and its accuracy appears to be as great as any other test. The test is based upon the inhibition by penicillin of the growth of a suitable group A β-hæmolytic Streptococcus—a 5 per cent suspension of washed sheep cells being used as an indicator. The penicillin to be tested is diluted to about 1 unit per ml. From this dilution quantities 0·20–0·10 are measured in 10 per cent steps, by means of a micrometer syringe, into 8 cm. × 1 cm. tubes containing 1 ml. of nutrient broth. To each tube is then added 0·2 ml. of a suspension of 500–700 × 10^6 organisms per ml. and 0·8 ml. of sheep cells. The tubes are then mixed by invertion and incubated in a water bath at 37° C. for approximately 3–3½ hr. They are then centrifuged and read for hæmolysis. A suitable standard and controls are included with each rack of tests.

The most critical factor in this test is the maintenance of the culture in a suitable state so that the suspension of organism does not contain any preformed hæmolysin. This is achieved by growing the culture on plain agar—and washing off with broth immediately before use.

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Colour of Red Blood Corpuscles

IN a recent communication[1], Dr. J. R. Baker has asked why a single red blood corpuscle looks yellow instead of pink. Whether a thinned-out red colour is pink or yellow depends on the extent of its absorption in the blue end of the spectrum. Let us for simplicity regard white light as composed of equal parts of red, green and blue. Imagine two coloured substances with extinction coefficients as follows :

	Extinction coefficients		
	Red	Green	Blue
Substance 1	0·3	0·7	0·7
Substance 2	0·3	0·7	7·0

At a certain thickness substance 1 transmits 5 per cent red, 0·1 per cent green and 0·1 per cent blue, while substance 2 transmits 5 per cent red, 0·1 per cent green and 0·00 per cent blue. At such thicknesses and above both substances transmit so little green and blue as to be red in colour. At a thickness about one fifteenth of this the proportions transmitted are—substance 1 : 74 per cent red, 67 per cent green and 67 per cent blue ; substance 2 : 74 per cent red, 67 per cent green and 0·1 per cent blue. The colour of the first substance is 201 parts white (equal parts red, green, blue) and 7 parts red, that is, pink, while that of the second is composed of red and green which additively combine to give the sensation of yellow. If a different light source having a different red : green : blue ratio is used the tints will naturally be modified. The yellow colour of red blood corpuscles seen singly merely indicates the presence of a substance of high extinction coefficient in the blue region of the spectrum. The identification of a coloured substance is more satisfactorily made on the basis of its *absorbing* rather than on its *transmitting* qualities.

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[1] NATURE, **152**, 331 (1943).

THE fact to which Dr. Baker directs attention can be readily understood if the absorption spectrum of hæmoglobin be considered in its entirety. In all the text-books attention is concentrated on the characteristic absorption bands in the yellow and yellowish-green, and the lower end of the spectrum is neglected. The very strong absorption in the violet, which helps to determine the colour of hæmoglobin at all concentrations, has therefore escaped the notice of many men of science.

A strong solution of oxyhæmoglobin absorbs completely all rays below the orange-yellow (585 mμ) and transmits nearly completely all those above it. The boundary (which corresponds to the upper margin of the α absorption band) is very sharp. The colour of such a solution is intense scarlet ; on progressive dilution it changes through vermilion to a somewhat dull orange, and eventually to the pale straw-yellow of a single corpuscle under the microscope. The changes in the absorption spectrum that accompany these changes of colour are as follows. First a dim zone of transmission appears in the green, at about 505 mμ ; its upper limit (the lower margin of the β band) is fairly sharp, but on further dilution its lower limit advances steadily through the blue-green and turquoise. Simultaneously with this extension a transmission zone in the yellow-green (560 mμ) appears, separating the α from the β band. The bands then become thinner and fainter, but at this stage absorption below the mid-blue (470 mμ) is still almost complete ; and even at very high dilutions, when the β band is invisible and the α band very faint, absorption in the violet, below 425 mμ, is quite conspicuous.

Roughly speaking, therefore, one may say that a dilute solution transmits all but the violet and ultramarine ; a solution of medium strength transmits the red, orange and much of the green and blue-green ; and a strong solution only the red and orange. It is easy to understand that these will appear respectively pale yellow, moderate orange and intense scarlet.

Reduced hæmoglobin shows, as is well known, a single absorption band in the yellow-green, instead of the two of oxyhæmoglobin. This has scarcely any effect on its colour ; its cherry-red hue at moderate dilutions, instead of the orange-vermilion of oxyhæmoglobin, is due to its much less vigorous absorption of the blue rays. But absorption in the violet is extremely strong, so that very dilute solutions have the same pale yellow colour as those of oxyhæmoglobin.

If, instead of diluting the solution, the depth of it through which the light passes is decreased, the results are exactly the same. It may be true of some dyes, as Dr. Baker says, that change of colour on dilution arises from molecular dissociation ; but in most cases the explanation is analogous to that just given for hæmoglobin. This phenomenon of dichroism is much more widespread than is generally supposed. The most striking cases, such as chlorophyll and chlorocruorin, are well known ; but the changes in hue on diluting methylene blue or copper sulphate (shift towards green) or erythrosin (shift towards orange) differ only in degree. Safranin, gentian violet, Bismarck brown and malachite green, which are respectively scarlet, violet, orange-brown and blue-green at moderate dilutions, are all deep ruby-red in concentrated solution.

The importance of dichroism in interpreting the results of colour tests on a microscopic scale is, as