THE COLOUR OF BLOOD IN SYRINGES AS A GUIDE TO HYPOXAEMIA

BY

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SUMMARY

The frequency with which blood of differing oxygen saturation in syringes may be distinguished from a fully saturated sample of the same blood was examined using both anaemic and normal blood. It is suggested that under suitable lighting conditions the examination of arterial blood in a syringe may provide a more reliable guide to the state of arterial oxygenation than the presence or absence of cyanosis.

The unreliability of cyanosis as a sign of arterial hypoxaemia is evident (Stadie, 1919; Comroe and Botelho, 1947; Medd, French and Wyllie, 1959; Kelman and Nunn, 1966a), but instrumental methods for estimating the oxygenation of arterial blood are by no means universally available.

Inspection of arterial blood in a syringe has apparent advantages over inspection of mucous membranes as a means of detecting arterial hypoxaemia. The colour of the blood is not masked by overlying tissues and it is easy to control the lighting conditions in which the syringe is inspected. There is no possibility of confusing arterial desaturation with that due to stasis.

A possible advantage is that a saturated sample of the same blood, for use as a standard of comparison, can be produced by shaking some of the blood with oxygen in a syringe. To investigate this an attempt has been made to determine the consistency with which blood of differing oxygen saturation levels may be distinguished from a fully saturated sample of the same blood. Data have been collected on the influence of haemoglobin concentration and lighting.

METHODS

Experimental design.

Well mixed venous blood samples were divided into 2-ml quantities in identical 5-ml glass syringes. The blood in one syringe in each batch was shaken with oxygen for 5 minutes. Previous experiment had shown that more than 99 per cent saturation could be produced by this means, even when the blood had a packed cell volume of 60 per cent and an initial saturation of less than 50 per cent. The other syringes were numbered and the blood in them was equilibrated for shorter lengths of time to produce blood of differing saturations. The bubbles were excluded from the syringes which were then capped.

The observers were three doctors with normal colour vision as tested by the Ishihara test. The same three observers took part throughout. They were given the first syringe which they were told contained blood which was fully saturated. They were then shown the numbered syringes, one syringe at a time, and asked to compare them with the first syringe to decide whether the blood was the same colour as, or darker than, the saturated blood. The blood was well mixed before being presented. If they decided that there was no difference, or if they were in doubt at the end of an observation period of 7 seconds, they were asked to record them as "equal". If they decided that the blood in the numbered syringe was darker, then they were asked to record it as "blue". The observers wrote their decisions on prepared cards without collusion.

The numbered syringes were shown in random order until each syringe had been inspected three times. The numbers on the syringes were not apparent to the observers.

When the observations had been completed the syringes were stored in iced water to await analysis. The time between the first observation and immersion in iced water did not exceed 6 minutes. The mean room temperature was 22.5°C, the range was 19.5 to 25.5°C. Measurements on the
blood samples were completed within 60 minutes of immersion in the iced water.

The experiment was performed in daylight which was shining through a frosted glass skylight on days when most of the sky was blue and was repeated in the light of a 60-watt tungsten filament bulb and in that of a 40-watt Ekco "White" fluorescent tube. This fluorescent lamp was chosen as a light source with colour rendering properties very different from those of daylight; its spectral power distribution is shown in figure 1. To limit variation in the quality of reflected light, the syringes were inspected in a white-walled box open above to the light source and in front to the observers. The intensity of the incident light was measured with a photometer (Avo light meter, model 3, Avo Ltd., London). The average intensity was 125 lm/sq.ft.

Blood of both low and normal haemoglobin concentration was inspected in each light source. The blood of low haemoglobin concentration, less than 10 g/100 ml, was obtained from patients with iron deficiency anaemia.

Analysis.

The haemoglobin concentration was measured by the cyanmethaemoglobin method. Po2 was measured using a microelectrode (IL system 113–S1, Instrumentation Laboratory Inc., Boston, U.S.A.). The electrode was maintained at 37.0 ± 0.1°C and was calibrated with gases of known oxygen tension. The measured Po2 was corrected for difference in output of the electrode from blood and gas at the same tension by a factor determined by tonometry. pH and base excess were estimated using an Astrup micro electrode and micro equilibration unit (Radiometer Corporation, Copenhagen, Denmark). The Po2 which would obtain at pH 7.4 with no base excess and a temperature of 37°C was calculated from nomograms of Kelman and Nunn (1966b). A standard dissociation curve (Severinghaus, 1966) was used to convert this Po2 into percentage saturation. The fall in saturation with time spent at room temperature (less than 6 minutes) was ignored. It was assumed that the percentage saturation of the blood sealed in an anaerobic environment was not altered by temperature changes and the small error due to change in dissolved oxygen at differing temperatures was ignored.

RESULTS

There was uniform agreement that blood of less than 85 per cent saturation was darker than fully saturated samples of the same blood (table I).

<table>
<thead>
<tr>
<th>Percentage saturation</th>
<th>No. of observations</th>
<th>No. distinguished</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>80–84</td>
<td>117</td>
<td>117</td>
<td>100</td>
</tr>
<tr>
<td>85–89</td>
<td>108</td>
<td>93</td>
<td>86</td>
</tr>
<tr>
<td>90–94</td>
<td>72</td>
<td>60</td>
<td>83</td>
</tr>
<tr>
<td>95–99</td>
<td>252</td>
<td>103</td>
<td>41</td>
</tr>
</tbody>
</table>

Effect of lighting.

Results obtained in daylight and in the light of a tungsten bulb were similar, but in the light of the particular fluorescent tube used there was a higher incidence of "blue" observations in the 95–99 per cent range (fig. 2).

Results using blood of normal haemoglobin concentration, excluding those obtained in fluorescent light (table II).

In this group the frequency with which blood of 85–94 per cent saturation was distinguished from a fully saturated sample was about 75 per cent. Above 94 per cent saturation the frequency
was less than 5 per cent. "Within observer" and "between observer" reliability are examined in this group.

"Within observer" reliability.

Table III deals with the reliability of the same observer in deciding on the colour of the same blood on three occasions. Clearly all three decisions are the same, or one is different from the other two; there is a simple dichotomy into consistent and inconsistent. The highest incidence of inconsistency was in the 85–94 per cent saturation range and was 42 per cent. The inconsistency rate above 94 per cent saturation was 12 per cent.

"Between observer" reliability.

Taking majority opinions of individual observers on a given syringe there is a dichotomy on agreement between observers, either all agree or one disagrees with the other two. Two conflicts of majority opinions occurred in this group; they were both in the 90–94 per cent saturation range (table IV).

Effect of haemoglobin concentration.

At each saturation level greater than 85 per cent, anaemic blood was more often distinguished from a fully saturated sample of the same blood.
TABLE V
Effect of haemoglobin concentration on the frequency with which blood was distinguished from fully saturated samples of the same blood (all lighting conditions).

<table>
<thead>
<tr>
<th>Haemoglobin concentration (g/100 ml) (mean and SD)</th>
<th>Percentage saturation (mean and SD)</th>
<th>No. of observations</th>
<th>No. “blue”</th>
<th>Percentage “blue”</th>
</tr>
</thead>
<tbody>
<tr>
<td>13.1 ± 2.2</td>
<td>97.1 ± 1.79</td>
<td>144</td>
<td>22</td>
<td>15</td>
</tr>
<tr>
<td>6.6 ± 1.2</td>
<td>97.9 ± 1.78</td>
<td>108</td>
<td>81</td>
<td>75</td>
</tr>
</tbody>
</table>

The frequency is significantly higher in the low haemoglobin group (P < 0.0005).

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than was blood of higher haemoglobin concentration (fig. 2). This effect was most marked in the 95–99 per cent range where the frequency was 75 per cent for anaemic blood but 15 per cent for blood of higher haemoglobin concentration. This difference is statistically significant (table V).

The design allowed each of three observers to see five samples of blood three times, a total of forty-five observations, in 6 minutes. It would be expected from the nomograms of Kelman and Nunn (1966b) that in 6 minutes at room temperature the fall in percentage saturation would not exceed 1 per cent absolute. If a fall in saturation occurred during the observation period sufficient to produce observable colour changes in the samples, then one might expect a higher incidence of “blue” observations the third time the samples were shown than when they were first shown. There was no significant difference between the frequency of “blue” observations at the first and third showing (table VI).

TABLE VI
The frequency of “blue” observations at the first and third showing.

<table>
<thead>
<tr>
<th></th>
<th>First showing</th>
<th>Third showing</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. “blue”</td>
<td>124</td>
<td>128</td>
</tr>
<tr>
<td>No. not “blue”</td>
<td>59</td>
<td>55</td>
</tr>
<tr>
<td>Total</td>
<td>183</td>
<td>183</td>
</tr>
</tbody>
</table>

$\chi^2 = 0.216; P > 0.6.$

DISCUSSION

The method by which varying oxygen saturations of the blood samples were obtained was quick but somewhat unpredictable. An attempt was made to obtain samples the saturations of which were evenly distributed between 80 and 99 per cent. Five samples had saturations of less than 80 per cent; these were uniformly recorded as “blue” by the observers and do not appear in the results. The number of samples at each saturation level is somewhat uneven (table I).

The similarity of the results obtained in daylight to those obtained in the light of a tungsten bulb is, at first sight, surprising. However, colour adaptation by the eye tends to maintain the colour constancy of objects seen under different qualities of illumination provided that the spectral energy distribution of the light sources is relatively smooth. The yellowness of the tungsten illumination and the blueness of daylight are largely compensated by corresponding degrees of specific yellow and blue adaptation in the two cases (Wright, 1964). For the same reasons, the results obtained in daylight are likely to be applicable to a wider variety of daylighting conditions than those used. It is possible that a fluorescent lamp which meets the Medical Research Council’s (1965) recommendations would give results closer to those obtained in the light of a tungsten bulb.

The effect of haemoglobin concentrations is the reverse of that which might have been anticipated in the view of the oft-repeated statement that the presence of cyanosis depends upon the absolute amount of reduced haemoglobin and cannot be detected unless there is at least 5 g/100 ml reduced haemoglobin in the capillary blood (evidence for this statement appears in papers written by Lundsgaard published in 1919). The results suggest that the change of colour with falling saturation of blood in a syringe is more marked when the haemoglobin concentration is low than when it is high (table V). An alternative explanation is that there is a systematic error in the method by which saturation was estimated.
such that the percentage saturation of anaemic blood is overestimated. This possibility is being explored.

The level of arterial oxygen saturation below which cyanosis can be detected on more than 95 per cent occasions has been estimated by several workers as about 75 per cent in traditional lighting (Stadie, 1919; Comroe and Botelho, 1947; Medd, French and Wyllie, 1959). In a study designed to evaluate the relative merits of a variety of fluorescent lamps, Kelman and Nunn (1966a) say that “under suitable lighting conditions it is possible for the average observer to detect arterial hypoxaemia in 97.5 per cent cases at a saturation level of about 90 per cent.” A scatter diagram is presented in which a cyanosis score is plotted against percentage saturation. There are six points representing blood samples of less than 90 per cent saturation from six patients, each of whom was inspected by three observers in the “suitable” light, making a total of eighteen observations. Cyanosis was not recorded on at least three of the eighteen occasions. This could be represented as a 17 per cent failure rate in the detection of cyanosis when the arterial saturation is less than 90 per cent.

It would seem that a test which involved a comparison of the colour of an arterial blood sample in a syringe with a sample of the same blood saturated by shaking with oxygen would provide a better guide to the state of arterial oxygenation than would examination of the patient for cyanosis when the haemoglobin concentration is greater than 10 g/100 ml and when the syringes are examined in daylight or in the light of a tungsten bulb. If the blood samples used in this study had been arterial samples from patients, the observers would have made a diagnosis of arterial desaturation in all the patients with an arterial saturation of less than 85 per cent, in three-quarters of those with arterial saturation in the 85-94 per cent range, less than 5 per cent of those with an arterial saturation of more than 94 per cent. When samples from anaemic patients were included, however, arterial desaturation would have been diagnosed more frequently when the estimated arterial saturation was greater than 94 per cent.

ACKNOWLEDGEMENTS
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REFERENCES

LA COULEUR DU SANG DANS LES SERINGUES COMME GUIDE DE L’HYPOXÉMIE

SUMMARY
La fréquence avec laquelle le sang à saturation différente en oxygène dans les seringues peut être distingué d’un échantillon complètement saturé du même sang fut examiné en utilisant le sang anémique et le sang normal. Il est suggéré que dans des conditions d’éclairage convenable, l’examen du sang artériel dans une seringue peut constituer un guide plus digne de confiance pour montrer l’état d’oxygénation artérielle que la présence ou l’absence de cyanose.

DIE BLUTFARBE IN SPRITZEN ALS HINWEIS AUF EINE HYPOXÄMIE

ZUSAMMENFASSUNG