given up expectorants in the treatment of bronchitis. In acute bronchitis—a condition which tends to spontaneous cure—we rely chiefly on rest in bed, an abundance of hot drinks, steam inhalations, and sedatives to control an irritating cough; in chronic bronchitis, on general measures as they affect physical condition, climate, environment, occupation, etc., and, since chronic bronchitis of any standing inevitably connotes bronchiectasis, we advise such patients as seem capable of it to practise postural drainage every morning and at intervals during the day. This can be achieved very simply by a patient stretching himself across a bed or couch with the trunk hanging steeply over the side and coughing till no more sputum is produced.

Lobar Pneumonia: Bronchopneumonia

In spite of the amazing success of sulphapyridine (M & B 693) and allied preparations in this disease there is still need for the well-established non-specific methods of treatment: measures to achieve rest and sleep, good nursing, a sufficiency of fluids, and oxygen. Special attention has always been directed towards the heart. Most doctors seem to visualize a heart poisoned by infection gradually dilating as it becomes flabbier till in the end it fails. Yet seldom, even in fatal cases, are the classical signs of congestive heart failure seen. Moreover, in a radiological study of 119 hearts in pneumonia by Davies, Hodgson, and Whitby (1935) definite enlargement was witnessed in no more than three. It is not cardiac failure which is to be feared; it is peripheral circulatory failure. This is due to loss of contractility of the muscular coat of the peripheral arterioles, with consequent stasis of the blood in the capillaries.

If this be the case, the uselessness of so-called "heart tonics" is manifest, even if they can ever be proved to have any stimulating effect on the myocardium. Certainly alcohol, whatever its value as a food or a sedative, has no such action, nor have strychnine and camphor, while strychnine does not appear to exert any effect on the poisoned peripheral vessels. Digitalis—in popular medical opinion the most potent of all "heart tonics"—is, after all, a poison, and its beneficial effects must be very convincing to warrant the further poisoning of an already poisoned organ. As a matter of fact, its effect in lobar pneumonia has been carefully investigated by many workers, among whom Wyckoff, DuBois, and Woodruff (1930) concluded that its routine use was dangerous and in large doses increased the mortality; while Cohn and Lewis (1935) in an analysis of 1,456 cases found that it did not influence the course of events. Whatever be the value of expectorants under any circumstances, they should logically have little place in the treatment of lobar pneumonia unless there is an associated bronchitis, for most of the exudate is absorbed and not expectorated.

All we have said about the heart, the circulation, heart tonics, etc., in lobar pneumonia holds also in bronchopneumonia. The value of such preparations as sulphapyridine in the individual case is, however, much more a matter of trial and error. Success or failure probably depends on the extent to which the pneumococcus is responsible for the infection, and this is difficult to assess.

Constipation

Incredible quantities of purgatives are ordered daily by doctors, in most cases needlessly and in many harmfully. In the first place to treat roughly and ignorantly a marvellous piece of mechanism like the intestinal canal, with its delicate epithelium, its wonderful secretory and absorptive apparatus, and its beautifully balanced rhythmic movements, is little less than an outrage. There is an ingrained belief in doctors and patients alike that manifold evils will result if the bowels are not opened once at least every twenty-four hours. Yet, as Gee insisted many years ago, this is not a natural law, and many people feel in better health when the bowels act once in every two or three days. There is little or no evidence to support the widely held opinion that auto-intoxication results from simple constipation: faeces are, after all, meant to dry in the distal colon, and toxic absorption is far more likely to derive from fluid faeces than from dry scybala. To quote Witts (1937): "Apart from mechanical distension, a constipated stool exerts little more influence on the patient in the colon than in the bed-pan." Again, in many people who complain

of constipation the faeces arrive normally at the pelvic colon, but subsequent evacuation is not adequately performed owing to loss of the conditioned defaecation reflex, generally caused by continued neglect to respond at once to the call to stool. It is, however, in the constipation met with in acute infections that purgatives may be definitely dangerous. Here constipation is chiefly due to dehydration, and to dehydrate further an already dehydrated patient seems hardly logical. Once more, though there has been of late a change of mind on the part of most surgeons as regards the preparation of patients for operation, the practice of preliminary purgation in all cases still obtains in some quarters. To dehydrate deliberately before operation and to use all kinds of ingenious means to rehydrate immediately after would seem to be the acme of illogical thinking.

We ourselves have less and less recourse to purgatives with the years. Much can be done by stressing the harmlessness of constipation. "The ill effects of constipation are almost entirely psychogenic and due to worry over disturbance of habit" (Witts, 1937), and anxiety about the action of the bowels is probably in itself a fruitful cause of constipation. Much, again, can be done by insistence on the necessity for cultivating a regular habit, diet, exercise, the provision of a low lavatory seat or the adoption of a squatting posture for defaecation, and the lubricant, liquid paraffin. There is seldom any urgency to open the bowels in acute infections under four or five days, and then this should be done by enemata and not by purgation. Enemata are almost certainly not used enough in the treatment of chronic constipation; a regular habit can often be regained by their daily use in constantly decreasing amounts.

Our thanks are due to Drs. John Parkinson, Donald Hunter, Hugh Dunlop, and Clifford Hoyle for their helpful criticism of various points we have tried to make in this very incomplete and discursive paper.

REFERENCES

- Beaumont, G. E., and Dodds, E. C. (1941). *Recent Advances in Medicine*, London.
Clark, A. J. (1937). *Applied Pharmacology*, London.
Cohn, A. E., and Lewis, W. H., jun. (1935). *Amer. J. med. Sci.*, **189**, 457.
Davies, D. T., Hodgson, H. G., and Whitby, L. E. H. (1935). *Lancet*, **1**, 791.
Evans, W., and Hoyle, C. (1933). *Quart. J. Med.*, n.s., **2**, 311.
— (1934). *Ibid.*, **3**, 105.
Hunter, D. (1938). *Practitioner*, **140**, 11.
Joll, C. A. (1932). *Diseases of the Thyroid Gland*, London.
Vaughan, J. M. (1932). *Lancet*, **1**, 122.
White, P. D. (1937). *Heart Disease*, New York.
Witts, L. J. (1930-1). *Proc. roy. Soc. Med.*, **24**, 543.
— (1937). *Lancet*, **1**, 427.
Wyckoff, J., DuBois, E. F., and Woodruff, I. O. (1930). *J. Amer. med. Ass.* **95**, 1243.

OBSERVATIONS ON THE ACID HAEMATIN METHOD FOR THE ESTIMATION OF HAEMOGLOBIN

BY

C. A. ASHFORD, M.A., Ph.D.

(From the Department of Physiology, University of Bristol)

The acid haematin (Sahli) method is one which is widely used for the estimation of haemoglobin, and is recommended (M.R.C. Report, 1943) for use when the Haldane method cannot be employed. Though not ideal, it has several points in its favour—e.g., the reagents are simple and independent of a supply of CO, and the colour obtained with 20 c.mm. of blood is strong enough to read easily in the Haldane type of haemoglobinometer tube. While the conversion to alkaline haematin has many advantages (Clegg and King, 1942) the colour produced in similar concentrations is much weaker, and consequently comparison, particularly visual, is more difficult. A serious disadvantage of the acid haematin method, however, is that the fluid is a suspension and not a true solution (Barcroft, 1928). Considerable sedimentation has usually been observed in 24 hours at room temperature in several hundred samples investigated in this department. Moreover, occasional samples of blood give suspensions of such turbidity that they are impossible to read.

A further disadvantage is the confusion which exists regarding the length of time necessary for colour development. This lack of uniformity is illustrated by the following examples: Myers (1924) and Beaumont and Dodds (1941) give 2 hours as the time necessary at room temperature, while Whitby and Britton (1939) give 30 to 40 minutes. Stewart and Dunlop (1937) say: "Compare after 2 minutes," while the University of Glasgow Standing Committee (1940), as in the original Sahli (1909) technique, recommend comparison after one minute. Smith (1941) considered 10 minutes a suitable time for clinical purposes, and Campbell (1942) reported agreement with the Haldane method by comparing after 7 or 8 minutes. No doubt other workers use different times. These and other points were commented upon briefly by Ashford (1942). Since complete colour development undoubtedly requires time, it follows that divergent findings must result from the use of different times before comparison with a permanent standard. The time would, of course, be immaterial if a sample of blood of known Hb content was similarly treated at the same moment as the unknown. When, however, a standard such as the Newcomer disk (1919) is used the result will be inaccurate unless comparison is made at the time for which it has been calibrated by the makers. It would appear that this instruction has not always been supplied, or, if it has, has been neglected by the user. It would be advantageous to have agreed uniform conditions, and certain recommendations in that direction are made in this communication.

The Time Relations of Colour Development

A. At Room Temperature.—In the following experiments a Hellige colorimeter was used, and the "standard" was either a coloured glass of the Newcomer type or, more usually, a solution of acid haematin made up some 18 to 24 hours previously and filtered before use if not absolutely clear. A stop-watch was started at the moment when the sample of blood (20 c.mm. or a proportionate amount) was delivered into 5 ml. of N/10 HCl and mixed. The standard being already in position, the fluid was transferred to the vacant cup of the instrument and readings taken as rapidly as possible. The temperature was that of the room (14° to 18° C.). In these and in many other observations no increase of colour was observed after 2 hours, and this value was accordingly adopted as "100%" and the intermediate figures calculated as fractions of it. A typical curve obtained with normal fresh human blood is reproduced (see Graph).

Graph showing rate of colour development at room temperature.
Fresh human blood.

It will be seen that the colour development is initially very rapid, reaching 75% in 1 minute and 88% in 5 minutes. Essentially the same kind of curve was obtained in a number of experiments with ox blood. (In a few experiments it has been noticed that the rate of alkaline haematin formation at room temperature is very much slower in ox than in human blood.)

Comment.—The rate is somewhat slower than that reported by Newcomer (1919), and it is presumed that his room temperature (not stated) must have been higher than those obtaining in this laboratory. Plesch (1941) claimed 88% colour development in 2 minutes; this is higher than either of our figures. It follows that if a solution is compared at 15 minutes against a standard calibrated for 5 minutes the result will be 4 to 6% too high, and after 2 hours some 12% too high. Moreover, owing to the varying time (50 seconds to 2 minutes in a series of observations) necessary to make dilution and comparison when using the simple haemoglobinometer technique, the time for reading should not be less than 20 minutes at room temperature, and the colour would then be only 95% of the final

(2-hour) development. It is difficult to see how times of 1 or 2 minutes can be employed with accuracy, owing to the rapidity of the increase of colour in the early stages. To obviate the delay and to obtain complete colour development in a shorter time the effect of temperature was studied.

B. Experiments at 40° and 100° C.—It was not practicable to maintain the stage of the colorimeter at 40° to 45° C. or higher. The samples were therefore usually measured into tubes containing the acid and fitted with air-condensers to prevent evaporation. They were immersed in the water-bath for the time shown in Table I, in

TABLE I.—Rate of Colour Development at Different Temperatures. (The Figures are the Readings in mm. of the Colorimeter Scale, the "Standard" being Constant in Each Experiment)

Species	Temperature °C.	Time t	Reading at Time t (R _t)	Reading after 5 min. at 100° C. (R ₁₀₀)	Ratio $\frac{R_t}{R_{100}}$
Human	Room	2 hours	21.35	17.00	1.26
		2 "	21.30	16.80	1.265
		10 min.	21.30	16.50	1.29
	40-45	10 "	21.40	16.80	1.27
		5 "		16.80	1.265
		5 "		19.15	1.25
Ox	Room	3 hours	24.00	18.80	1.27
		2 "	23.90		
		5 min.	24.60		
	40-45	5 "	24.50		
		15 "	23.80	19.20	1.24
		15 "	23.60		

Where the experiment was carried out solely at 100° the value of R_t used is that obtained at room temperature.

which two examples of results obtained with human and ox blood are given. Table II similarly reports an example of the course of the reaction at 40° to 45° C. The tubes were rapidly cooled in melting ice before making the comparison. In all experiments a sample measured with the same pipettes was allowed to stand for

TABLE II.—The Rate of Colour Development at 40° to 45° C. Ox Blood. (Readings of the Colorimeter Scale in mm.)

Temperature °C.	Time t	Reading at Time t (R _t)	% of Final Reading	Reading after 5 min. at 100° C. (R ₁₀₀)	Ratio $\frac{R_t}{R_{100}}$
Room	2 hours	29.92	100.0	23.3	1.28
	5 min.	30.30	98.0		
	10 "	30.25	98.5		
42-45	15 "	30.10	99.3		
	20 "	29.90	100.0	23.9	1.25
	5 "	30.60	97.6		
40-42	10 "	30.40	98.4		
	15 "	30.10	99.3		
	20 "	29.90	100.0	23.8	1.25

at least 2 hours at room temperature, and this value was used as the final, or 100%, figure. It thus appears that incubation in a water-bath at 40° to 45° C. for 10 to 15 minutes gives results very close to that figure. As this was in a large volume of fluid in which the attainment of the raised temperature would be slower, there is little doubt that under the usual conditions of the estimation, when 20 c.mm. of blood is delivered into less than 1 ml. of acid, the reaction is at least 99% complete in 10 minutes. This technique has been adopted, with satisfactory results, for many rapid determinations in this laboratory. When the tubes fitted with condensers were immersed in a boiling-water bath for 5 minutes and cooled in melting ice before reading, an increase of colour of about 25% was observed. This is indicated in Tables I and II by the ratio R_t/R₁₀₀, since the concentrations are inversely proportional to the colorimeter readings, the standard being at the same mark throughout each individual experiment. It was found also that this increased colour production was complete in 3 to 4 minutes. This finding is a further reason for the standardization of conditions and calibrations. It also conflicts with the statement of Berman (1919) that boiling for one minute over a free flame gives the same degree of colour as 20 minutes at room temperature. As the fluid obtained at 100° C. is often rather turbid it is not suitable for use as a routine procedure.

Choice of a Standard

There is no doubt that for occasional routine estimations a permanent standard of the Newcomer glass type, properly calibrated and used under standard conditions, is the method of choice. Moreover, the tube employed should be rectangular in shape, since the standard has a plane surface; and it is an advantage if two pieces of glass, between which the unknown is placed, are employed. Furthermore, the tube should be graduated in terms of g. Hb per 100 ml. (or the Haldane scale), owing to the varying meanings which may be attached to the expression "100% Sahli" (see Ashford, 1942).

When a large number of estimations are to be made and a glass standard suitable for use in a colorimeter is not available, it is preferable to use a standard of blood of known Hb content similarly treated with acid. The standard made by dilution from a stock 3% solution of acid haematin (Cohen and Smith, 1919) has not been found entirely satisfactory, being usually more turbid than the fluid from a fresh blood, and the turbidity tends to increase with the age of the strong solution. As this is a thick suspension it is unlikely that the state of dispersion of the solution diluted from it would be identical with that of a freshly made suspension. The procedure therefore adopted was to prepare the acid haematin standard by appropriate dilution with N/10 HCl, from ox or human blood whose oxygen capacity had been determined when fresh by the Barcroft method, and which had been stored in the refrigerator, just before starting to draw the series of blood samples. This technique is justified by the observation that the rate of formation of acid haematin was unaffected by the storage of the blood, at any rate up to 2 weeks.

It has been further shown that it is permissible to use ox blood as standard when human blood is under test. Palmer (1918) stated that this was not so; but Cohen and Smith, who studied several species, did not agree with him. An example of our experiments is shown in Table III. It will be seen that

TABLE III.—Comparison between Samples of Ox and Human Blood. Ratios of Haemoglobin Content Calculated (a) from Total Oxygen Capacity, and (b) from Direct Colorimetric Comparison after Conversion to Acid Haematin

Species	g. Hb per 100 ml.	Ratios	
		(a)	(b)
Ox No. 2	12.35	1.00	1.00
" " 3	14.60	1.18	1.22
" " 4	13.00	1.05	1.03
Human No. 1 ..	16.35	1.32	1.36
" " 2 ..	12.20	0.99	1.02
" " 3 ..	10.50	0.85	0.855
" " 5 ..	9.20	0.745	0.72
" " 6 ..	10.40	0.84	0.83
" " 7 ..	11.05	0.895	0.91
Mean of human figures		0.94	0.95

the ratios calculated from oxygen capacity and by comparison after conversion to acid haematin are very similar. The experiments have included "normal" and weaker samples of blood; none have been made in the presence of abnormal pigments—e.g., COHb or MetHb.

Discussion

The disadvantages of the acid haematin method have been summarized by Clegg and King (1942). Wu (1922) showed that the colour obtained depended on the plasma proteins, but concluded that in all normal samples variations in protein and lipid content were insufficient to cause an error. In fact, his figures showed that the amount of plasma could be increased 3 or 4 times above the normal ratio without causing an appreciable change, though there was a 20% deficit in colour when plasma-free Hb was used. His results were obtained with sheep's blood; if applicable to human blood they would suggest that the method can be used accurately over a wide range of anaemic bloods (cf. Table III). Nevertheless Wu favoured the conversion to alkaline haematin because abnormal pigments such as COHb, MetHb, and CyanHb gave a depth of colour in acid up to 15% above that of HbO₂. It does not appear likely, however, that the amounts of these pigments which may be present in human blood would appreciably affect the colour.

Summary

Formation of acid haematin from ox and human whole blood reaches completion at room temperature (14° to 18° C.) in 2 hours; the same depth of colour is obtained in 10 to 15 minutes at 40° to 45° C. The latter procedure is recommended for routine technique, and the permanent standard should be calibrated accordingly.

Heating at 100° C. for 4 to 5 minutes causes an increase of some 25% in colour. Boiling should therefore be avoided.

A standard obtained by dilution from a 3% stock solution of acid haematin was not found satisfactory. An alternative procedure is suggested.

Ox blood can be used as standard when human blood is being assayed.

My thanks are due to Prof. R. J. Brocklehurst for his interest in this work.

REFERENCES

- Ashford, C. A. (1942). *British Medical Journal*, **1**, 163.
 Barcroft, J. (1928). *The Respiratory Functions of the Blood: Haemoglobin*, **2**, 17, Camb. Univ. Press.
 Beaumont, G. E., and Dodds, E. C. (1941). *Recent Advances in Medicine*, 10th ed., Churchill, London.
 Berman, L. (1919). *Arch. Intern. Med.*, **24**, 553.
 Campbell, R. D. (1942). *British Medical Journal*, **1**, 88.
 Clegg, J. W., and King, E. J. (1942). *Ibid.*, **2**, 329.
 Cohen, B., and Smith, A. H. (1919). *J. biol. Chem.*, **39**, 489.
 M.R.C. Report (1943). *British Medical Journal*, **1**, 209.
 Myers, V. C. (1924). *Practical Chemical Analysis of the Blood*, 2nd ed., Henry Kimpton, London.
 Newcomer, H. S. (1919). *J. biol. Chem.*, **37**, 465.
 Palmer, W. W. (1918). *Ibid.*, **34**, 119.
 Plesch, J. (1941). *British Medical Journal*, **2**, 859.
 Sahli, H. (1909). *Lehrbuch d. klin. Untersuchungen Methode*, 5th ed., p. 846, Leipzig.
 Smith, W. (1941). *British Medical Journal*, **2**, 926.
 Stewart, C. P., and Dunlop, D. M. (1937). *Clinical Chemistry in Practice Medicine*, 2nd ed., Livingstone, Edinburgh.
 University of Glasgow Standing Committee on Laboratory Methods (1940). *Notes on Clinical Laboratory Methods*, 4th ed., John Smith, Glasgow.
 Whitty, L. E. H., and Britton, C. J. C. (1939). *Disorders of the Blood*, 3rd ed., Churchill, London.
 Wu, H. (1922). *J. Biochem.*, Tokio, **2**, 173.

LOCAL APPLICATION OF SULPHANILAMIDE POWDER IN RADIOTHERAPY

BY

ALEXANDER A. CHARTERIS, M.B., Ch.B.,
D.P.H., F.R.F.P.S.

Radium Therapist and Donald Research Scholar for Cancer,
Western Infirmary, Glasgow

Sepsis presents a constantly recurring problem in radiotherapy, and its prevention or treatment merits the most careful consideration. Descriptions have previously been given of the antiseptic action of certain acridine compounds, and the success of proflavine in radiotherapy has been stressed (Charteris, 1937; Young and Charteris, 1940). In view of the increasing interest in local application of this substance, it is gratifying to find that my early estimate of its efficacy remains unchanged, and that its proper use makes various radiotherapeutic procedures possible and safe. This is especially true as regards superficial ulcerating growths, also tumours in the nose and its accessory sinuses, and in the realm of gynaecology. I have used proflavine oleate 1% in liquid paraffin, and the original preparation has been improved by the addition of enough oleic acid to produce complete solution.

There is a further important field for antiseptics, however, in the form of powder preparations where the parts must be kept dry, and this refers particularly to the after-treatment of patients, in contrast to their preparation and actual irradiation, when proflavine in an oily vehicle is usually excellent. For dry applications sterile sulphanilamide powder was chosen. It has been used in a large number of cases since early in 1942, and proved most satisfactory. There are three main kinds of application.

Methods of Application

The first and most general of these is in the care of unbroken skin undergoing a dry reaction after radiotherapy. If moist desquamation is to be avoided the skin must be protected against friction and pressure, besides being kept clean and dry. For the latter purpose powder dressings are excellent, and not only is sulphanilamide powder suitable in this sense but it also eliminates the risk of chronic pustules forming. A special use is found in the case of reactions about the vulva or anus, or in any place where apposition of the parts makes a more severe reaction likely, apart from any local variation in tissue sensitivity: regularly changed dry applications on gauze will at least limit desquamation in such situations if carried out two or three times in the day, according to circumstances.

Where it has been necessary to subject skin to something approaching maximal irradiation in order to deliver an adequate "tumour dose," moist desquamation is inevitable, and its treatment presents the dual problem of minimizing discomfort and diminishing post-radiational stigmata so far as is possible.