STUDIES ON THE BACTERIOLOGY OF
HYPOPYON ULCERI.—The Conjunctival Flora of Healthy
Coal Mine-workers

BY

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Introduction

THE work recorded in this paper is the first of a series of investigations carried out under the auspices of the W. H. Ross Foundation for the Study of Prevention of Blindness. The work was initiated owing to the fact that over half the cases of hypopyon ulcer attending the Eye department of the Royal Infirmary of Edinburgh occur in mine-workers (coal or shale), which appears to be an unduly high percentage. The economic importance of hypopyon ulcers in miners is considerable. In severe cases the eye may be lost, and in all cases a scar remains, seriously interfering with vision, and thus the earning capabilities of the worker.

Although certain ulcers may be purely traumatic in origin, it is generally agreed that the vast majority of cases are infective. Accordingly it was felt that a suitable line of research would be bacteriological. As a preliminary to a bacteriological study of established cases of hypopyon ulcer it was considered advisable to undertake a field study of the conjunctival flora of a large number of healthy mine-workers. In this paper the bacteriological examination of some 658 coal mine-workers will be reported. A parallel study of shale mine-workers will be reported in a separate communication.

Numerous workers have, of course, studied the conjunctival flora (see Duke-Elder, 1938), both in health and disease, and its nature is very well known. I have not been able to find records, however, of any study quite comparable to this, dealing with a specialised class of worker comparatively subject to corneal ulceration and infection.

Methods

Preparation of cultures.—Men were examined at the pit-head, usually coming off their shift. On one or two isolated occasions surface-men had to be examined during the shift, but no examina-

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tions were made before men began work. Cultures were taken from the conjunctival sacs before the men washed, thus obviating any watering due to soap suds.

Cultures were taken from both right and left conjunctival sacs by pulling down the lower lid and drawing a platinum loop from the caruncle laterally. The loop was immediately rubbed over the surface of a blood agar slope contained in a small round screw-topped bottle (see Mackie and McCartney, 1938). These bottles can be strongly recommended for field work of this type, their advantages over cotton-wool stoppered test tubes being as follows: they can be stored for some time without risk of the medium becoming desiccated; contamination with aerial organisms is virtually unknown; they will stand upright on a table and do not need to be supported in racks or boxes; they are very strong and seldom break even if dropped on a stone floor; the screw top is easily detached and flaming of the neck is not really necessary; the layer of medium is comparatively thin and if the bottle is held up to a strong light any colonies showing haemolysis are readily detected; the surface is broad enough to make it possible to secure isolated colonies; in many cases further plating is unnecessary, suspicious colonies being picked and subcultured directly. Blood agar in screw topped bottles forms the ideal medium for these reasons, as well as for ease of manipulation in a rather crowded space. The men, naturally, do not wish to be detained for long, and in actual practice each complete examination does not occupy more than 30-45 seconds.

On return to the laboratory the blood agar cultures were incubated at 37° C. for 48 hours and then examined. With experience it was frequently possible to determine the organisms present by naked-eye examination only, sometimes films were stained, and sometimes subcultures were prepared for further tests.

Classes of workers examined.—(a) At each pit approximately 40 surface workers were examined. These men perform the most varied tasks, some are labourers, bricklayers, masons, or blacksmiths. Others are engineers, electricians, and winding-men. Others again are employed on the picking-tables sorting the coal, while others push hutches (small trucks) of coal away from the cage. None of these men works below ground.

(b) Below-ground workers fall into three classes, oncost-worker, miner, and brusher. Oncost-workers are employed in the transport of the coal. Some work near the coal face, others at the pit bottom. These workers spend most of their time pushing hutches, they never hew coal and are thus not liable to receive flying spicules in the eye. (c) The term "miner" technically refers to the man who "gets" the coal away from the face. In three of the four collieries visited the coal is actually removed from

the face by a mechanical coal cutting machine. The miner in these pits is known as a "stripper," and has to use a pick to break up the coal dislodged by the cutter. In the fourth pit all the work is done by pick, the miner here being called a "hewer," Miners are, of course, very liable to receive a flying splinter of coal in the eye. (d) The brusher is also known as a stone-miner. He is not primarily concerned with coal at all but trims up the rock exposed by the miner. These workers are liable to receive small fragments of stone or coal in the eye. Miners and brushers constitute the main number of those injured in the eye in any one pit, and correspondingly the majority of cases of hypopyon ulcer.

Mines visited.—Examinations were carried out at mine A (189 men), W (149 men), V (160 men) and mine L (160 men), all in the Lothians area of central Scotland. The coal at mines A and V was "soft," and "hard" at W and L. Mines A and V were situated comparatively close to each other, as were W and L. The managements were, however, quite separate and employees did not mix, at any rate during working-hours. Mine A was examined in the late summer and early autumn, mine W in the winter, and mines V and L in the spring.

TABLE I

Examination of 40 Healthy Students, showing percentage of persons harbouring various organisms.

ORGANISMS GROWING IN PROFUSE OR MODERATE AMOUNT.

<i>Staphylococcus albus</i>	-	-	-	-	-	-	-	40
Diphtheroid bacilli	-	-	-	-	-	-	-	25

ORGANISMS GROWING IN ANY AMOUNT.

<i>Staphylococcus citreus</i>	-	-	-	-	-	-	-	2·5
<i>Staphylococcus chromogenicus</i>	-	-	-	-	-	-	-	—
α cocci {	<i>Streptococcus viridans</i>	-	-	-	-	-	-	2·5
	<i>Pneumococcus</i> (group 4)	-	-	-	-	-	-	—
	<i>Pneumococcus</i> (type 1, 2 or 3)	-	-	-	-	-	-	—
	<i>Diplo-bacillus of Morax</i>	-	-	-	-	-	-	—
	<i>Micrococcus catarrhalis</i>	-	-	-	-	-	-	—
	Haemophilic bacilli	-	-	-	-	-	-	—
	Anthracid bacilli	-	-	-	-	-	-	2·5
	<i>B. coli</i>	-	-	-	-	-	-	—

Controls.—A control series was afforded by the examination, in October and November, of 40 healthy students. The results in Table I show that apart from the usual staphylococci, diphtheroids and harmless anthracoids, *streptococcus viridans* was the only potential pathogen found. This organism was only found in one case. The results of this examination are altogether of a different order from those to follow. It is expected that further studies in this series will deal with other groups of workmen.

Results

At the outset of the investigation it was thought that it might be possible to compare one mine with another and link up any differences in flora with some varying working condition. This is, however, manifestly impossible. A short experience of mining makes it obvious that no two mines have exactly similar working conditions. Although the coal may be roughly of the same type there are countless variant seams and faults which make minor differences. Again, the conditions of dust, moisture, ventilation and warmth vary from pit to pit. Moreover, the various pits furnished results which are of the same order, although there are, of course, minor variations here and there.

It is felt that the scope of the investigation has been large enough to furnish a reliable index of the conjunctival flora of coal-miners. A wide variety of organisms, commensals and pathogens were isolated and these will be discussed *seriatim*. Following that, the findings in the individual mines will be tabulated and referred to briefly.

Organisms isolated.—Organisms are recorded as occurring in such and such a percentage of patients, without reference to whether they were isolated from one or both eyes.

The smallest unit in each case consisted of the class of worker; thus the findings in the miners, brushers, oncost-workers, and surface-workers of each pit are recorded (see Tables III, IV, V, VI). In certain cases the percentage of various organisms in the whole pit is noted; while for certain organisms the percentage present in the whole 658 men is recorded.

Staphylococcus albus.—At the outset it was realised that this organism was present in some quantity in almost every eye. The presence of the organism has therefore only been considered worthy of record if the growth was profuse or moderate in amount. With this standard in mind the organism was found to occur in from 18 per cent. to 60 per cent. of cases, but the commonest figure was between 30 per cent. and 40 per cent. The organisms

may be regarded as harmless commensals and their presence as probably of little significance.

Diphtheroid bacilli.—These organisms also were widely prevalent and are recorded only if present in profuse or moderate amount. Figures varied from 11.6 per cent. to 56 per cent., but more usually from 30 per cent. to 50 per cent. Morphologically, these organisms were usually club-shaped, although bacillary forms were always present. The colonies sometimes showed a narrow zone of haemolysis. Diphtheroids were not tested for any further properties. The presence of diphtheroid bacilli is probably of little significance.

The following organisms occurred less commonly, and their presence in any quantity is recorded.

Pigmented staphylococci.—*Staphylococcus citreus* was sometimes present, and figures varied from complete absence up to 20.8 per cent., but few groups had a percentage higher than 10-15. A few strains of *staphylococcus chromogenicus* were isolated. These organisms resembled *staphylococcus aureus* but differed in the following tests: they were not haemolytic; they did not liquefy gelatin, or ferment mannite. These pigmented staphylococci are generally regarded as harmless commensals.

Green producing or α cocci.—Cocci producing α haemolysis were quite frequently encountered. The accompanying table (2) shows that from 17.42 per cent. to 21.2 per cent. of persons in the different mines harboured these organisms, with an average of 19.35 per cent. Various tests were carried out to distinguish between *streptococcus viridans* and *pneumococcus*. In the first place morphology was of little value. The commonest appearance was of chains of short to moderate length composed of oval cocci. Occasionally certain strains had a more definitely pneumococcal appearance. Usually, however, it was not possible to tell from morphology whether subsequent tests would prove the organism to be *streptococcus viridans* or *pneumococcus*.

Colonial appearances were not very helpful either. Sometimes typical pneumococcal colonies were encountered but more usually the colony was rough and rather granular, and it was impossible to say definitely whether the organism was streptococcal or pneumococcal. All α cocci were grown on boiled blood and a green colour watched for, to exclude the presence of a β haemolytic coccus. It is thus evident that reliance had to be placed on tests other than morphology and colonial appearances. Thus the bile test was carried out by adding one part of bile or sodium taurocholate solution to 5-10 parts of broth culture. Inulin fermentation was carried out using Hiss's serum water. In the case of organisms suspected to be pneumococcal a considerable quantity

of culture was injected intraperitoneally into a mouse, and typing carried out.

Streptococcus viridans.—An *a* coccus which was not bile-soluble and did not ferment inulin was labelled *streptococcus viridans*. Such organisms occurred in 11.25 per cent. of all persons examined.

Pneumococcus.—In all, strains of *pneumococcus* were isolated from 54 persons (8.1 per cent. of total); 43 of these strains presented the typical reactions of bile solubility and inulin fermentation. Eight strains fermented inulin but were bile insoluble. These were regarded as rough pneumococci. Three strains were bile soluble but did not ferment inulin. As quite a number of pneumococcal strains have been shown not to attack inulin no hesitation was felt in labelling these accordingly. The interesting observation was made that whereas the classical bile soluble and inulin fermenting strains were distributed over all mines, seven out of eight of the bile insoluble inulin fermenters occurred in mine W.

All suspected pneumococcal cultures were injected into mice, which were killed after 24 hours. On one occasion only (out of 54 strains) was the mouse found dead after this period. On six other occasions definite capsulated pneumococci were found in the mouse peritoneal exudate. In the remainder, however, examination of the exudate failed to show any pneumococci. Typing was carried out by mixing drops of exudate with agglutinating rabbit sera for types 1, 2, and 3. In the case where the mouse died the organism proved to be a definite type 3 pneumococcus. In the other six cases where diplococci were seen in the exudate no reaction occurred with these sera and they were labelled group 4. Those remaining pneumococci, that were destroyed in the peritoneal cavity, were also labelled group 4, in the sense that they certainly were not types 1, 2, or 3. It might be more accurate, however, to call these rough, avirulent and untypable pneumococci.

It is difficult to assess accurately the importance of the presence of *a* cocci in the eye. With regard to the more virulent pneumococci their significance is obvious. They must be regarded as potential pathogens. The rough untypable pneumococci must also be considered of importance. Although avirulent for the mouse it is possible that they may regain their virulence in a traumatised eye (see Duke-Elder, 1938). *Streptococcus viridans* must also be viewed as a potential pathogen, as it may occur in cases of corneal ulcer (personal observations).

Diplobacillus of Morax.—This organism was detected regularly in almost all the different groups of workers. Altogether it was found in 24 persons (out of 658) but the distribution was not

regular over all the pits. Thus at pits L, V, and W it occurred in 2·3 per cent. of all workers, whereas at pit A in 6·76 per cent.

The organism was tested for its ability to liquefy Löffler's serum at 37° C., to grow on agar at 37° C. and room temperature, and for liquefaction of gelatin. Serum was liquefied by all strains while none grew on agar at room temperature nor liquefied gelatin. It was found that six strains grew quite luxuriantly on agar at 37° C. and did not lose this property on repeated subculture. Five of these particular strains occurred in workers at mine A.

TABLE II.

Colliery	A.	V.	W.	L.	Combined results
Number of men examined	189	160	149	160	658
	per cent.	per cent.	per cent.	per cent.	per cent.
Total <i>a</i> cocci present -	21·2	19·22	17·42	20·46	19·35
<i>Streptococcus viridans</i> -	10·6	9·30	11·39	14·26	11·25
<i>Pneumococcus</i> - -	10·6	9·92	6·03	6·2	8·1

None of the workers examined complained of any conjunctival irritation and certainly no person with a marked degree of angular conjunctivitis was noted. It is possible, of course, that as the examination was of necessity a rapid one certain minor degrees may have been missed.

These figures show, however, that the organism is quite generally present in the conjunctival sac of mine workers and may on occasions reach a high percentage. As far as could be determined in the majority of cases the presence of the organism did not appear to evoke any conjunctivitis. Evidence will be presented in a further study that the organism may be carried temporarily. Certain Continental workers have recorded the presence of the organism in healthy conjunctival sacs. Thus Pillat (1922) has found it in 22 per cent. of persons, and v. Pelláthy (1932) in 9·5 per cent. In this country, however, it has generally been accepted that the organism is a pathogen and does not occur in healthy persons.

The presence of *diplobacillus of Morax* in the conjunctival sacs with such regularity must be regarded as of great importance, the more so in view of the fact that the organism is a well recognised causal agent of corneal ulcer.

Micrococcus catarrhalis.—This organism was isolated from 15 persons (out of 658), or 2·25 per cent. All strains grew on agar

at 37°C. The majority of strains were tested for their fermentation reactions, which were negative for glucose, saccharose, and maltose. The organism is probably a harmless commensal and little or no importance is attached to its presence. Various authors have, however, recorded its occasional presence in cases of conjunctivitis (see Duke-Elder, 1938).

Haemophilic bacilli.—These organisms were isolated from 10 persons (out of 658), or 1·5 per cent. All strains were typical Gram-negative bacilli which grew on blood agar but not on ordinary agar, and would thus normally be labelled *B. Koch-Weeks*. The organism would appear either to be carried as a harmless commensal, or to cause a mild chronic infection, as certainly no case of typical “pink-eye” was encountered. The organism is generally accepted as a pathogen and some importance should be attached to its presence. It should, however, be noted that it is not possible to differentiate between this organism and *B. influenzae*. It is possible the organisms isolated were

TABLE III
Examination of Colliery A Mine-Workers, showing percentage of persons harbouring various organisms.

	Miners	Brushers	Oncost	Surface
Total number examined	60	50	39	40
ORGANISMS GROWING IN PROFUSE OR MODERATE AMOUNT.				
<i>Staphylococcus albus</i> -	43·3	18	57·2	33·3
Diphtheroid bacilli -	50	26	39	30
ORGANISMS GROWING IN ANY AMOUNT.				
<i>Staphylococcus citreus</i> -	10	10	20·8	—
<i>Staphylococcus chromogenicus</i> - - -	1·7	4	—	—
<i>Streptococcus viridans</i> -	3·3	16	18·2	7·5
<i>Pneumococcus</i> (group 4)	13·3	6	10·4	12·5
<i>Pneumococcus</i> (type 1, 2, or 3) - - -	—	—	—	—
<i>Diplo-bacillus of Morax</i>	6·7	6	13	2·5
<i>Micrococcus catarrhalis</i>	5	4	—	—
Haemophilic bacilli -	3·3	—	10·4	—
Anthracoid bacilli - -	—	—	—	—
<i>B. coli</i> - - - -	—	—	2·6	—

not true *B. Koch-Weeks* strains, but rather influenzal strains of little or no significance in ocular pathology.

B. coli.—This organism was only isolated from 5 persons, *i.e.*, 0.75 per cent. As this organism may sometimes be pyogenic its occasional presence may be of some importance.

Other organisms.—Little importance need be attached to the occasional presence of anthracoid bacilli or *micrococcus tetrigenus* as they are generally devoid of pathogenic action. With regard to the former, however, it should be remembered that anthracoid organisms are commonly found in that rare condition of ring-abscess of the cornea.

The findings in individual mines.—Having now discussed the various organisms the results of each mine may be given separately.

Mine A (Table III).—Two main facts are evident from these figures. First, as already mentioned, there is a higher percentage

TABLE IV
Examination of Colliery V Mine-Workers, showing percentage of persons harbouring various organisms.

	Miners	Brushers	Oncost	Surface
Total number examined	40	40	40	40
ORGANISMS GROWING IN PROFUSE OR MODERATE AMOUNT.				
<i>Staphylococcus albus</i> -	20	40	35	35
Diphtheroid bacilli -	50	45	37.5	47.5
ORGANISMS GROWING IN ANY AMOUNT.				
<i>Staphylococcus citreus</i> -	2.5	15	17.5	7.5
<i>Staphylococcus chromogenicus</i> - - -	—	—	—	—
<i>Streptococcus viridans</i> -	7.5	5	10	15
<i>Pneumococcus</i> (group 4)	7.5	22.5	5	5
<i>Pneumococcus</i> (type 1, 2, or 3) - - -	—	—	—	—
<i>Diplo-bacillus of Morax</i>	7.5	2.5	2.5	—
<i>Micrococcus catarrhalis</i>	—	—	2.5	—
Haemophilic bacilli -	—	2.5	2.5	—
Anthracid bacilli -	5	10	2.5	5
<i>B. coli</i> - - - -	—	2.5	—	2.5
<i>M. tetrigenus</i> - -	—	2.5	—	—

a cocci {

15

27.5

15

20

of *diplobacillus of Morax* than in any other mine. Second, comparing the various groups it is evident that the oncost-workers' conjunctival flora is more profuse than that of any other of the A workers.

Mine V (Table IV).—The percentage of *Morax diplobacilli* here is substantially smaller than at mine A although 7·5 per cent. of the miners harboured the organisms. The oncost-workers' flora is not more profuse than that of the other groups.

TABLE V

Examination of Colliery W Mine-Workers, showing percentage of persons harbouring various organisms.

	Miners	Brushers	Oncost	Surface
Total number examined	39	34	36	40
ORGANISMS GROWING IN PROFUSE OR MODERATE AMOUNT.				
<i>Staphylococcus albus</i> -	39	34·8	47·6	60
Diphtheroid bacilli -	54	11·6	56	32·5
ORGANISMS GROWING IN ANY AMOUNT.				
<i>Staphylococcus citreus</i> -	13	14·5	11·2	12·5
<i>Staphylococcus chromogenicus</i> - - -	—	—	—	—
α cocci {	<i>Streptococcus viridans</i> -	15·6	22·4	2·5
	<i>Pneumococcus</i> (group 4) -	—	11·2	7·5
	<i>Pneumococcus</i> (type 3) -	2·6	—	—
	<i>Diplo-bacillus of Morax</i>	2·6	2·9	2·5
	<i>Micrococcus catarrhalis</i>	—	5·6	—
	Haemophilic bacilli -	—	2·8	—
	Anthraxoid bacilli -	2·6	2·8	2·5
	<i>B. coli</i> - - -	—	—	2·5
	<i>M. tetragenus</i> . -	—	2·8	—

Mine W (Table V).—In this mine the oncost group of workers' flora is distinctly more profuse than that of the others.

Mine L (Table VI).—In this mine the profuse flora of the oncost-workers of mines A and W is not found, these men presenting a comparatively scanty flora.

TABLE VI

Examination of Colliery L Mine-Workers, showing percentage
of persons harbouring various organisms.

	Miners	Brushers	Oncost	Surface
Total numbers examined	40	40	40	40
ORGANISMS GROWING IN PROFUSE OR MODERATE AMOUNT.				
<i>Staphylococcus albus</i> -	30	20	42.5	20
Diphtheroid bacilli -	32.5	45	40	37.5
ORGANISMS GROWING IN ANY AMOUNT.				
<i>Staphylococcus citreus</i>	10	5	5	7.5
<i>Staphylococcus chromo-</i> <i>genicus</i> - - -	—	—	—	—
<i>Streptococcus viridans</i>	17.5	12.5	2.5	25
<i>Pneumococcus</i> (group 4)	10	2.5	10	2.5
<i>Pneumococcus</i> (type 1, 2, or 3) - - -	—	—	—	—
<i>Diplo-bacillus of Morax</i>	—	2.5	—	2.5
<i>Micrococcus catarrhalis</i>	5	10	—	2.5
Influenzal bacilli - -	—	2.5	—	—
Anthracid bacilli - -	15	5	2.5	—
<i>B. coli</i> - - - -	—	2.5	—	—

Discussion

On general principles of bacterial ecology it was thought that there might be a tendency for an organism, once having gained access to a group of workers to spread throughout the unit. It was possible definitely to substantiate this supposition on two occasions. Thus in the case of *pneumococcus* although only eight bile insoluble, inulin fermenting, strains were isolated, seven were found in the workers at mine W. Secondly, there were isolated only six strains of *diplobacillus of Morax* growing on agar, yet five of these occurred in mine A.

From the above results it is evident that a wide range of organisms occurs in the conjunctival sacs of coal mine-workers, and generally speaking the results in all groups, whether above or below ground, are of the same order. In two pits, however, the oncost group of workers had a noticeably more profuse flora than the other workers. Apart from this there was no evidence

that workers below ground harboured a more profuse flora than did those who worked on the surface.

All groups of workers frequently harboured such pathogenic or potentially pathogenic organisms as *pneumococcus*, *streptococcus viridans*, *diplobacillus of Morax*, haemophilic bacilli, and *B. coli*. Anthracoid bacilli were also encountered. Of these organisms the first mentioned three are of particular importance, all being well recognised aetiological agents of hypopyon ulcer.

The presence of these organisms is not, however, of equal importance in the various groups of worker. Only the miners and brushers are commonly exposed to risk of corneal injury. The other workers although harbouring a similar flora rarely suffer corneal trauma. Correspondingly one finds that of any series of cases of hypopyon ulcer in mine-workers the great proportion is constituted by miners or brushers (personal observations).

It has been suggested that in certain cases of hypopyon ulcer the infective agent may enter the cornea on the traumatising particle. It is difficult to see how this could obtain in coal mine injuries as unhewn coal is probably quite sterile (personal observations). It is considered that the most reasonable explanation of the pathogenesis of hypopyon ulcer in miners is as follows:—The conjunctival sac frequently harbours pathogenic organisms and any damaging spicule of coal or stone may allow these organisms to enter and cause infection. Hypopyon ulcer occurs most commonly, therefore, in those exposed to most risk of trauma, that is the miners and brushers.

In conclusion, these studies serve to emphasise the importance of shielding the eyes of those exposed to risk of corneal injury, and if such an injury does occur the care with which it should be treated.

Summary

1. The conjunctival flora of 658 healthy coal mine-workers has been examined, representing 4 different pits.
2. In two pits the oncost-worker evidently harboured a more profuse flora than the other workers.
3. Apart from this, below-ground workers did not harbour a flora essentially different from that of surface workers.
4. The following potentially pathogenic organisms may be found in all groups of workers in significant quantity: *streptococcus viridans*, *pneumococcus*, *diplobacillus of Morax*, haemophilic bacilli and *B. coli*.
5. *Streptococcus viridans* occurred in 11.25 per cent. of persons.

6. Fifty-four strains of *pneumococcus* were encountered (representing 8.1 per cent. of the workers), but only one of these fell into a main type (type 3). The bulk of the remainder proved avirulent to the mouse, but showed typical reactions otherwise.

7. The distribution of *diplobacillus* of *Morax* was somewhat irregular, occurring in 2.3 per cent. of all workers in pits L, V, and W, and in 6.76 per cent. at pit A.

8. Haemophilic bacilli were isolated from 1.5 per cent. of persons, and *B. coli* from 0.75 per cent.

9. It is considered that the conjunctival flora of coal mine-workers is potentially dangerous and that the source of infection in hypopyon ulcer is from organisms already present in the conjunctival sac. Those most liable to contract the disease are those most exposed to corneal trauma—the miners and brushers.

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