available for the serosal transfer step of the transport mechanism; third, a DNA-mediated inhibitor of the second step of the iron transport is produced which decreases the serosal transfer of iron. Mice with the mutant gene, *sla*, offer an important system for testing these hypotheses as well as for studying the biochemistry of the active transport mechanism for iron in the intestinal mucosa of the mouse.

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Distribution of HbCO in Human Erythrocytes following Inhalation of CO

THE distribution of carbon monoxide in erythrocytes of persons exposed to high concentrations of gas for short periods was investigated using a modification of the staining technique described by Betke and Kleihauer¹. The experiments consisted of examination of citrated human blood carboxylated with pure CO and then decarboxylated with oxygen, and capillary blood taken at intervals following inhalation of CO as the pure gas or from tobacco smoke.

The method used in this study was as follows: 50 μ l. 1.25 per cent w/v aqueous sodium nitrite was mixed with 50 μ l. blood and stood under nitrogen for 10 min.; 50 μ l. 1 per cent w/v aqueous potassium cyanide was then added and the mixture was then left for 1 min. Thin, even smears were prepared and the slides were stood vertically without shaking in a freshly prepared mixture of 80 ml. 96 per cent ethanol, 16 ml. 0.2 M citric acid and 5 ml. 30 per cent hydrogen peroxide for 3 min. They were then washed in methanol and stained in 0.05 per cent w/v aqueous gentian violet for 1 min. The slides were examined by light microscopy with a green filter.

Fig. 1. Photomicrograph showing human erythrocytes stained for the presence of carboxyhaemoglobin in peripheral blood 1 h after inhalation of 500 ml. of CO.

Fig. 2. Photomicrograph showing human erythrocytes from peripheral blood of a pipe smoker stained for the presence of carboxyhaemoglobin.

It was observed that when 100 per cent CO was passed into human citrated blood for varied times, carboxylation did not affect all erythrocytes in the same manner. At low concentrations of carboxyhaemoglobin, few cells showed any staining, but those that did were more densely stained at the periphery than at the centre. As the uptake of CO proceeded the proportion of stained cells increased, as did the evenness of staining, until full carboxylation was observed. Staining started at the periphery and increased gradually until the whole cell was stained. On passing oxygen through the carboxylated blood the reverse was noted with the cells losing stain from the periphery leaving a more intensely stained centre. Individual cells lost stain at different rates. It was also observed that whereas uptake of CO by erythrocytes took place rapidly, the complete decarboxylation process took several hours.

Experiments *in vivo* consisted of a study of capillary blood samples taken 1 h after inhalation of 500 ml. of pure CO and from persons who were both cigarette and pipe smokers. Fig. 1 is a photomicrograph of capillary blood taken 1 h after inhalation of the pure gas. The blood sample was stored at 4° C for 96 h before staining. It can be seen that the rapid redistribution of O₂ and CO between erythrocytes at 37° C reported for experiments *in vitro*¹ had not occurred, either during body circulation or during storage of the sample. When tobacco smoke is inhaled the picture is initially similar with the stained cells having dark peripheries; as normal smoking continues throughout the day the picture alters with a mixed stained cell population appearing, the majority of the cells now having more intensely stained centres as decarboxylation of the individual erythrocytes proceeds. Fig. 2 is a photomicrograph of capillary blood taken at 1600 h from a pipe smoker who had smoked freely since 0900 h. Comparison of the proportion of the types of stained cells undergoing carboxylation and decarboxylation in such a preparation gives an indication of the time that has elapsed since inhalation of CO. These observations suggest that: (1) following inhalation of CO only the erythrocytes exposed to the gas in the lungs are carboxylated; (2) redistribution of the carboxyhaemoglobin between erythrocytes during subsequent circulation does not occur; (3) the hypoxic insult to an individual erythrocyte will not be related to the carboxyhaemoglobin saturation as determined on whole blood.

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