steady blood levels, and it appears to be inevitable that in some patients the plasma salicylate level will rise dangerously; weekly estimations are no guarantee that this will not occur.

Previous reports state, and experience with this case confirms, that marked hyperpnoea should be taken as a warning of impending dangerous toxic reactions; it should be an indication for a reduction in dose. The plasma level is not an absolute guide, as it is evident that the toxic level varies greatly from patient to patient. Graham and Parker (1948) have found that severe drowsiness and excitement commonly appear at levels of 35 to 55 mg. per 100 ml. Intravenous salicylate administration to patients with carbon dioxide retention and respiratory acidosis has been shown (Wégria *et al.*, 1955) to reduce the arterial $p\text{CO}_2$, and has been suggested as a possible method of treatment of such cases; the use of salicylate-induced hyperpnoea in this way would seem to be dangerous, as the blood levels which may be required or involuntarily achieved to produce a therapeutic effect differ little from those which in some subjects are dangerously toxic.

In treatment of established salicylate encephalopathy, general anaesthesia, with the use of a relaxant and controlled respiration, may be necessary, and was effective in the above case in arresting the tonic spasms; elimination of salicylate in the urine may be encouraged by the administration of intravenous sodium bicarbonate. Bicarbonate was formerly recommended to combat the supposed acidosis, but its main action is to promote renal excretion of salicylate (Parker, 1948; Reid, 1948). It may, however, cause a further rise in pH (Rapoport and Guest, 1945); but, theoretically, inhalation of high CO_2 gas mixtures might be expected to correct the metabolic disturbance. A high rate of urine formation should be maintained by an adequate fluid intake (either intravenous or oral).

Summary

A case of acute rheumatism, complicated by encephalopathy attributed to sodium salicylate therapy in ordinary dosage, and associated with a plasma salicylate level of 50 mg. per 100 ml., is described.

Hyperpnoea should be taken as a warning sign of impending dangerous toxic effects of salicylate therapy.

Encephalopathy occurring in these circumstances can be treated successfully by general anaesthesia, with the use of a relaxant and controlled respiration, intravenous administration of sodium bicarbonate, and the maintenance of a high fluid intake.

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AN EVALUATION OF THE MICROSPECTROGRAPHIC DIAGNOSIS OF APLASTIC ANAEMIA

BY

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Our attention has recently been drawn to a paper by Kobayashi and Watanabe (1955) describing differences in the spectroscopic absorption curves of the haemoglobin in individual erythrocytes of leukaemia and aplastic anaemia. They suggest that their technique may be of some help in the differential diagnosis of these two conditions. We have in this laboratory some experience of cytospectrophotometry and its attendant errors, and we consider that the absorption curves published by them show enough inaccuracies to invalidate their conclusions. As the medical treatment of these two conditions may be diametrically opposite, we felt that it was essential to publish, as soon as possible, preliminary results confirming our doubts about the value of this method of diagnosis.

Absorption Curves of Red Cells

Fig. 1 shows the absorption curves of normal red cells redrawn from the paper by Kobayashi and Watanabe. A close examination of these curves reveals considerable inaccuracies. We have regularly plotted absorption spectra (4,000–7,000 Å) from small areas of individual red cells, both normal and abnormal, from various mammalian species (man, rat, guinea-pig, camel), and have always obtained curves having their maxima at identical wavelengths (4,150 Å, 5,420 Å, and 5,750 Å) (Keohane and Metcalf, 1956). These agree with the figures obtained by us and by many others for solutions of haemoglobin (Sidwell *et al.*, 1938; Jope, 1949). The differences in the chemistry of haemoglobins of different species are differences in the globin part of the molecule, and do not affect the visible absorption spectrum, which is entirely due to the haem radical.

Many early workers in this field found it difficult to obtain the 4,150 Å (Soret) absorption with intact erythrocytes (Adams, 1938; Keilin and Hartree, 1941), and others obtained inaccurate maxima for the 5,420 Å and 5,750 Å absorption (Cordier and Coudier, 1950). These errors have been shown to be due to scatter in the apparatus (Jope, 1949; Keohane and Metcalf, 1956). Elimination of this scatter corrects the error and improves the consistency of the curves. As the wavelengths of the absorption maxima of haemoglobin are constant it can be assumed that the peaks demonstrated above 4,500 Å in Fig. 1 are simply fluctuations due to experimental error and give a measure of the accuracy of the method. Other workers have failed to demonstrate the α and β (5,420 and 5,750 Å) absorption of intracellular haemoglobin (Jope, 1949; Metcalf, 1951). This is not surprising, as the percentage absorption of light at this wavelength is of the same order as the accuracy of photographic photometry ($\sim 10\%$). We have adopted a photometric technique in order to be able to obtain the α and β absorption maxima consistently and accurately in individual cells (Keohane and Metcalf, 1956).

The type II curve of Kobayashi and Watanabe (Fig. 2) shows decreased absorption in the Soret region but considerable absorption in the 4,500–5,000 Å region, a wavelength band in which haemoglobin should have no absorption. This spurious absorption is easily produced by using

FIG. 1.—Absorption curves of single R.B.C. (Type I). Ordinates: Optical density. Abscissae: wavelength in $m\mu$. (After Kobayashi and Watanabe.)

a mountant which mismatches the refractive index of the cell, with consequent loss of light by refraction. Decreased absorption at $4,150 \text{ \AA}$ is frequently due to focusing errors, as even apochromatic objectives show appreciable change of focus with wavelength in the violet part of the spectrum.

Assuming that the same apparatus has been used to produce Figs. 1 and 3, Type 3 curves cannot be true absorption curves.

FIG. 2.—Absorption curves of single R.B.C. (Type II). Ordinate: Optical density. Abscissa: Wavelength in $m\mu$. (After Kobayashi and Watanabe.)

The variation in absorption throughout the spectrum is less than the errors in the technique as deduced from Fig. 1. Such "curves" are obtainable in many ways. Perhaps the most obvious of these is by the selection of the very thin centre of biconcave erythrocytes where the quantity of haemoglobin is too small to be detected by the apparatus. Experiments which we have performed (unpublished), with a view to discovering the limitations of refracting microscopes in microspectrophotometry, show that spurious "curves," with absence of peaks and changes in absorption intensities, can be obtained by varying the condenser aperture and the wavelength at which the microscope is focused. As a result of these findings the earlier work of one of us (W. K. M.) was not extended.

FIG. 3.—Absorption curves of single R.B.C. (Type III). Ordinate: Optical density. Abscissa: Wavelength in $m\mu$. (After Kobayashi and Watanabe.)

The Soret absorption band of individual red cells can be demonstrated very simply by illuminating the microscope with monochromatic light derived from a mercury arc with filters or from a monochromator (Huggins, 1865; Wilkins and De Carvalho, 1953). The intense absorption of the haemoglobin in the cells in the violet region can be clearly seen. This absorption falls away very rapidly in the blue and green. We have examined smears of red blood cells in this way from a wide variety of haematological diseases (including myeloid leukaemia and aplastic anaemia), and in no case was the Soret band absent, although the absorption is somewhat diminished in hypochromic anaemia owing to the well-recognized diminution in the intracellular haemoglobin concentration in this disease.

This simple experiment is sufficient to show that the Soret band is present in these haematological diseases, and a more formal microspectrographic analysis will be published in due course.

Summary

Accurate absorption measurements have shown that intracellular and extracellular haemoglobin have identical absorption maxima at all wavelengths in the visible spectrum. Observations of erythrocytes in monochromatic violet light confirm the presence in them of the Soret absorption band in a number of haematological disorders, including aplastic anaemia.

The differences in absorption between "leukaemic" and "aplastic" red cells described by Kobayashi and Watanabe are, we believe, due to experimental errors in their technique.

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The Department of Scientific and Industrial Research is to replace the present Fuel Research Station at Greenwich by a new research station at Stevenage. Work has already begun on the new station, the estimated cost being about £750,000. The station should be completed early in 1959. Among the work to be transferred to Stevenage is that on atmospheric pollution and its prevention. It is hoped to provide a fuller scientific service in this field for the Ministry of Housing and Local Government, to assist it in making the administrative decisions necessary under the Clean Air Act regulations.