Light Transmission Patterns in Occluded Tissue: Does Rouleaux Formation Play a Role?

Mark P. McEwen, Karen J. Reynolds

Abstract— **There is evidence from the literature that suggests erythrocyte rouleaux, seen to form under favourable conditions (such as reduced blood flow) in vitro, may also occur in vivo, leading to a possibility of rouleaux influencing the transmission of light through tissue. This pilot study investigates the transmission of light through occluded tissues of human subjects, horses and sheep with a view to greater understanding of rouleaux formation in vivo, and its effect on light transmission through tissue.**

The paper supports the evidence that occlusion may cause rouleaux formation in vivo, but the association between rouleaux formation and light transmission changes during occlusion is complex, and occlusion may have effects on light transmission beyond the formation of rouleaux.

***Index Terms*—red blood cells, light transmission, rouleaux, occlusion**

I. INTRODUCTION

ROULEAUX (Fig. 1) are aggregates of red blood cells (RBCs) that normally form in the blood of humans and some other animals in vitro at low shear rates [1-4], and they separate as shear rate increases [5]. When blood is flowing in vitro and the flow is suddenly stopped, the majority of individual rouleaux form within 10 seconds of flow cessation [6]. Beyond the first 10 seconds, larger aggregates continue to form as rouleaux collide into each other and stick together following collision [7].

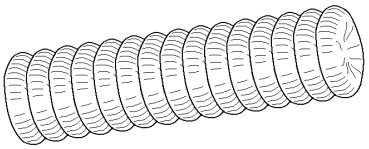

Fig. 1. A rouleau of red blood cells.

While rouleaux formation is clearly visualised in vitro, this is not easy in vivo. However some studies have generated results which provide evidence that rouleaux can form in vivo, particularly when blood is at stasis or flowing slowly. Several researchers have investigated the presence of rouleaux in blood using ultrasound, finding that ultrasound echogenicity of human blood appears to increase as a result of rouleaux formation [8-10] (which results in the presence of effectively larger particles in blood). Images recorded from rats have provided further evidence for the formation of rouleaux in vivo [11-13].

Rouleaux formation in vitro has been studied in detail by a number of authors using optical methods [6, 14-17] Syllectometry, as originally described by Zjilstra [15, 18], is a technique in which blood is illuminated, and subjected to shear flow. Light transmission (or reflection) through the blood is recorded, when flow is abruptly stopped, and plotted against time as a syllectogram.

A syllectogram reveals a distinctive pattern resulting from the behaviour of the RBCs [19]. With human blood, an initial rapid decrease in light transmission results from the disalignment of the RBCs, previously aligned during flow. This is followed by a subsequent increase in light intensity, rapid at first (as rouleaux form in the blood) and then slowing down to a plateau (with increasing aggregation of the cells). When rouleaux formation is negligible (eg sheep [2]) or is so strong that rouleaux are present in flowing blood (eg horse [20]) little or no increase in light intensity, corresponding to the formation of rouleaux when blood flow stops, may be expected.

A role for rouleaux has been proposed in photoplethysmography [14, 17] and in the time course of light transmission through tissue during blood flow occlusion. Differences in the vasculature and the blood of different species enable the influence of these factors on the transmission of light through tissue to be investigated. This pilot study aimed to investigate light transmission through tissue during blood flow occlusion in animals of three different species, with different degrees of rouleaux formation. Specifically, the paper compares the transmission of light through the occluded tissues of humans, sheep and horses. The Aggregation Index for human, sheep and horse blood is 0.09, 0.01 and 0.30 respectively [2], indicating that while sheep blood does not readily form rouleaux, horse RBCs aggregate very strongly.

II. METHODS

A. Ethics

Ethical approval was obtained from the Flinders Clinical Research Ethics Committee for all testing involving human subjects - who all gave informed written consent. Ethics approvals were obtained from the Flinders University Animal Welfare Committee and the Institute of Medical and Veterinary Science Animal Ethics Committee for all testing

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conducted on sheep and horses.

B. Test Subjects

11 volunteer human subjects, eight sheep and five horses were involved in this study. In the case of sheep and horses, tests were conducted on anaesthetised animals to avoid unwanted movement. To reduce the impact on animal wellbeing, only animals being anaesthetised for other purposes were involved.

C. Light transmission measurement

A custom device, containing a Light Emitting Diode (LED) probe and a light sensor probe, was used to measure the transmission of light through tissue (Fig. 2). The nominal centre wavelengths of the LEDs used were 660, 810, and 940 nm. Note, the custom device also contained 8 other wavelengths of light as part of a bigger study, but these are not included in the current paper.

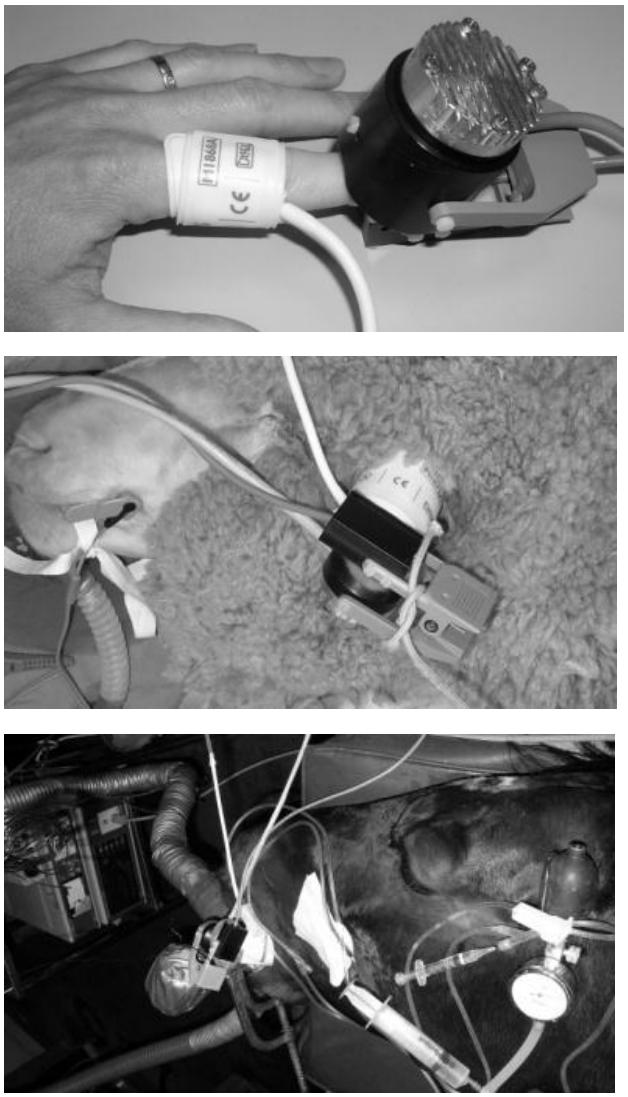

Fig. 2. Measuring light transmission during occlusion through: Top - a human finger; Middle – a sheep ear; Bottom – a horse tongue.

D. Anatomical measurement sites

When measuring light transmission through human tissue, the finger was used as the measurement site (Fig. 2), as is usual practice in pulse oximetry. Human patients rested in a sitting or supine position during testing. The external ear and the tongue were the most convenient measurement sites for testing animals. In the case of horses with dark fur, the signal-to-noise-ratio was very low when the ear was used, hence the tongue, with its absence of fur, was used for all horses (Fig. 2).

E. Occluding blood flow

To achieve the condition of blood flow occlusion, a blood pressure cuff was used. The LED and light sensor probes were positioned at a measurement site and the occlusion cuff was positioned proximally to the probes, adjacent to the measurement site (Fig. 2). When performing tests on human fingers, the occlusion cuff was inflated around the proximal end of a finger, without distorting the finger. However, when the cuff was inflated around an animal's ear, the ear could fold - possibly damaging the ear and preventing complete blood flow occlusion. Therefore to maintain the natural curved shape of the ear, a bung consisting of some silicone wrapped around a piece of rubber hose (Fig. 3) was placed inside the ear and the occlusion cuff was placed outside the ear, such that when the cuff was inflated, the ear was squeezed between the bung and the cuff - resulting in the occlusion of blood flow without folding of the ear.

Fig. 3. Cuff and bung for measuring light transmission through a sheep ear during occlusion.

When using the tongue as the measurement site, to maintain the natural, approximately flat, shape of a tongue when occlusion pressure was applied, "tongue clamps" (Fig. 4) were placed over an occlusion cuff when it was positioned around the proximal end of a tongue. Although called clamps, these devices were not used to apply any force to the tongue. They were positioned over occlusion cuffs, and adjusted to reduce the clearance between the tongue, cuff and clamp before occlusion was applied, to minimise distortion of the tongue when the cuff was inflated.

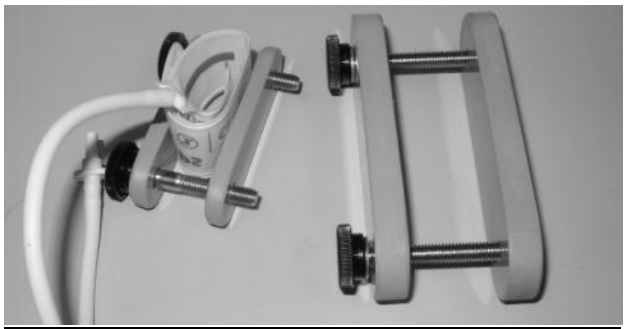

Fig. 4. Tongue 'clamps' for maintaining natural tongue shape during occlusion.

Occlusion cuff pressures between 200 and 300 mmHg were used to occlude blood flow. This range is well above

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the normal systolic blood pressure for humans, sheep and horses [21-23]. These pressures are well tolerated by test subjects for short periods - being equivalent to the pressures at the bottom of a 3 to 4 metre deep swimming pool.

F. Occlusion procedure

Occlusion tests were performed, involving the following steps:

- The light transmitting through tissue was measured for approximately 10 - 20 seconds under normal perfusion conditions
- The pressure cuff was quickly inflated from zero to 200 – 300 mmHg using a hand operated pump, and the intensity of light transmitting through tissue was measured for approximately 60 seconds
- The pressure cuff was deflated and the intensity of light transmitting through tissue was measured for another 20 - 30 seconds

G. Analysis of results

Individual light transmission signals were observed. To make comparisons of the light transmission changes during occlusion for the different species, the mean light intensity measured during occlusion was determined and plotted for each species. This involved dividing the value of light intensity at all times during the occlusion period, by the value of light intensity at the point occlusion was applied (normalising), and multiplying the result by 100, then calculating the arithmetic mean value (and standard deviation) of light transmission at all times during occlusion for all the signals recorded for each species.

III. RESULTS

A. Light transmission signal patterns - human

Measurements of the light transmission through the fingers of human subjects during blood flow occlusion resulted in signals which generally followed the pattern of initial sharp decreases, followed by a relatively rapid exponential type increase, steadying to an approximately linear increase/decrease in transmission (Fig. 5).

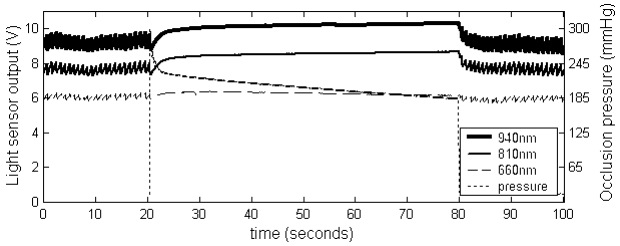

Fig. 5. Light transmission through a human finger during occlusion – note exponential increase following initial decrease.

In some cases, signals did not match this pattern, and the initial exponential increase was not observed. Close examination of the signals revealed that the pattern of light transmission during occlusion was not affected by the timing of the application of occlusion pressure with respect to the cardiac cycle.

B. Light transmission signal patterns - sheep

Signals measured from sheep also showed some variability. Fig. 6 shows a signal measured from a sheep ear. In general, the exponential type increase of Fig. 5 (human) was not observed.

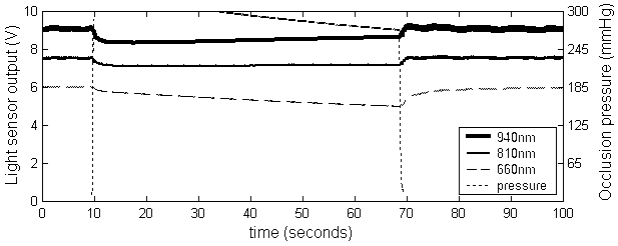

Fig. 6. Light transmission through a sheep ear.

C. Light transmission signal patterns - horses

Signals measured from horses also varied but in general, the exponential type increase of Fig. 5 (human) was not observed.

D. Mean transmission signals

Fig. 7 shows the mean and standard deviations of light transmission at the three wavelengths recorded during occlusion from human subjects (left), sheep (centre) and horses (right). Time zero corresponds to the point of occlusion. Table I compares the basic features of those signals, including the slope of the mean signals during the latter part of occlusion periods.

TABLE I
DIFFERENCES I N MEAN LIGHT TRANSMISSION BETWEEN SPECIES

	Human	Sheep	Horse
Rouleaux formation:	Moderate	No	Strong
Exponential type transmission change at start of occlusion:	Increase	Decrease	No
Slope of 660 nm transmission 20-50 s after occlusion onset (%/s):	-0.19	-0.22	-0.31
Slope of 810 nm transmission 20-50 s after occlusion onset (%/s):	0.05	-0.01	0.02
Slope of mean 940 nm transmission 20-50 s after occlusion onset (%/s):	0.07	0.03	0.06

IV. DISCUSSION

The mean light transmission signals for the different species (Fig. 7) show a number of features:

- All showed an immediate decrease in light transmission at the start of occlusion.
- Following this initial decrease, the transmission of all colours of light increased in decaying exponential fashion for a short time through human tissue, and decreased in decaying exponential fashion for a short time through sheep tissue. Mean signals from horses did not have obvious exponential type components.
- Signals beyond the first few seconds in all species were approximately linear. In this region, the red (660 nm) light transmission decreased in all three species (see

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Table I), while infrared (940 nm) light transmission increased slightly. 810 nm light transmission remained approximately constant in sheep and horse tissue, whilst increasing in human tissue, as occlusion time increased.

The mean pattern seen in human subjects was in agreement with that reported by Shvartsman and Fine [16, 17, 24] who stated that "the transmission always grows during a certain initial time interval, which is different for different wavelengths of incident radiation, and then, in an asymptotic time interval, it monotonously grows or falls, depending upon the wavelength of incident light". It should be noted however, that not all signals recorded from human subjects in our study clearly displayed this pattern.

Dobbe et al [7] described two phases of aggregation; an initial phase of one-dimensional (as in Fig. 1) rouleaux formation with a time constant of 1-3 s, followed by three-dimensional (3-D) aggregate formation during which rouleaux connect end-to-end as well as side-to-end, creating larger aggregates. In normal human blood, the formation of these aggregates is a slower process with a time-constant of about 10–25 s.

The decaying exponential increase seen in the mean signals from human subjects could be due to the initial formation of rouleaux following blood flow cessation. This early increase in transmission was not observed in the mean signals for sheep (which do not form rouleaux), neither was it observed in horses, whose blood shows a very strong tendency to form aggregates. This may be interpreted as an indication that rouleaux formation was not responsible for the decaying exponential-type increase seen in the mean signals from humans. However the extremely strong tendency of horse RBCs to aggregate means that rouleaux do not disperse fully even at high shear rates [20]. Thus it is feasible that horse blood is aggregated to some degree during normal blood flow in which case halting blood flow may not result in a rapid process of rouleaux formation.

Following the initial exponential changes in light transmission, further changes were more linear (Fig. 7). These changes (decrease in red (660 nm) and increase in infrared (940 nm) light transmission) may be at least partly due to deoxygenation of the blood, during occlusion, as oxygen moved from haemoglobin in the RBCs to the nearby tissues. Light at 810 nm is absorbed approximately equally by oxygenated and reduced haemoglobin (an isobestic wavelength), hence would not be expected to change due to deoxygenation. Fig. 7 and Table I show that 810 nm transmission changed little in sheep or horses after the first 20 seconds of occlusion, however there was a small increase in 810 nm light transmission in the mean signals from humans. Continued rouleaux formation and aggregation may be an explanation for the presence of this mean increase.

There was considerable variation in the time course of light transmission through tissue during occlusion, as indicated by the large standard deviation compared to the overall change in mean transmission during occlusion (Fig. 7).

Mechanisms that could have contributed to the large variation in light transmission included:

- The transfer of occlusion pressure to deep blood vessels. Although occlusion pressure was applied to the outside of tissue quickly, it may not have been transferred to the deeper blood vessels at the same rate for all individuals due to variations in tissue viscoelasticity.
- Vasoactive mechanisms, such as capillary dilation. Gregory and Mars showed that capillaries can dilate in response to compressed air massage of muscles [25]. If capillaries can dilate in response to tissue being occluded, there could be a movement of blood from within large vessels to capillaries. Such a movement of blood could enable light to more easily transmit through large vessels, whilst decreasing the transmission of light through capillaries, which may result in a change in overall light transmission through tissue.
- Differences in the rate of rouleaux formation between individuals. Aggregation is affected by some plasma protein concentrations [26], by the blood haematocrit [27], and is increased in a number of diseases [28].

This work has not considered all the possible differences between humans, sheep and horses. It is possible that species dependent parameters, other than those considered here, may be involved in the differences found, in the measurements of light transmission, from humans, sheep and horses.

V. Conclusion

Decaying exponential type increases in the mean light transmission recorded for human subjects, but absent in signals from sheep and horses, may be a consequence of rouleaux formation. Other research has provided evidence that rouleaux may form in vivo, but light transmission measured during blood flow occlusion did not appear to be a reliable indicator of this phenomenon, due to considerable variations in the pattern of light transmission observed during occlusion. The evidence from this work indicates that occlusion may cause rouleaux formation in vivo, but a definitive relationship between rouleaux formation and light transmission changes during occlusion has not been identified, and occlusion may have effects on light transmission beyond the formation of rouleaux.

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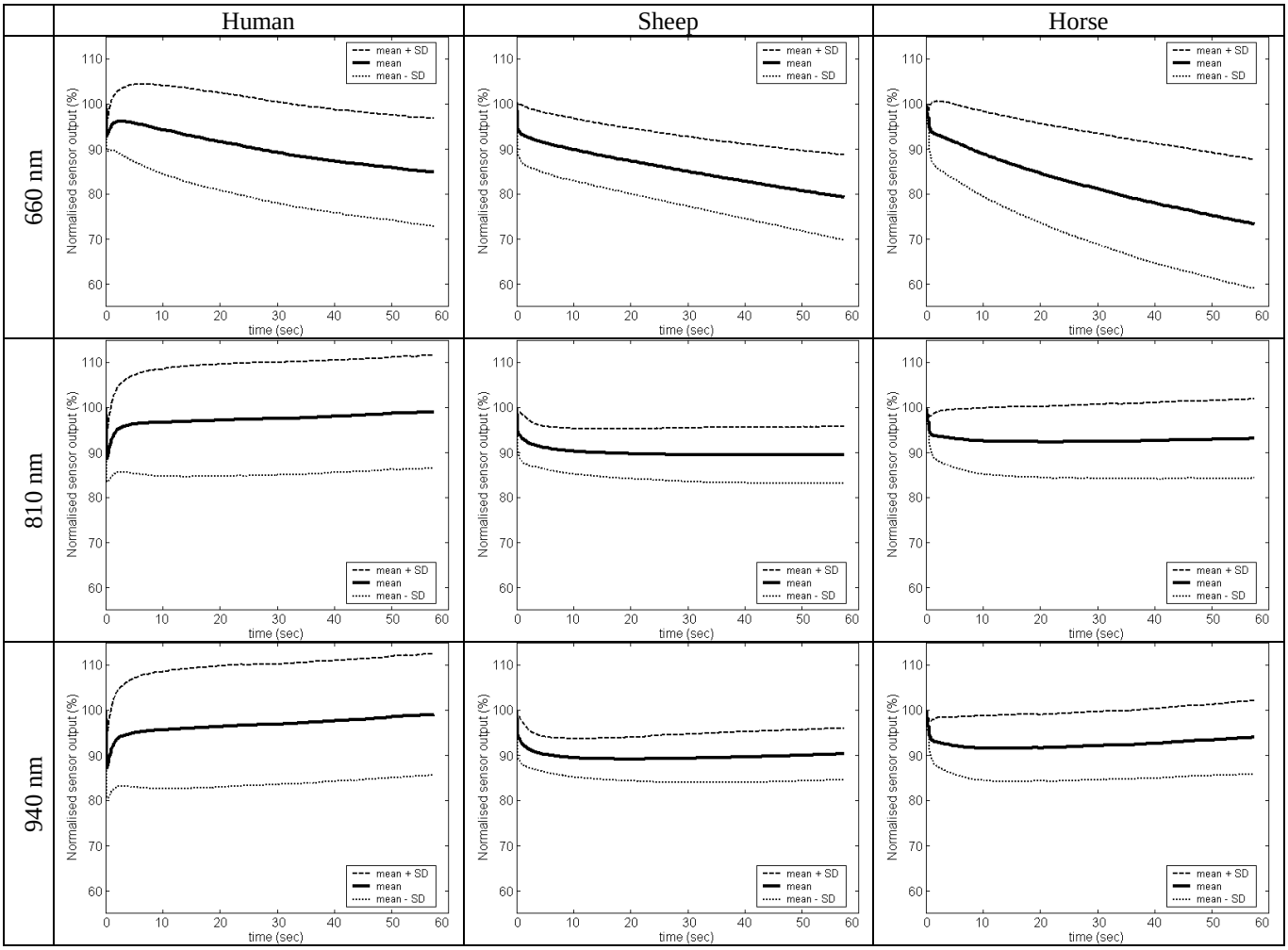

Fig. 7. Mean light transmission during occlusion for human subjects (left), sheep (centre) and horses (right).

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