Remarks

ON THE

CLINICAL AND BACTERIOLOGICAL ASPECTS OF AN EPIDEMIC SIMULATING INFLUENZA,

WHICH HAS RECENTLY OCCURRED IN THE EAST HERTS DISTRICT.

BY

R. A. DUNN,
M.D., B.S., D.Hy., D.P.H.;
M.O.H. East Herts and Essex
Combined District.

and M. H. GORDON,
M.A., M.D., B.Sc.

DURING last winter there spread through this district an epidemic which was thought to have been influenza. It was not until the beginning of January that my attention was drawn to what appeared to be some anomalous cases. The first was a girl whose hands were desquamating, and who had previously had an irritable rash and sore throat. Both the history of the case and the character of the peeling led me to exclude scarlet fever. Very shortly after this a medical practitioner wrote to me about a family named E., whose illness he at first diagnosed as influenza, but subsequently suspected to be scarlet fever. The cases were very puzzling. One had what appeared to be scarlet fever, others influenza, whilst another developed cerebro-spinal symptoms. On the death of the latter I made inquiries of the neighbouring practitioners so as to ascertain if they might have had patients under their care with similar symptoms. Some four or five cases which might have been cerebro-spinal fever were thus brought to my notice. Following on these several rash cases were reported to me which closely resembled scarlet fever; also cases of nephritis. Not feeling satisfied that this was an outbreak of scarlet fever I took swabs from the throats of six patients who had recently had these rashes, and sent them for bacterioscopic examination to Dr. Gordon. He failed to find either the streptococcus scarlatinae or the bacillus of Pfeiffer. Next a child died after three weeks' illness with symptoms which might have been due to tuberculous meningitis. A *post-mortem* examination of the head revealed extensive basic meningitis, very dilated ventricles, but no tubercles. Some cerebro-spinal fluid was obtained, together with the choroid plexus, and these were examined by Dr. Gordon. He found some diplococci to be present which closely resembled the gonococcus in morphology, and which did not stain with Gram. Owing to *post-mortem* contaminations this organism could not be isolated.

We next turned our attention to the bacterioscopic examination of the tonsillar and nasal mucus. As a result a non-Gram-staining diplococcus was frequently found.

At the same time I continued to investigate the disease further, and, as a result, arrived at the following conclusions: First, that an extensive outbreak of disease had been, and still was to a less extent, prevalent.

Further, that this disease was not localized to any one town or village, or group of villages, but had extended throughout the district (and probably much further), attacking nearly every household.

Lastly, that one disease alone was present, although various members of the same family were simultaneously exhibiting symptoms of dissimilar diseases. Amongst this large number of persons attacked the majority were only slightly ill, and considering the disease to be only a bad cold or mild influenza, frequently did not seek medical advice. In a certain number of cases the disease was more severe, and was accompanied by a rash. (In most instances this rash was scarlatinial in character.) A less number of patients developed irregular cerebro-spinal symptoms. A few patients were notified as typhoid fever, others again as diphtheria, and I suspect that several deaths in babies which were attributed to bronchitis and convulsions were in reality due to this cause.

Having now briefly described the outbreak I propose to give various of the symptoms more in detail. I should add here that I did not personally attend any of the cases, but that I obtained my information mainly from the medical practitioners in the neighbourhood or from the patients or their relatives.

The onset was nearly always sudden—the few exceptions were in those patients who, previous to the onset, were suffering from coughs, colds, etc. Sometimes there were

rigors or a feeling of chilliness. The temperature as a rule was not high, usually 99° to 101° . Sore throat, often severe and sometimes simulating diphtheria, was a very common symptom. Enlarged cervical glands, especially those along the posterior border of the sterno-mastoid, were equally common. Stiff neck was a very fairly common symptom. It was often slight. In some cases it may have been due to the enlarged glands, but more often appeared to be dependent on a myalgia. Headache (severe and sometimes persistent) and "aches and pains all over" were present in most of the cases. Drowsiness and photophobia were noted in some. Nasal discharge was frequently present; sometimes it was very profuse. Sneezing and running at the eyes were not uncommon. Constipation was met with in a large number of cases, and in some retching or actual vomiting occurred. Peeling was met with on one or two occasions even in the absence of any rash. Herpes, on the whole, was less frequent than I should have expected. It seemed more common in certain districts. Much weakness and debility were the usual sequelae to the disease, which lasted from one to four weeks.

The above symptoms were those usually met with in the mild "influenzal" type of the disease. This type was the rule amongst the adults attacked with hardly an exception. The type met with amongst children was certainly more severe, and all the worst cases have occurred in the young.

I now propose to describe those cases which presented a scarlatinial rash. The onset, as in the previous cases, was sudden. The illness usually commenced with vomiting, headache and sore throat, occasionally with rigors. The temperature was not as a rule very high, about 101° to 102° . The pulse was frequent, often about 120. In several of the cases admitted to hospital it was noted to be small and irregular. The rash appeared within twenty-four hours. It did not invariably commence on the chest, but was often first noted along the posterior border of the sterno-mastoid, and then rapidly spread over the rest of the body. It consisted of an erythematous blush and punctate spots. Most of the erythema quickly subsided, leaving a closely set punctate rash, which was often noticed to be more discrete about the thighs and knees, approaching nearer to the rash of röteln. A large number of the patients complained of its marked irritability, and some had a local oedema. The rash was of short duration, not usually lasting more than from one to three days, and was followed by desquamation, which as a rule commenced early, sometimes before the rash itself had disappeared. Its character varied considerably. In some cases it was slight and branny, whilst in others the skin peeled off in strips. In some of the cases I noticed a well-marked circumoral pallor. The patients were often light-headed for one or two nights, especially if the rash were well marked. In nearly all the cases the tongue was thickly coated at the commencement. It peeled off the anterior half within twenty-four hours, and frequently left the typical "strawberry" appearance. Sordes were present in severe cases. The throat was injected, sometimes covered with thick muco-purulent discharge, and occasionally something suspiciously like diphtheritic membrane. The cervical glands were enlarged, especially those along the posterior border of the sterno-mastoid. In some these subsequently suppurated. Constipation was frequently met with. Haematuria and albuminuria were present in several of the patients. (It was noted on more than one occasion that the blood was present in larger proportion to the albumen than was expected.) Giddiness was common. A rheumatic condition of the joints was not common. Tenderness and even wasting of the muscles was, however, not infrequent. Conjunctival injection, though slight, was often met with. Profuse nasal discharge nearly always occurred. It commenced two or three days after the appearance of the rash, and lasted only a few days. Several of the patients developed a temporary ear discharge. Recovery was slow and tedious. A very discrete papular rash was sometimes noticed a week or so after the preliminary scarlet rash. Sudaminal rashes were also occasionally met with. Besides the above rashes, purpura was noted in four of the cases. In two of these it appeared late in the disease, and was associated with haematuria; in a third it was accompanied by melaena and a mild "typhoid condition." Epistaxis was reported in a few of the patients. In three children a true urticaria was described. One of them died rapidly with cerebro-spinal symptoms. All three were friends and lived in the same village, but there was an interval of four to six weeks between the individual attacks. Some parents described to me a rash "something like measles," but I did not personally see any true morbilliform eruption.

The description of the cerebro-spinal cases is not so easy, as I have not had the same opportunity of watching the progress of the disease. In many my suspicions were only aroused on receiving the death returns.

The onset varied considerably. In some cerebro-spinal symptoms were the first to attract attention, whilst in others these symptoms followed on those of an influenzal or typhoid condition. Severe headache was a constant symptom. Its position differed in different patients. It was nearly always associated with pain in the back of the neck and stiffness. It was inclined to persist some time after convalescence was established. Pain in the lumbar region was frequently present. Vomiting was an early and constant symptom. Constipation, though usually present, was not invariable. The tongue was thickly coated, and there were sordes on the lips and teeth. The patient was light-headed, and in severe cases unconscious. If the case proved fatal, the end was often ushered in by convulsive fits. The temperature was very erratic; in some it was never high, whilst in others it was 104° or higher before death. The pulse has been noted to be quite regular throughout in some of the cases. Discharge from the ear occurred in several of the cases. In some it was of old standing, and probably gave entrance to the infection. Copious muco-purulent discharge from the nose was nearly always present. The patient usually lay curled up in a state of general flexion. In only a few cases has marked retraction of the head or opisthotonos been noticed. With regard to other nervous symptoms and paralyses, there has been no settled type. Dilatation of one or both pupils seems to have occurred fairly frequently. In severe cases incontinence of urine and faeces was present. This persisted for a considerable time, and was due to a definite paralysis. The other paralyses appeared to be due to trophic changes in the muscles, which showed marked wasting. It is of some interest to note that in two cases the cerebral symptoms followed closely on a tonsillotomy.

It should be borne in mind that every degree of severity was met with, from one of a few days' illness with slight retraction of the head to one with marked cerebro-spinal symptoms, lasting many weeks, and interspersed with relapses. The above classification is therefore purely arbitrary.

Besides the above, several deaths occurred in quite young children, the cause of death being given as convulsions often associated with bronchitis and pneumonia. These children appear to have been ill for only a short time, and as the illness was not thought to be serious medical assistance was not sought for until the patient was moribund. In two such cases a swab from the nose showed a non-Gram-staining diplococcus to be present.

Lastly, there was the typhoid type of the disease. Four patients were notified to me as enteric fever, but, in spite of the fact that in two of them the Widal reaction was present, I am inclined to doubt the diagnosis, owing to the subsequent progress of the cases. One or two others were described to me as "typhoid-like," but without any rise of temperature.

It would seem desirable now to deal with the clinical differential diagnosis—the bacteriological will be considered more especially later. The diseases to which this epidemic have borne the greatest resemblance are: (1) Influenza; (2) scarlet fever; (3) epidemic cerebro-spinal fever. It will be noticed from the cases quoted that, taken individually, it would have been nearly impossible to have distinguished this disease from one of the above three. Taking the cases collectively, however, one is forced to a different conclusion.

Influenza.—The diagnosis of the disease in many cases, especially amongst the adult patients, was undoubtedly compatible clinically with that of influenza. There are few symptoms or sequelae for which influenza has not been held responsible. In my opinion, however, the number of rash cases, occasionally followed by nephritis, the association of cerebro-spinal symptoms, the frequency of stiff-neck, and the severity of the disease amongst children and its mildness amongst adults, are strong points against influenza. The absence of Pfeiffer's bacillus amongst the number of cases especially examined for it are confirmative of this opinion.

Scarlet Fever.—Taken individually, many of the younger patients bore a striking resemblance to scarlet fever. The rash, sore throat, circumoral pallor, vomiting, strawberry tongue, desquamation, and occasional onset of nephritis were extremely suggestive. On the other hand, the absence of much fever and the irritability of the rash appear to be points against scarlet fever. But what was to me far more convincing was the association of the different types of the disease in the

some invaded household. On several occasions the adult had an "influenzal" attack, and infected the child, who within a few days developed a scarlatinal type, or vice versa. Moreover, several of these rash cases were nursed at home, and in no single instance did another member of the same family contract a scarlatinal type. Further, at least two patients with scarlatinal rashes were known to have previously had undoubted scarlet fever. And in not one of six typical scarlatinal rash cases could Dr. Gordon obtain the streptococcus scarlatinae.

Cerebro-spinal Fever.—For some little time I was under the impression that the epidemic under consideration was one of cerebro-spinal fever. Although it corresponded to it in certain particulars—such as its proclivity to attack children and its association with influenzal symptoms as described by Dr. Bruce Low in 1899—still the mildness of the epidemic, the comparatively small number of cases with typical cerebro-spinal symptoms, the infrequency of the two usual concomitant rashes—namely, purpura and herpes—led me to doubt my provisional diagnosis. Further, the fact that no organism having all the characteristics of the meningococcus could be obtained from the numerous cultures which have been made from the nasal or pharyngeal discharges.

The symptoms of those patients who had rashes did not appear to me to correspond with either those of "fourth disease" or röteln, apart from the fact that the majority of those attacked had no eruption.

There is, however, a striking resemblance to certain epidemics which have previously occurred in England, and which were reported by Dr. Bruce Low,¹ notably that of Raunds, Northamptonshire, in 1891, that of Market Rasen, Lincolnshire, in 1891, and that of Laxfield, Suffolk, in 1894, and more recently to that of Oswestry, Shropshire, in 1903, which was investigated by Dr. Reginald Farrar² on behalf of the Local Government Board.

The one feature which has stood out pre-eminently in this investigation has been the consistent presence of a non-Gram-staining diplococcus. From the middle of February to the middle of April it was discovered in almost every case which was examined bacterioscopically.

All the cases in the table I have prepared are recent. The only one anterior to December, 1904, refers to a patient whose illness commenced in August, 1904. It must be borne in mind that the cases quoted are only a small proportion of all which occurred. They are therefore useless for gauging either the case mortality or the incidence of the disease on the population. For example, a large number of the cases are reported from Hertford, the reason being that here the material was nearer to hand. It is also fairly certain that they include chiefly the severer cases, as it was these which were especially brought to my notice, many through the death returns. The object of their publication has been to bring out prominently the more common symptoms. That many cases never came under medical treatment the following statements seem to show:

Dr. Patch of Hertford on March 2nd was examining some 20 young adults for admission into the Volunteer service. He found 5 of them had scarlatiniform rashes. Of these all 5 had slight sore throat, 4 had nasal discharge, 4 had headache, 3 had stiff-neck, and 1 had herpes. None of them were being medically treated. Again, the schoolmaster at Birch Green schools stated to me that "there had been a considerable amount of slight sickness amongst the children generally. The duration was usually about a week. The symptoms chiefly noticed were sore throat, cough, and profuse discharge from the nose. Sickness and stiff-neck were not uncommon, together with enlarged glands." The schoolmaster at Birch-anger, Essex, told me that "the larger part of the school here have suffered from apparently severe colds. Several had sore throats. Several were sick before the cold. They were generally all right within a few days. Two had severe breaking out about the mouth. One child was stated to have had a fit. Most of those who were ill were drowsy. Headache was common."

In conclusion, my best thanks are due to the kindness and assistance of the various local medical practitioners for permission to visit their patients, and for the material with which they furnished me. And especially are they due to Messrs. Lloyd-Patch and Lawson-Smith, who in addition have allowed me to make use of numerous excellent notes.

I regret that, owing to want of space, it has not been found possible to have printed the tables which had been prepared. These demonstrated, amongst other things, the various symptoms which had occurred in each of 162 patients, the ages of

the patients, in many cases the spread of the disease, and the towns and villages in which it occurred.

SUMMARY OF RESULTS.

I must therefore be content to give the following summary of results:

Of 162 patients of whom notes were made, a non-Gram-staining diplococcus was found in 63, 24 of which were obtained in pure culture, and tested by Dr. Gordon.

Of these 162 patients 84 were males and 78 were females.

"	"	98 had a temperature below 102°, and 21 a temperature above this.
"	"	98 had sore-throat.
"	"	96 had nasal discharge.
"	"	39 had conjunctivitis.
"	"	60 had cough.
"	"	49 had a scarlatinal (irritable in 32), 2 a purpuric, 3 a papular, 3 a morbilliform, and 3 an urticarial rash.
"	"	49 had desquamation.
"	"	29 had herpes.
"	"	85 had vomiting.
"	"	81 had stiff-neck.
"	"	41 had enlarged glands.
"	"	103 had cephalic pain.
"	"	46 had cervical pain.
"	"	50 had spinal pain.
"	"	10 had thoracic pain.
"	"	14 had abdominal pain.
"	"	42 had general pain.
"	"	11 had oedema.
"	"	20 had albuminuria.
"	"	9 had haematuria.
"	"	23 had rigors.
"	"	37 had delirium.
"	"	62 had drowsiness.
"	"	68 had constipation.
"	"	6 had diarrhoea.
"	"	31 had retraction of the head.
"	"	13 had hyperaesthesia.
"	"	9 had dilatation of the pupils.
"	"	15 had convulsions.
"	"	11 had photophobia.
"	"	19 had vertigo.
"	"	12 had otorrhoea.
"	"	10 had pareses.
"	"	19 had unconsciousness.
"	"	10 had Kernig's sign.
"	"	16 died.

BACTERIOLOGICAL REPORT.

The following is Dr. M. H. Gordon's report on the bacteriological aspect of the Hertford outbreak, together with an account of some characters by which various Gram-negative cocci may be differentiated and identified:

On February 10th, 1905, three swabs were received from Dr. Dunn, with the request that they should be examined for streptococcus scarlatinae. The swabs had been taken off the throats of patients suffering from an infectious disease then epidemic in the Hertford district. From the clinical standpoint the precise nature of the disease in question was uncertain, and as a proportion of the cases had developed a scarlatiniform eruption, it was thought possible that the outbreak was in reality one of scarlet fever. I was therefore asked to determine if the bacteriological evidence supported this view or not.

From dilutions of the four swabs cultures were made on a series of blood-serum tubes, and after forty-eight hours' incubation at 37° C., submitted to microscopical examination, with the result that in no case was a streptococcus presenting the morphology characteristic of *S. scarlatinae* (conglomeration, spindle-shaped and bacillary forms) present. Nor was *B. diphtheriae* found.

In view of the negative result with respect to *S. scarlatinae*, the next material was examined not only for that micro-organism, but for *B. influenzae* and also, at Dr. Dunn's request, for micrococcus meningitidis. The material in question, which arrived on February 14th, consisted of two swabs (a) from the throat of a case named B., and (b) from the nares of a case P.

It will be remembered that both the influenza bacillus and the meningococcus fail to stain by Gram's method. As by far the majority of the bacteria found both in health and disease in nasal and pharyngeal mucus give a positive result with this stain, the negative reaction to Gram of the two pathogenic micro-organisms in question is of special differential value when identifying them in the secretion of the upper respiratory passages. Accordingly, coverglass pre-

parations were made from each of the two swabs, and stained with Gram, using a dilute watery solution of fuchsin as counterstain. As a result, the influenza bacillus was not observed, but amongst a majority of micro-organisms positive to Gram, some free Gram-negative cocci were seen to be present in both cases.

Cultures from dilutions of the two swabs were made on blood serum tubes as before, and, in addition, surface plate-cultures were made both on blood agar and on ascitic agar. The outcome was negative as regards *S. scarlatinae*, *B. diphtheriae*, and *B. influenzae*. On the other hand, both swabs yielded numerous colonies on all three media of a micrococcus negative to Gram's stain. After some difficulty, owing partly to the opacity of the ascitic agar, and partly to the parasitic nature of the coccus, which at this stage refused to grow either on ordinary agar, or on glycerine agar, the micro-organism was isolated in pure culture from the first case (B.), and examined, with the result that the cultural characters of the coccus were found to be different from those of the meningococcus in two respects. In the first place, the colonies on ascitic agar were much more opaque, and more granular than those of the meningococcus, and secondly, the coccus grew on gelatine at 20° C., a temperature at which the meningococcus quite fails to develop.

Reference to the literature of Gram-negative cocci showed that these two respects in which the Gram-negative coccus in question differed from the meningococcus were identical with those in which the so-called micrococcus catarrhalis had been found to differ from the meningococcus. I therefore reported to Dr. Dunn that while a Gram-negative coccus had been found belonging to the category of the meningococcus, the coccus in question was culturally distinct from the meningococcus, and that it closely resembled *M. catarrhalis*.

Before proceeding further it is advisable to refer briefly to the history of *M. catarrhalis*. This micro-organism was originally found by Seifert³ in the sputum and nasal secretion of those affected by a small epidemic of infectious bronchitis. From the clinical point of view the epidemic in question resembled influenza, but the disease was milder in type. Later the same coccus was encountered by Pfeiffer, who gave it the name of catarrhalis. Pfeiffer⁴ witnessed its presence in large numbers in the secretion of the bronchioles and alveoli of cases of bronchopneumonia in young children, and he observed it to be often associated with the influenza bacillus. *M. catarrhalis* was sometimes found to be intracellular, and in a proportion of the cases so marked was this feature that the secretion of the respiratory passages bore a remarkable resemblance to gonorrhoeal pus. The coccus was not found to be pathogenic for animals. Subsequently Ghon, Pfeiffer, and Seder⁵ described *M. catarrhalis* in further detail. The importance of this micro-organism, however, arises not solely from the pathological conditions with which its presence in the respiratory passages has been found to be associated, but also, and perhaps chiefly, from the fact that not infrequently in the past *M. catarrhalis* has been confounded with *M. meningitidis*.

The prime necessity of excluding *M. catarrhalis* when examining nasal secretion for the meningococcus has been emphasized recently in a valuable paper by Lord,⁶ who, after carefully examining all available reports of the finding of the meningococcus in nasal secretion, showed that out of 53 such statements on record no fewer than 49 cannot be accepted as correct, because the Gram-negative coccus present was not differentiated from *M. catarrhalis*. It may be added that Lord himself mentions having found *M. catarrhalis* in the sputum of 12 cases of ordinary bronchitis occurring amongst his out-patients. Finally, while the present paper is being written, an account has been published by Bezancon and de Jong⁷ of the bacteriological character of the sputum during an epidemic that occurred in Paris between November, 1904, and February of the present year. The epidemic in question was clinically diagnosed as influenza, but *B. influenzae* was not found to be present. On the contrary, in all of 25 cases examined, the sputum contained a Gram-negative coccus closely allied to, if not identical with, *M. catarrhalis*.

Having found, therefore, that in both the Hertford cases referred to a Gram-negative coccus was present in large numbers, and that in the instance in which this organism was isolated in pure culture and examined its characters corresponded with those of *M. catarrhalis*, a bacteriological examination was made forthwith of a large number of further cases occurring in course of the same epidemic. The result of this investigation has been that Gram-negative cocci have almost invariably been found present in the nasal and pharyngeal

mucus of those infected by the Hertford disease. The clinical and epidemiological aspects of this outbreak have been fully dealt with by Dr. Dunn. The bacteriological evidence may be summed up as follows:

In cases examined at an acute stage of the attack cover-glass preparations of the nasal mucus in particular showed Gram-negative cocci to be present in large numbers, far in excess of other micro-organisms, and *pari passu* with the decline of the symptoms, the abundance of these cocci in the mucus

Fig. 1.—Nasal secretion of a case of true influenza showing Pfeiffer's bacillus in large numbers. The influenza bacillus was readily cultivated from this material. Stain, Gram-fuchsin. Magnification, 1,000.

diminished. As regards other micro-organisms present in the mucus, the two with which the Gram-negative cocci were most frequently associated were Hoffmann's bacillus and the pneumococcus. On the other hand, *B. influenzae* and *B. diphtheriae* were but rarely met with.

Owing to the abundance of material supplied me by Dr. Dunn, it has been possible to isolate the Gram-negative coccus in pure culture from no less than 24 different cases of the Hertford disease, and in each of these 24 instances the characters of the coccus have been submitted to detailed

Fig. 2.—Nasal secretion of a case of the Hertford disease, showing *M. catarrhalis* in almost pure culture. Stain, Gram-fuchsin. Magnification, 1,000.

observation. A special feature of the present investigation is that all these 24 Gram-negative cocci have been examined in respect of their action upon certain selected carbohydrates of well-known composition but easily decomposable. Through the kindness of Drs. Andrewes and Horder of St. Bartholomew's Hospital, I have also been able to obtain pure cultures of the meningococcus from three different cases of cerebro-spinal meningitis, and to examine them in the same way. It may be said at once that although the biochemical side of Gram-negative cocci appears to have hitherto escaped atten-

tion, the results of the present investigation have clearly shown that these carbohydrate tests form a simple and welcome addition to our means of differentiating the micro-organisms in question, and thus promise to be of material assistance in enabling us to form a correct view of the significance of the pathological conditions in association with which certain of these cocci are found to occur. I may add that the system on which the carbohydrate tests have been applied in the present instance to Gram-negative cocci is identical with

Fig. 3.—Nasal secretion of a second case of the Hertford disease. The intracellular arrangement of *M. catarrhalis* is here seen. The rash was very marked in the present case. Stain, Gram-fuchsin. Magnification, 1,000.

that employed by me recently when investigating a large number of the Gram-positive cocci—namely, streptococci and staphylococci in the same way. Full particulars of the latter inquiry will be contained in the next issue of the report of the Medical Officer of the Local Government Board.

The following are the methods of examination.

Methods employed in examining material from the Hertford cases:

(a) *Microscopical Examination*.—Whenever opportunity offered, cover-glass preparations of nasal or pharyngeal mucus or of sputum were examined microscopically. The uniform procedure has been to employ

Fig. 4.—Nasal secretion of a third case of the Hertford disease. The occasional tendency of *M. catarrhalis* to be grouped in tetrads is marked in the present instance. Stain, Gram-fuchsin. Magnification, 1,000.

Gram's stain, in applying which the times found to yield the best results were $\frac{1}{2}$ of a minute in the aniline gentian violet and in the iodine solutions respectively. In decolorizing the preparation in alcohol—the crisis of Gram—the practice has been to fill three watch-glasses with alcohol and to pass the preparation through each in turn, constantly moving it about when immersed. It is of great importance to remove the preparation from the alcohol at the exact moment when no more violet is given off, and to at once wash it in water. Unless this end point is faithfully observed the stain is apt to lose its differential value. In order to bring out those micro-organisms which have parted with their stain to the alcohol—the Gram-negative bacteria—the prepara-

tion after being washed in water is placed for three to five seconds in a watch-glass containing 0.5 per cent. of fuchsin dissolved in water. By this procedure the contrast between the micro-organisms positive and negative to Gram is well seen, the former being stained violet, the latter scarlet.

(b) *Cultures*.—In a large number of cases the primary culture was made on blood-serum in the same manner as for the bacteriological diagnosis of diphtheria. But while the Gram-negative cocci met with in the Hertford outbreak developed well on serum, it is preferable to

Fig. 5.—Nasal secretion of a fourth case of the same disease. The whole of the dark mass is composed of *M. catarrhalis*. The present patient had the scarlatiniform rash intensely developed. Stain, Gram-fuchsin. Magnification, 1,000.

The specimen of *M. catarrhalis* present in the nasal mucus of the above four cases (Figs. 2-5) was isolated in pure culture and tested.

cultivate primarily on plates of nutrose ascitic agar, for by this means the organisms referred to may be at once isolated in pure culture. Nutrose ascitic agar, a convenient short term for which is "nasgar," is a medium originally suggested by Wassermann for the gono-

Fig. 6.—Culture of *M. catarrhalis* on agar, one day at 37°C. Stain, Gram-fuchsin. Magnification, 1,000.

coccus. The nasgar used in the present inquiry was made as follows:

A.—Ascites fluid, 15 c.cm.

Distilled water, 35 c.cm.

Nutrose, 1 gram.

Put in a flask and bring to a boil constantly shaking till the fluid boils. Filter.

B.—Ordinary peptone agar.

Mix one part of A with two parts of B, steam for thirty minutes, filter, and put in tubes.

In examining primary cultures on nasgar plates a convenient plan is to make an impress-preparation from a crowded part of the plate. On applying the Gram-fuchsin stain to the impress-preparation, colonies of Gram-negative cocci are at once obvious. It is hardly necessary to

remark that impress-preparations made in this way give much more information than smear specimens made with the platinum needle.

(c) *Subcultures*.—The medium used when testing the capacity of various Gram-negative cocci for decomposing various carbohydrates was prepared according to the following formula, for which I am indebted to Dr. A. C. Houston:

Lemco	...	...	1 per cent.
Peptone	...	...	1 "
Sodium bicarbonate	...	0.1	"
Carbohydrate	...	1	"

And 10 c.cm. per cent. of a 10 per cent. watery solution of ordinary solid litmus.

The point to be observed is whether the micro-organism under examination produces an acid reaction in the case of a

Fig. 7.—Peritoneal exudation of guinea-pig dead three days after intraperitoneal injection with *M. catarrhalis*. The intracellular arrangement of the coccus is here well seen. Stain, methylene blue. Magnification, 1,000.

given carbohydrate when cultivated in the lemco medium to which that carbohydrate has been added in the proportion of 1 per cent.

Some of the more delicate Gram-negative cocci—for example, the meningococcus and the gonococcus—may fail to

Fig. 8.—Nutrose-ascitic-agar plate culture inoculated with an artificial mixture of *M. catarrhalis* and *M. meningitidis*, and incubated for two days at 37°C. The dark colonies with rough edge and halo are colonies of *M. catarrhalis*. The larger, smooth, transparent colony in the middle of the photograph is a colony of the meningococcus.

(The above photographs were taken by Mr. Albert Norman.)

develop in this lemco medium, but in such cases I have succeeded in obtaining satisfactory growth by adding to the culture a few drops of sterile raw ascitic fluid. The ascitic fluid which I used for this purpose had by a happy thought of Dr. Drysdale been received at once into sterile test tubes at the time that it was drawn off from a patient. After being filled direct from the patient in this way the sterility of the

tubes of ascitic fluid had been proved by incubating them for several days at 37° C. These tubes of ascitic fluid had been subsequently stored in the pathological department of St. Bartholomew's Hospital and Dr. Andrewes kindly allowed me to make liberal use of them.

In examining the action of various Gram-negative cocci on different carbohydrates, I have found it advisable, after inoculating the tubes, to allow them to incubate at 37° C for at least a week. In all cases control tubes were incubated side by side with the inoculated tubes for the purpose of comparison. In this way even small changes in the reaction of the medium were readily perceived.

Some of the less delicate Gram-negative cocci bleach the litmus culture, and it may be difficult at times to decide off-hand whether this bleaching is accompanied by an increase of acidity or of alkalinity. In such cases a convenient means of deciding the point is to shake the tube well, so as to aerate the medium, and to then leave the tube at the room temperature for twenty-four hours, by which time the colour of the litmus will be found to have reasserted itself.

The Hertford cases in which a bacteriological examination has been made fall into two groups. In the first series, comprised of 11 cases, although a Gram-negative coccus developed on serum tubes inoculated with mucus from the nares or pharynx, the organism was not isolated. In the second series, which included 24 cases, the Gram-negative coccus was isolated in pure culture and examined. Some of the latter cases, in which the coccus was present in large numbers, were at an acute stage of the disease at the time that the bacteriological examination was made, and showed the scarlatiniform eruption particularly well developed. Several cases were repeatedly examined with positive result.

CHARACTERS OF GRAM-NEGATIVE COCCI ISOLATED FROM THE HERTFORD CASES.

Altogether three different kinds of Gram-negative cocci have been obtained from the Hertford cases. Their characters were found to be respectively as follows:

1. The first of the three types was met with far more frequently than either of the other two, and was cultivated from no less than 22 of the 24 cases. For this cause, and also by reason of its great abundance in cases examined at the height of the attack, this particular coccus is especially to be identified with the present outbreak. From the fact that in the majority of instances it grew well on ordinary agar at 37° C., and also on ordinary gelatine at 20° C., it would seem that the present coccus is closely allied to, if not identical with, *M. catarrhalis*. It is important to note that all 22 examples of the coccus produced an alkaline reaction when cultivated in the lemco-litmus fluid, containing either glucose, galactose, maltose, or saccharose. As regards pathogenicity, I have found that, although the coccus is apparently non pathogenic when injected subcutaneously into mice or guinea-pigs, nevertheless, when administered intraperitoneally, it is capable of producing in both animals a fatal peritonitis. In such fatal cases the coccus is found to be markedly intracellular, being enclosed for the most part in leucocytes of the peritoneal exudation.

Having recently obtained a coccus which I have been unable to distinguish from the present one from the sputum of a case of chronic bronchitis, in which it was present to over 100,000 but under 1,000,000 per c.c.m., I have little doubt that this coccus obtained so uniformly from the Hertford cases is identical with that found by Seifert, Pfeiffer, Lord, and others, and generally known as *M. catarrhalis*.

2. The second type of Gram-negative coccus was obtained from 2 cases only, and in both it was associated with *B. diphtheriae*. As the result of the bacteriological examination, these 2 cases were regarded as cases of diphtheria rather than of the Hertford disease. Although the present coccus grew well on ordinary agar at 37° C., on which it rapidly formed an opaque confluent growth, greyish-white in colour, it quite failed to develop on gelatine at 20° C. In all the four carbohydrate tests, namely, glucose, galactose, maltose, and saccharose, this coccus rapidly produced a strong acid reaction, thus forming a complete contrast to No. 1. When injected subcutaneously into mice and guinea-pigs the coccus produced no lesion, but when injected intraperitoneally it was fatal to both animals, and was found to be markedly intracellular in the peritoneal exudation.

3. The third type of Gram-negative coccus was obtained from only 1 of the 24 Hertford cases, but on both of two occasions when the nasal secretion of this case was examined the coccus was found present therein in large numbers. Coccus

No. 1 was also present in this nasal discharge, but it was much less abundant than Coccus No. 3. The latter grew well on ordinary agar at 37° C., on which its growth was much less granular, and less opaque than that of Cocci Nos. 1 and 2. As regards shape, smoothness, and transparency, the young colonies of the present coccus on nasagar bore a very close resemblance to colonies of the meningococcus on the same medium. Older colonies of the coccus on agar or on nasagar, however, differed from those of the meningococcus in that they became faintly but distinctly yellow. When first isolated, the coccus refused to grow on gelatine at 20° C., but latterly a slow but unmistakable growth has been obtained on this medium. As regards carbohydrates, the coccus produced an acid reaction when cultivated in the presence of glucose or maltose, but an alkali reaction in the case of galactose and saccharose. As in the case of Cocci Nos. 1 and 2, subcutaneous injections were negative, but when injected intraperitoneally, the coccus was found capable of producing a fatal peritonitis in mice and guinea-pigs, and the cocci were markedly intracellular in the peritoneal exudation.

These three types include all the Gram-negative cocci obtained from the Hertford cases. For the purpose of comparison I will now give the result of an investigation that I have made in the same way of the meningococcus, and some other Gram-negative cocci.

THE CHARACTERS OF GRAM-NEGATIVE COCCI OBTAINED FROM OTHER SOURCES.

4. *The Meningococcus*.—All three cultures of the meningococcus which have been examined were obtained from the pathological department of St. Bartholomew's Hospital, and had been isolated from cases of cerebrospinal meningitis. Two of them had been recovered during life by lumbar puncture, and the third was obtained from the meninges *post mortem*. In all three instances there was good growth on nasagar of circular or oval, smooth, clear, slightly blue colonies.

From their smoothness the colonies of the meningococcus on nasagar suggest to the eye the young colonies of coli on that medium rather than the colonies of staphylococcus albus. On the other hand, the colonies of the Gram-negative Cocci Nos. 1 and 2 on nasagar bear a close resemblance to the rough opaque white colonies of staphylococcus albus. Of the three specimens of the meningococcus examined, two refused to grow on ordinary agar, but the third grew fairly well on this medium. All three absolutely failed to grow on gelatine at 20° C. Two of the meningococci were apt to soon lose their vitality, and it was necessary to subculture them every two days on nasagar; the third meningococcus, however, was much less delicate, and lived for quite a week on nasagar. Although the two more delicate meningococci died rapidly on nasagar, they lived for over a week in broth to which raw sterile ascitic fluid had been added. All three specimens of the meningococcus gave identical reactions in the carbohydrate tests, in applying which a few drops of sterile raw ascitic fluid were found to be necessary in the case of the two more delicate specimens, though the third meningococcus grew well enough in the carbohydrate medium without the addition of ascitic fluid.

The reactions produced were as follows: Glucose acidified, galactose acidified, and maltose acidified; in the case of saccharose, on the other hand, no acid was produced, but an increase of alkalinity. As in the case of the preceding three cocci, subcutaneous injections into mice and guinea-pigs gave a negative result, but when injected intraperitoneally a proportion of both animals succumbed to a peritonitis. The intracellular disposition of the meningococci in the peritoneal exudation was even more marked than in the case of the preceding cocci.

5. *The Gonococcus*.—Through the kindness of Dr. Andrewes I recently had the opportunity of examining the gonococcus with the same carbohydrate tests as have been applied to the preceding cocci. The gonococcus which I examined had been isolated by Dr. Andrewes from the urethral pus of a typical case of gonorrhoea by means of a blood agar plate. I found that the gonococcus that developed in large numbers on this plate was far more delicate than any of the previous Gram-negative cocci, and I failed to get growth even on the nasagar medium unless a little fresh human blood was previously smeared over it. The colonies of the gonococcus on blood nasagar were more transparent and smaller than those of the preceding cocci. No growth occurred on gelatine at 20° C. Three colonies of the gonococcus were isolated from the plate and tested in the carbohydrate media to which a few drops of

the raw sterile ascitic fluid were added. At the end of a week at 37° C. acid reactions had appeared in the case of glucose and galactose, but in the case of saccharose and maltose the reaction of the medium was unchanged. All three colonies responded in the same way to these carbohydrate tests.

6. *A Gram-negative Coccus from the Normal Urethra*.—This micro-organism was isolated from mucus present in a sample of normal urine and was found to grow readily on ordinary agar at 37° C., and on ordinary gelatine at 20° C. When examined in the carbohydrate tests the coccus rapidly produced an acid reaction in all four, namely, in glucose, galactose, maltose, and also in saccharose.

The Chief Characters of the Six Gram-negative Cocci.

Number.	Source.	Indifferent Specimens Examined.	Different Colonies Tested.	Growth on Naggar at 37° C.	Growth on Gelatine at 20° C.	Carbohydrates. Production of an Acid Reaction in			
						Glucose.	Galactose.	Maltose.	Saccharose.
1	(<i>M. catarrhalis</i> .) Nasal and pharyngeal secretion in 22 cases of the Hertford outbreak. Sputum chronic bronchitis	23	65	Opaque granular	Positive	—	—	—	—
2	Nasal secretion of 2 Hertford cases in both of which <i>B. diphtheriae</i> also present	2	4	Opaque granular	Negative	+	+	+	+
3	Nasal secretion of 1 Hertford case in which <i>Coccus</i> No. 1 also present	1	4	Clear, smooth, later becomes yellowish	Negative at first, positive later	+	—	+	—
4	(<i>Meningococcus</i> .) Lumbar puncture 2 specimens; meningitis post-mortem 1 specimen	3	9	Clear, smooth	Negative	+	+	+	—
5	(<i>Gonococcus</i> .) Urethral pus	1	3	No growth unless blood added to medium	Negative	+	+	—	—
6	Mucus from normal urethra	1	2	Opaque, somewhat granular at centre but smooth at edge	Positive	+	+	+	+

CONCLUSION.

It follows from these observations that while the virulence for mice and guinea-pigs of the various Gram-negative cocci examined appears to be of little, if any, value for differentiating them, the effect of these cocci upon glucose, galactose, maltose, and saccharose, respectively, is of much value for this purpose. Owing to the special pathogenic significance of the meningococcus, gonococcus, and possibly also of *M. catarrhalis* and other individuals of the group, it is advisable in future, when examining a Gram-negative coccus, to test it, amongst other things, in all the four carbohydrate media before considering that its identity has been established. If mistakes are to be avoided, this procedure would seem to be particularly desirable—I may even say necessary—when examining nasal or pharyngeal secretion for the meningococcus, or when examining discharge from the urogenital tract, or other material, for the gonococcus.

REFERENCES.

- ¹ Paper read before the Epidemiological Society on January 20th, 1899.
² Memorandum by Dr. Farrar to Local Government Board, September, 1903.
³ *Flugge, Micro-organismen*, Band II, 1896, pp. 154-5. ⁴ Loc. cit.
⁵ *Zeitschr. f. klin. Med.*, Band XLIV, 1901. ⁶ *Centralbl. f. Bakt. Originale*, Band XXXIV, 1903, p. 641. ⁷ *Bull. Soc. Méd. Hôp. de Paris*, March 2nd and 10th, 1905.

OLD AGE.—The London Vegetarian Association has arranged a meeting at which the question what period of life should be termed old age will be discussed by a number of gentlemen between the ages of eighty and ninety, who are still engaged in literary, medical, agricultural, and other work. The chair will be taken by Mr. C. P. Newcombe, who is an octogenarian, and other speakers will be Professor Mayor, Mr. Joseph Wallace, and others. The meeting will be held in the Memorial Hall, Farringdon Street, on October 13th.

RATS IN RELATION TO PLAGUE.

By BRUCE SKINNER,
Lieutenant-Colonel R.A.M.C.

SINCE the publication in the BRITISH MEDICAL JOURNAL of May 6th, p. 994, of a paper on Plague and the Geographical Distribution of Rats, and, indeed, since the following remarks were drafted, I have become indebted to Lieutenant-Colonel Leishman for a reference to a paper by Dr. Rudolf Abel relating to Rats and Plague.¹ This paper contains a record of research into the ancient accounts of plague epidemics extending back to that described in 1 Sam., chap. v and vi. These records associate rodents and many other animals with plague. Dr. Abel comments on the want of reference to rats in relation to the plague epidemics which have occurred in Europe since 1730, and regards this as pointing to the fact that improved sanitation is probably the reason why Europe has since that date been less liable to plague epidemics. He suggests that perhaps *M. rattus* was less predisposed to plague than *M. decumanus*, and that as *M. decumanus* "belongs to the species that now in India and China suffers so much from plague, the rats in South and East Europe ought to have played a much more important part in plague epidemics, which is not the case." "Also," he says, "we are not justified in assuming that *M. rattus* shows less predisposition for plague than *M. decumanus*, as the matter has not been settled by experiment." He concludes that his historical survey shows that the most serious plague epidemics known which ravaged Europe in olden times without special participation of the rats and mice warn us that these rodents must not be regarded as the first cause in the epidemiology of plague.

Dr. Abel has not conceived the possibility that *M. decumanus* may be responsible for the comparative freedom of Europe from plague, as previously explained, evidently because he is under the impression that this species is the rat of the areas of endemic plague. We know, however, that *M. rattus* is the indigenous species of the plague endemic areas; and we are justified in inferring that if the latter species causes or spreads plague, he does not do so in Europe because he is now a very subordinate occupant of that area.

Some further observations on the habits of the different species of rats are, however, required, especially in their relation to plague.

The following extract from a classical writer is not without interest in its bearing on the part played by rats in its dissemination:

Neither are they (the Iberians) the only sufferers in being tormented with vast swarms of mice, from which pestilential diseases have frequently ensued. This occurred to the Romans in Cantabria, so that they caused it to be proclaimed that whoever would catch the mice should receive rewards according to the number taken, and even with this they were scarcely preserved, as they were suffering besides from want of corn and other necessities.²

Although the word mice is used we may accept it in its broadest sense as a family name, as the differentiation of the family into genera and species seems to be modern. It does not necessarily mean, as I have seen suggested, that the Romans were unaware of the existence of rats.

One point in the above quotation which especially merits attention is that the Romans employed the modern unscientific method of dealing with the mouse family when plague was prevalent.

We in the present day are aware that long-tailed rats suffer from plague. But we are not aware that they initiate plague. Analogy rather points to the certainty that these rats contract plague from some extraneous source, as animals cannot be said to initiate any specific fever.

That source has not yet been discovered. But inasmuch as it must be something outside the rat, it would appear more scientific to adapt our defensive measures to our immediate surroundings rather than to destroy those animals whose misfortune it is to suffer in common with ourselves from pestilence. Further, in our country at least, rats in towns are valuable scavengers.

There are certain seasons when rats betake themselves to houses. For instance, in England, the short-tailed rat, when he has adopted a country life, lives entirely out-of-doors from spring to autumn; in winter he resorts to corn-ricks, bean-stacks, barns, and houses.

The town rat lives usually in sewers. Where there is a *rus in urbe*, some of the town rats may be classed as to habits with the country rat. The great plague epidemics of England raged during the period when the rat lived out-of-doors.