounced effect in reducing the degree of aggregation and there is a corresponding fall of blood viscosity at low shear rates. The observation

| TABLE 2: Effect of treatment with warfarin on blood rheology in a pony |
|------------------|------------------|
| Day              | Before | 2   | 3   | 4   | 5   | 6   |
| PCV (litres litre⁻¹) | 0·39   | 0·37 | 0·43 | 0·37 | 0·39 | 0·38 |
| Blood viscosity (cp)    | 3·26   | 3·02 | 3·94 | 3·11 | 3·17 | 2·99 |
| Plasma viscosity (cp)     | 1·46   | 1·38 | 1·42 | 1·29 | 1·36 | 1·29 |
| Relative viscosity (ln [ηr]) H⁻¹ | 2·08 | 2·12 | 2·40 | 2·42 | 2·24 | 2·25 |
| Fibrinogen (mg ml⁻¹)       | 3·16   | 2·37 | 4·23 | 4·34 | 4·18 | 3·29 |
| RBC flexibility (% min⁻¹) | 30·0   | 26·0 | 24·7 | 28·5 | 27·5 | 24·1 |
| MCV (fl)          | 47·5   | 48·4 | 48·0 | 48·8 | 49·2 | 48·0 |

See Table 1 for key
that erythrocyte flexibility is increased in untreated horses with navicular disease suggests that the balance may be critical. Only a relatively small, but significant, increase of erythrocyte flexibility is necessary to produce a clinically adverse increase of Rouleaux, aggregation and low shear rate viscosity. This could account at least in part for the venous congestion at the extremities of the forelimbs, which would reduce blood flow through the capillaries and arteries and cause ischaemia, as suggested by Colles and Hickman (1977). This is supported by the significant changes of erythrocyte flexibility and plasma viscosity that were found in horses being treated with warfarin and during the trial of this drug in ponies. How warfarin produces this effect is not known.

The advantage to the horse of having smaller and very flexible erythrocytes appears to be that they facilitate a more rapid exchange of oxygen. Decreasing the flexibility of human erythrocytes lowers the rate at which oxygen is taken up by haemoglobin in the cell (Sirs 1968). There is an apparent contradiction in the rheological behaviour of horses' blood in that, although the red cells are more flexible, the blood viscosity at high shear rates is comparable, or slightly higher, than for man at the same haematocrit. This is because the shape of horse erythrocytes is more asymmetric than human cells, which increases the effective viscosity. The viscosity of plasma is sensitive to the fibrinogen concentration for the same reason.

Amin and Sirs (1982) have reported that the shape factor, K, for horses is 3.8, compared to 3.3 in man, and this balances the change in erythrocyte flexibility. It can be shown that, at high shear rates, the slope of a plot of the natural logarithm of the relative viscosity against haematocrit depends on the product of the shape factor and reciprocal of the erythrocyte flexibility. This is consistent with the increase of $\ln(\eta H^{-1})$ shown in Table 2, as the flexibility decreases. The constancy of the MCV supports the view that this is not due to a change of shape. So over all, in six days of the trial, there would not be time for erythropoiesis to affect the cells, there was no change of shape, the erythrocyte flexibility decreased and the plasma viscosity fell. The most likely explanation of the action of warfarin, consistent with these observations, is that it acts indirectly on some factor in the plasma that can modify erythrocyte flexibility.

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