varied, owing evidently to lack of homogeneity in the material. This experiment was done in July. A much more elaborate repetition was begun in January, when heavily infected dust was obtained by passing a vacuum-cleaner over the bed-clothes of four patients known to have haemolytic streptococcal infections. This dust was filtered through gauze to remove coarse particles, yielding a dense fine grey powder, of which 20 mg. was kept in each of many tubes either in the dark or exposed to north daylight, and cultivated quantitatively from time to time. Neither in tubes nor in Petri dishes in which their contents were subsequently spread out as far as possible did this dust cease to yield haemolytic streptococci in culture until April 2, the experiment having been begun on Jan. 18. Winter conditions may have accounted partly for this long survival, but a more important reason was probably the nature of the material itself; ultra-violet light failed repeatedly under various conditions to kill more than a proportion of the streptococci in it, and it may be that dust in this artificially concentrated form is more protective to contained bacteria than the looser aggregations of natural dust.

The main interest of this experiment was the survival period in the dark. The original number of living haemolytic streptococci per gramme of dust was 204,000; succeeding counts were 176,000 on Jan. 25; 5,900 on March 19; 8,700 on April 2; 2,700 on May 4; 700 on June 4; and 300 on August 2, when the last available tube was cultivated. The three colonies in this last culture were all of Group A, and that sent to Dr. Elliott proved, as did a subculture from the plate of June 4, to be of Type 11. This type was responsible for the epidemic in progress in the ward when the dust was collected six and a half months earlier (195 days). This is the longest survival on record.

Discussion

These imperfect observations are placed on record in order to draw attention to the possible importance of good natural illumination as a hygienic safeguard, and in the hope that they may lead to further study of this subject. Although good lighting is universally recognized as desirable, it has never, so far as I am aware, been insisted on as a prime necessity in wards for septic surgical cases. This study suggests that in such wards it has an important part to play, particularly if no special measures (such as the oiling of bed-clothes and vacuum dust extraction) are taken to prevent the atmospheric diffusion of dust. It has been shown that haemolytic streptococci naturally present in dust will survive for over six months in the dark. It was noticeable that dark corners on the floors of infected wards were always more liable to yield dust containing haemolytic streptococci than more open situations; one dark recess beneath a bookcase was repeatedly sampled, and never failed to yield them. Prolonged ward epidemics with long intervals between fresh cases are readily explicable in such conditions. It was also found that dust on or close to windows never contained haemolytic streptococci, whether exposed to direct sunlight or not. Whether this is an effect of light, or is partly or wholly explicable on other grounds, can only be settled by further observations.

The quantitative study of bacteria in natural dust *in vitro* is beset by difficulties, and it is not claimed that direct proof of the disinfectant action of light on this material has been obtained. Of the action of light on haemolytic streptococci in another natural medium there can be no question; dried films of pus are of the same nature as infected dry particles liberated from a wound dressing, and form an unexceptionable test object. These are sterilized within a few days by diffuse north daylight passing through two layers of glass, whereas in the dark the streptococci in them remain viable for weeks. My observations on this point differ from those of C. R. Smith, since no glass was interposed between his cover-slip films and the northern sky of California. Preoccupation with the ultra-violet part of the spectrum has led to a common belief that only direct sunlight is usefully bactericidal; it must now be recognized that ordinary diffuse daylight, even on a cloudy day and even in winter in England, can be lethal to bacteria, and that glass is no absolute bar to this effect. The conditions governing this type of light effect would evidently repay further investigation from several points of view.

Summary

In wards where there are patients with haemolytic streptococcal infections dust may contain these organisms in large numbers, particularly near infected patients' beds.

Haemolytic streptococci were found to be most numerous in floor dust, and were absent from many specimens of dust in the same wards collected from sites on or close to the windows. They were more often found in dust from exceptionally dark wards than in comparable specimens from normally lit wards.

Haemolytic streptococci of Group A, Type 11, in naturally infected dust survived in small numbers in the dark at room temperature for 195 days.

Ordinary diffuse daylight is bactericidal to haemolytic streptococci. The interposition of glass does not prevent this effect, and it occurs even under winter conditions in England.

These facts suggest the possibility that good natural lighting may be a factor in preventing the atmospheric spread of infection in surgical wards and elsewhere.

I am indebted for valuable technical assistance in this work to Miss P. M. Waterworth. Her services and the equipment of the laboratory in which most of the work was done were made available by the generosity of "Bundles for Britain," which organization provided a fund under the control of Mr. Rainsford Mowlem for laboratory work in connexion with his surgical unit. I am also indebted to the medical superintendent of the hospital for permission to publish these findings, and to Mr. Mowlem and other members of the surgical staff for allowing me to refer to cases and conditions in their wards.

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A NOTE ON THE TRANSMISSIBILITY OF HAEMOLYTIC STREPTOCOCCAL INFECTION BY FLIES

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During the investigations described in the preceding paper by Prof. L. P. Garrod, when explanations were being sought for cross-infections in surgical wards, attention was directed to the possibility that these might be conveyed by flies. Wounds and septic dressings attract flies, and in some circumstances cannot altogether be protected from them.

In Sept., 1942, when flies abounded in this hospital, it was decided to catch some and cultivate them. They were trapped actually in a Petri dish containing blood agar, one half of the plate being spread with three drops of 1 in 3,000 gentian violet, as recommended by Fleming, and simply incubated in the plates, their busy wanderings on the surface of the medium being relied on to inoculate it with whatever bacteria might be on their feet. After overnight incubation the flies were found dead.

The flies were caught in two surgical wards where there were cases with streptococcal infections; a control series was caught in the laboratory. Of 27 flies caught in these wards 3 gave sterile plates, 9 gave cultures containing haemolytic streptococci, three of which proved to be of Group A, while the remainder grew a variety of other organisms, including coagulase-positive staphylococci and coliform bacilli; 2 plates were overgrown with *Proteus*. Of cultures from 22 flies caught in the laboratory 5 were sterile, 2 overgrown with *Proteus*, and the remainder grew a variety of unidentified organisms; there were no haemolytic streptococci in any culture.

Two of the three Group A strains isolated from ward flies proved to be of Type 4. Since a close check was being kept on all wound and throat infections in these wards, all Group A streptococci being typed by the kind co-operation of Dr. S. D. Elliott, it is possible to trace a hypothetical relationship between a carrier condition in these flies and infections in the human population of the wards. Type 4 had been identified only once previously—in a sore throat in a nurse ten days before the infected flies were caught; it is possible that this nurse conveyed the infection to a wound in which it escaped recognition. During the following two months Type 4 was recovered from eight further persons, six having wound and two throat infections. All these cases occurred in one of the two wards studied, and it was in this ward that both of the Type-4-carrying flies had been caught. After this stage two patients—one admitted to each ward—were found to have a Type 4 wound infection on admission, and 15 further cases in the two wards during an ensuing period of three months from that time are therefore not necessarily traceable to the earlier cases.

These observations do not prove the transmission of haemolytic streptococcal infection by flies, but they strongly suggest its possibility. When infection spreads otherwise inexplicably from one part of a hospital to another this agency should perhaps be considered.

Acknowledgments are due, as in the preceding paper, to the fund by which this study of wound infections was supported, and to Mr. Rainsford Mowlem. The work was done under the direction of Prof. L. P. Garrod, to whom we offer our thanks.

THE HALDANE HAEMOGLOBINOMETER

I. IRON, OXYGEN, AND THE BRITISH STANDARDS INSTITUTION COLOUR STANDARD

BY

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AND

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IN COLLABORATION WITH

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For more than 40 years the Haldane-Gowers haemoglobinometer has been in general use in this country, and for most clinical purposes it has been a satisfactory instrument. When, however, its results, changed by means of the traditional conversion factors into units of oxygen capacity and thence into grammes of haemoglobin, are compared with those of other methods in other countries, a curious discrepancy is apparent which has led to much confusion. Added to this, there is now a demand for a high degree of accuracy, resulting from the widespread adoption of the haemoglobin level as an index of nutrition and of haemoconcentration in shock. Judged by this more exacting requirement, it has become only too clear that the instrument as generally used may give dangerously inaccurate and variable results, and that a complete investigation of its efficiency is urgently needed.

The establishment by Donaldson, Harding, and Wright (*J. Path. Bact.*, 1943, **55**, 205) of a permanent B.S.I. Haldane colour standard, together with a means for accurate comparison at the National Physical Laboratory, has, for the first time, provided the essential fixed point on which such an investigation must be based. We have taken advantage of this standard in certain observations, which, though they have no claim to the desired completeness, may serve to guide further work. The problem of haemoglobinometry has been solved to their own satisfaction by a succession of workers during the past 60 years, but it still exists in all its complexity. We have at least avoided this one pitfall, of supposing our results to be final.

The first step in the present investigation has been an attempt to determine the mean oxygen capacity and iron content of blood of a colour equal to 100% of the B.S.I. colour standard. This attempt, which has met with unexpected difficulties, is here briefly described. The variability of the results of haemoglobin estimation by the Haldane-Gowers method under different conditions and in the hands of a large number of trained observers will be dealt with in a second communication. Finally, the puzzling systematic discrepancy between the results of this method and those of others, bound up as it is with the history of the Haldane standard, will later be discussed in the form of a review of the literature.

Method of Investigation

Material.—Blood was taken by venepuncture, a tourniquet being applied for the minimum time necessary to produce adequate venous distension and being released at the instant of puncture. The blood was heparinized, and within a few minutes of collection was divided into the required number of sub-samples, the vessel being shaken continuously to ensure even sampling.

The subjects were healthy adults (members of the hospital staff and medical students), 29 being males and 20 females. Unfortunately these were not evenly distributed in chronological order, most of the females being towards the end of the series—a fact that has complicated analysis of the results. The figures obtained in one other case, including an obviously erroneous iron estimation, have been omitted.

The following determinations were made on each blood sample and (except for iron determination) on the day of collection.

1. *Oxygen Capacity* (H. M. Jope).—This was estimated by the constant-volume van Slyke method, all measurements being made with carefully calibrated apparatus. Duplicate or triplicate estimations were made and the mean figure taken. In 20 blood specimens the CO capacity was also determined as a check on oxygen measurements. In addition 11 estimations of oxygen capacity have been made by Prof. C. G. Douglas, using the Haldane blood-gas apparatus, in parallel with the van Slyke estimations. The results of the two methods are in good proportional agreement, though the Haldane method gives results averaging 0.35 ml. % lower.

2. *Iron Determinations* (J. R. P. O'Brien and P. Quelch).—Haemoglobin iron was estimated by the Klumpp titanous chloride method, the results being the average of duplicate determinations. The method was standardized against pure iron wire, and all apparatus was carefully calibrated.

3. *Ultra-violet and Visible Absorption Spectra* (E. M. Jope).—A Hilger E3 medium quartz spectrograph and a Spekker photometer with ribbon filament lamp were used. The plates were matched on a photo-electric microphotometer. The results in the visible range were checked by two estimations made in parallel with the National Physical Laboratory spectrophotometer. To save space only the ultra-violet readings are quoted, and these have been expressed as a percentage of the B.S.I. standard on the basis of six determinations in parallel with the N.P.L. comparator.

4. *Haemoglobin Estimations* (B. Amos and R. G. Macfarlane).—The Haldane-Gowers haemoglobinometer was used. B.S.I. standards, diluting tubes, and pipettes were employed, and the necessary correction factors were applied. Each estimation was the mean of independent determinations by both observers, except for a period when one was not available. About half-way through the series both observers made many observations in parallel with colorimetric determinations by the National Physical Laboratory to eliminate personal errors, and this procedure was followed by rather higher readings being obtained from this point onwards. At the time of each estimation the observers were in ignorance of any other estimation on that blood sample—a matter of importance in view of the great susceptibility of this method to bias. In addition to the estimation of colour by the Haldane-Gowers method, six samples of blood were sent to the National Physical Laboratory for direct comparison with the colour standard by means of the special comparator. The figures so obtained were then