adjusted, though in the previous year 68 eye accidents had been recorded.

Section 49 of the Factories Act, 1937, provides that "in the case of any such process as may be specified by regulations of the Secretary of State (now the Minister of Labour), being a process which involves a special risk of injury to the eyes from particles or fragments thrown off in the course of the process, suitable goggles or effective screens shall, in accordance with any directions given by the regulations, be provided to protect the eyes of the persons employed in the process."

Compensation

I cannot enter here into the important question of compensation for industrial workers who are the subject of eye injuries, but several ophthalmic surgeons have kindly directed my attention to two points of some practical importance. One is that delay in the payment of compensation for an eye injury often has a prejudicial effect on the patient's general health, irrespective of any question of malingering; the other is that in weighing up the effect of the loss of one eye in a patient the ophthalmic surgeon should take pains to ascertain whether this has been the "master eye." If this is so, the effects on residual vision will be more serious than if the remaining eye is the "master eye."

Conclusion

It is encouraging to know that modern methods of prevention have greatly reduced the risk of eye lesions from chemical and other processes and that the results of medical research and progress are promptly applied. Eye accidents in mines, quarries, and factories constitute by far the largest proportion of cases of impaired eyesight or of blindness among industrial workers. Their prevention is largely a question of periodical medical examination, of education, of the use of protective appliances in industry, and of prompt ophthalmic treatment.

Ophthalmic surgeons will find in industrial eye-work not only a most interesting special branch of ophthalmology but also opportunities for investigation which may still further prevent impaired eyesight or blindness among industrial workers.

I must express my thanks to Sir John Parsons, Lieut.-Colonel Sir Stewart Duke-Elder, Mr. N. Bishop Harman, Mr. Spencer Walker, Miss Ida Mann, Dr. J. C. Bridge (H.M. Senior Medical Inspector of Factories), and Mr. E. W. Murray of the Home Office Industrial Museum for help and advice in the preparation of this lecture.

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HAEMOGLOBINOMETRY

BY

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The need has recently become apparent for a rapid method for the determination, with sufficient accuracy for clinical purposes, of total blood pigment in samples of whole blood (cf. Campbell, 1941). Such determinations should include not only oxyhaemoglobin and reduced haemoglobin, but also the abnormal pigments methaemoglobin and sulphaemoglobin, which are at times encountered in patients such as those to whom sulphonamide drugs have been administered, and CO-haemoglobin. The method should be suitable for performance under conditions in which facilities are limited.

I have for some time past been using a method of total blood-pigment determination in which all haem pigments present are converted into pyridine-haemochromogen, and the intensity of absorption of the latter around 550 m μ is measured upon the Hilger-Nutting photometer. The results so obtained possess a high degree of accuracy, and have the added advantage that they may be referred to a pure crystalline chemical substance—haemin—by means of which the instrument is calibrated. This method is based upon the procedure described by Roets (1940) in South Africa, but the less refined technique which he describes still possesses its main advantages, and is suitable for clinical use in the circumstances outlined above.

I have lately received several independent inquiries concerning the pyridine-haemochromogen method, and the purpose of this note is briefly to set forth its principal features.

The methods available for the determination of haemoglobin may be classified as follows:

Type of Method	Remarks
1. Gasometric: O ₂ or CO capacity	Precise, but requires specialized apparatus
2. Colorimetric: CO-haemoglobin	Rapid. Standard: stock CO-Hb solution. Requires source of coal-gas or CO
Acid haematin	Erratic. Standard: a coloured glass (Newcomer) or acid haematin from similar blood. Plasma constituents affect colour
Alkaline haematin	Less erratic than preceding. Standard: a similarly treated standardized blood
Cyanhaemoglobin, etc.	Colorimetric comparison with standards prepared from standardized stock blood
3. Mechanical aids	A variety of machines provided with coloured glass disks to correspond to concentrations of one or other Hb derivative
4. Chemical: Iron content	Precise but tedious. Assumes non-haemoglobin iron to be negligible
5. Spectrophotometric	Elaborate, requiring expensive and bulky apparatus
6. Pyridine-haemochromogen	Roets (1940) and present article. Rapid. Precise when using spectrophotometric apparatus. Suitable accuracy for clinical purposes when using hand spectroscope or colorimeter. Standard: pure crystalline haemin

It is not my intention further to discuss methods 1 to 4. Some general consideration may be given to the spectrophotometry of haemoglobin pigments.

When light passes through a solution of any substance which obeys Beer's law, and the depth of layer and incident

intensity remain constant, light absorption is proportional to the concentration. Greater sensitivity may be obtained by operating with monochromatic or nearly monochromatic light (obtained by coloured glass filters) of wavelength corresponding to the peak of an absorption band shown by the substance under examination.

With the advent in recent years of cheap photo-electric cells, various designs of photo-electric colorimeter have appeared, all operating on this principle. It is necessary, however, in order to use such instruments for quantitative determination, to calibrate them in terms of known concentrations of the substance to be measured, and, unfortunately, haemoglobin pure enough for such a purpose is obtainable only with the utmost difficulty. Alternatively, one can accept a figure in the literature for some function of pure haemoglobin, such as oxygen capacity, and on this basis analyse the solutions used for calibration, but such a procedure is obviously less satisfactory than one employing known amounts of a pure standard. By using crystalline haemin—a pure stable substance the structure of which is accurately known—as reference standard and converting it into pyridine-haemochromogen, instruments may be calibrated to measure the absorption in the region of 550 to 555 m μ , which embraces the peak of the very intense α band of the haemochromogen.

The advantages of utilizing the absorption band of a haemochromogen have been set forth by Dénes (1932) and Deutsch (1934), but in both the methods proposed by these authors globin-haemochromogen and not pyridine-haemochromogen was the substance produced. Pyridine-haemochromogen, as used in Roets's (1940) method, has also been employed for the determination of haematin in liver catalase by Zeile and Hellström (1930), Keilin and Hartree (1936), and Stern (1937), the last-named describing a simple adaptation of the hand spectroscope to form a spectrocolorimeter.

Method

A 1 in 500 dilution of blood in decinormal sodium hydroxide is made, using a standard or calibrated pipette. This dilution may, of course, be made in two steps, if it is preferred, as in the worked example quoted below. After a few minutes at room temperature a convenient volume, such as 10 c.cm., is measured into a small flask and one-fifth of the volume of pyridine is added. Upon mixing by gentle rotation the conversion of the haem pigments into the alkaline parahaematin is completed within a few seconds; hence there is no necessity to allow the original blood dilution to stand for 10, 20, or even 40 minutes before proceeding, or alternatively to heat in a boiling-water bath as is required when the alkaline-haematin method is used.

A pinch of good-quality sodium hydrosulphite (Na₂S₂O₄) is now added to the mixture, and the contents of the flask are swirled gently until the solid has dissolved. The immediate colour change to pink denotes the conversion of the pigments into pyridine-haemochromogen. Without undue delay (to guard against reoxidation) the solution is transferred to a photo-electric colorimeter cell and the light absorption in the region 550 to 555 m μ is measured, using a suitable filter (e.g., Ilford spectrum filter No. 605—yellow-green). From the calibration factor, obtained by using known weights of pure haemin instead of blood and proceeding as above, the haemoglobin content of the blood is then calculated.

Molecular weight of haemin, C₃₄H₃₂O₄N₄FeCl₄ = 651.4

Iron content of haemoglobin = 0.334%

$\therefore$ Molecular weight of haemoglobin = 66,890

$\frac{\text{Haemoglobin}}{\text{Haemin}} = \frac{66,890}{4 \times 651.4} = 25.66$

Should it be contended that the error involved in calculating the molecular weight of haemoglobin from its iron content is undesirably large, the results might alternatively be expressed in terms of haemin or haematin. The extinction ϵ of pyridine-haemochromogen prepared from crystalline haemin and using

a photo-electric absorptiometer with Ilford No. 605 filter and 1-cm. cell was found to be 0.108 at a concentration of 1 mg. per 100 c.cm.*

Should no photo-electric colorimeter be available, the concentration of the pyridine-haemochromogen solution may be determined by one of the alternative methods, such as colour comparison in a colorimeter with a standard prepared from haemin (standard solutions of haemin in alkali should be kept in the dark) or dilution while viewing with a hand spectroscope until the intensity of the α absorption band matches that of a standard, as described by Roets (1940). This last method is certainly the most simple and rapid, although not quite so accurate as the use of a colorimeter. Pure haemin is easily prepared according to Gattermann (1937), but should be recrystallized as follows:

5 grammes of haemin is dissolved in 15 c.cm. of pyridine, and 40 c.cm. of chloroform is added. The solution is passed through a No. 3 or No. 4 sintered-glass filter to remove any protein and is run into a mixture of 250 c.cm. acetic acid + 5 c.cm. saturated NaCl + 4 c.cm. concentrated HCl, which is maintained at water-bath temperature. After cooling to room temperature the crystals are filtered and washed successively with 50% acetic acid, water, alcohol, and ether, and then dried.

I have used the pyridine-haemochromogen method with equal success for specimens of plasma and urine containing haemoglobin, myohaemoglobin, or mixtures of these with their derivatives. It is only necessary to adjust the initial dilution so that the concentration of pigment in the final solution falls within the comfortable range for measurement.

Example.—1 c.cm. of blood was diluted to 100 c.cm. with decinormal sodium hydroxide. To 1 c.cm. of this solution was added 3 c.cm. of the alkali, then 1 c.cm. of pyridine and, after mixing, a pinch of sodium hydrosulphite. In a 1-cm. cell the reading on the Hilger-Nutting photometer at 552 m μ was 0.573. The calibration for this instrument had been found to be 0.62. Hence:

$$\begin{aligned} \text{Hb per c.cm. of blood} &= 0.573 \times 0.62 \times 500 \\ &= 177.6 \text{ mg. per c.cm.} \\ &\text{i.e., } 17.76\% \end{aligned}$$

The same result was obtained whether the determination was completed within five minutes or after allowing the mixture with alkali to stand for one hour and that with pyridine for a further half an hour.

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* This figure was kindly determined for me by Dr. C. J. O. Morris, to whom my thanks are due. Two specimens of haemin were used, the one prepared by me from ox blood, the other by the late Sir Patrick Laidlaw from sheep's blood, and the values obtained were found to differ by less than 1%. Dr. Morris has used the pyridine-haemochromogen method for the determination of haemoglobin in more than 100 samples of blood and has found the method to be eminently satisfactory.

The Ministry of Health announces (Circular 2549) that it has been arranged for persons undergoing vocational training at the Ministry of Labour's centres to be referred, where necessary, to hospitals in the Emergency Hospital Scheme for a specialist's opinion as to (a) their physical suitability for the training or (b) any possible harmful effect and the advisability of modifying or even discontinuing the training. Where the hospital staff are remunerated on the "occupied-bed" basis these cases will be treated in the same way as Service cases. Attendance at the hospital, whether for examination or out-patient treatment, is to be, so far as possible, at a stated date and time for each case. Patients will contribute towards the cost of maintenance and treatment in hospital unless the latter is for a war or war service injury. Medical officers are to be appointed to the training centres, and reference to E.M.S. hospitals will normally be made by them.