suggested that errors may arise from changes of light transmission and refractive index when hydrosulphite is dissolved in solution.

An alternative method, primarily developed for measuring the rate of dissociation in red blood cells, has been used to examine this problem further. A buffer solution of oxyhaemoglobin at pH 9.1, in equilibrium with oxygen at a pressure of 10–40 mm mercury, is rapidly mixed and at the same time diluted 1:20 with a similar buffer solution equilibrated with carbon monoxide at 800 mm mercury. Dissociation of the HbO_2 occurs with rapid uptake of carbon monoxide, under conditions similar to the displacement method of Gibson and Roughton⁵. The relative ratio of the concentrations, $[\text{O}_2]/[\text{CO}]$, after mixture, however, is now such that the rate is determined by $k_4/4$ to within 2 per cent. In practice this is within the experimental errors and no significant difference in the rate of egress can be detected with a four-fold change of oxygen tension or a three-fold change of carbon monoxide concentration. A comparison of this method with the hydrosulphite technique has revealed no significant error. The method can also be applied to measurements at lower pH.

With HbII(A), the electrophoretically fast component, the value of k_4 at 20° C and pH 9.2 was 40.8 ± 7 per cent by the sodium hydrosulphite method, and 38 ± 7 per cent by the dilution technique. At pH 7.4, k_4 was 44.5, higher by a factor of 1.1 in accord with the observations of Gibson and Roughton⁵. The activation energy at both pH values was $19.5 \text{ kcal} \pm 7$ per cent. Similar measurements on an electrophoretically pure form of HbI(B), at pH 9.2, gave a value of $k_4 = 45$ at 20° C by both methods. The activation energy in this case was $16.5 \text{ kcal} \pm 7$ per cent. At pH 7.4, using the dilution technique, k_4 at 20° C was 54.8 and the activation energy had increased to 19 kcal.

The values of k_4 for HbII(A) arc, within experimental error, the same as those obtained previously^{4,5}. In the case of HbI(B), however, an interesting discrepancy has occurred. The value at pH 9.2 is not the same as that obtained by Kernohan⁴, though the value at pH 7.4 is only slightly lower. On the other hand, it had been assumed that the values of k_4 obtained by Gibson and Roughton⁵ were those of HbII(A), though while at pH 7.1 only activation energies of the order 20 kcal were obtained, at pH 9.1 two distinct values occur one at 16.5 kcal and another at 20 kcal. It now seems probable that the 16.5 kcal measurements were made on HbI(B) samples. A similar difference in the rate of egress with pH change has been observed for this haemoglobin within intact red blood cells, so that the discrepancy is unlikely to be associated with the method of preparation of the haemoglobin solution.

The differences at pH 9.2 do not affect Kernohan's conclusion that the shift of the oxygen equilibrium curves observed by Huisman *et al.* is associated with differences in k_4 , but the question arises as to whether HbI and HbB are identical, or whether there are two forms of the electrophoretically slow HbI(B) sheep haemoglobin.

JOHN A. SIRS

Department of Physics,
St. Mary's Hospital Medical School,
London, W.2.

¹ Harris, H., and Warren, F. L., *Biochem. J.*, **60**, 29 (1955).

² Helm, H. J., van der Vliet, G. van, and Huisman, T. H. J., *Arch. Biochem. Biophys.*, **72**, 331 (1957).

³ Huisman, T. H. J., Vliet, G. van, and Sebans, T., *Nature*, **182**, 171 (1958).

⁴ Kernohan, J. C., *J. Physiol.*, **155**, 580 (1961).

⁵ Gibson, Q. H., and Roughton, F. J. W., *Proc. Roy. Soc., B*, **143**, 310 (1955).

⁶ Gibson, Q. H., and Roughton, F. J. W., *J. Physiol.*, **145**, 32P (1959).

⁷ Kernohan, J. C., *J. Physiol.*, **145**, 32P (1959).

⁸ Roughton, F. J. W., Legge, J. W., and Nicolson, P., *Haemoglobin (Barcroft Memorial Conference)*, 67 (Butterworths Scientific Publications, London, 1949).

⁹ Dalziel, K., and O'Brien, J. R. P., *Biochem. J.*, **49**, xlvii (1951).

HISTOLOGY

A Simple Dye Test for Skin Graft Viability

PATENT blue V dye (alphazurine 2G), which was previously used in evaluations of cutaneous burns¹, has also been found useful in the evaluation of the viability of allografted skin before and including the process of rejection. In small animals, dye injected intravenously provides immediate colouring of the skin over an intact vasculature.

The autograft after revascularization will be coloured light blue, the same as the surrounding skin. With increased vascular permeability as occurs in second degree burns or early rejection, the dye is deposited extravascularly as a dark blue stain, enabling the observer to locate the area of vascular disruption. With coagulation necrosis and thrombosis as found in third degree burns, or total allograft rejection, no tissue colouring occurs, and this demarcates the non-viable skin.

In very small animals, the dye can be injected intraperitoneally, but a 5–10 min lag period must be allowed for the full dye pattern to develop. Dye patterns should be read shortly after the skin has reached its maximum colour, as patent blue will in time diffuse into non-vital areas. This latter phenomenon provides a "reciprocal pattern" for confirmation while the intravascular dye is being rapidly metabolized by the liver and excreted through the kidneys.

This test may be repeatedly carried out without disturbing the graft to follow the process of rejection or its suppression. The histological changes occurring during rejection are not delineated in detail by this technique, but sequential information concerning the vascularization of the graft is obtained, and this bears directly on survival.

There are many possible clinical applications of this technique, but care should be taken in using this experimental agent in debilitated patients.

ROBERT W. BRIGHT
ALFRED J. DEFALCO

Laboratory of Experimental Surgery,
Department of Clinical Investigation,
Naval Medical Research Institute,
National Naval Medical Center,
Bethesda, Maryland.

¹ Randolph, J. G., *et al.*, *Surgery*, **56**, 193 (1964).

PHYSIOLOGY

Structure and Function of the Sarcolemma of Skeletal Muscle

THE functions of the sarcolemma of skeletal muscle are still uncertain, because of our lack of knowledge of its structure and detailed distribution over the entire surface of the muscle cell. Most of our knowledge is derived from electron microscope investigations and the sarcolemma is regarded as a semi-permeable unit membrane about 100 Å thick, which directly encloses the contents of the cell. This membrane is probably bimolecular and lipoprotein in character, and similar to that which is thought to invest most animal cells and many of their cytoplasmic organelles. Immediately external to the sarcolemma is a layer about 500 Å thick; this is an extracellular deposition of material of moderate density (the so-called basement membrane¹) which gradually diminishes in density as it extends from the sarcolemma into extracellular space and merges with the ground substance of the endomysial connective tissue. Because of its extreme thinness, the sarcolemma is difficult to observe in its longitudinal section; on the other hand, its characteristic unit membrane structure can usually be recog-