THE FILTER PASSER OF INFLUENZA.

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THE PROPERTIES OF FILTRABLE VIRUSES.

Although it is over twenty-four years since Loeffler and Frosch demonstrated that the virus of foot and mouth disease can pass through the Berkefeld filter, and following on that "key discovery" the infective principles of a large number of other diseases of unsolved etiology have been found to be "filtrable" in the same manner, the progress of knowledge with regard to the active pathogenic agencies here involved has been remarkably slow. That the infective principles in question are not mere enzymes, but living though extremely minute micro-organisms, is evident from the fact that under favourable circumstances these filtrable viruses are capable of endless multiplication; and further, that when submitted to conditions unfavourable to elementary forms of life, they perish. The filtrable viruses, however, possess certain properties which suggest that the minute living organisms comprising them are somewhat different from the ordinary pathogenic bacteria; thus while the resistance of these viruses to heat is lower, their resistance to glycerine is very much higher than that of most bacteria.

While the label "invisible" or "ultramicroscopic" has been applied to these filter-passing viruses there is reason to suspect that this term is premature until the possibility of rendering them visible by staining or other methods has been explored a great deal further than appears to have
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been the case up to the present time. Particularly significant is the information that has been obtained concerning viruses such as those of vaccinia, variola, poliomyelitis, and molluscum contagiosum where the presence of a definite filter-passing micro-organism has been ascertained. In the case of all these viruses the micro-organisms present are described as minute bodies, round or oval in shape, from 0.1 to 0.3 of a micron in diameter, occurring chiefly as isolated individuals, or in groups, but occasionally in the form of diminutive diplococci, and rarely in short chains. These "micro-micro-organisms" fail to stain by Gram's method; indeed, so resistant are they to ordinary stains that it is only by preliminary fixation, by then using a hot mordant, and finally by applying the most powerful of all bacterial stains, namely hot carbolfuchsin freshly prepared, that chief success has been obtained so far in demonstrating their presence. An alternative to this method of Loeffler (introduced originally for the staining of flagella) is to fix the film preparation for twenty-four hours or longer in methyl alcohol or sublimate alcohol, and then to apply Giemsa's stain for twenty-four hours, using preferably several changes of the stain. It is important, however, to note that when a film of filter-passing virus has been stained successfully, these minute micro-organisms may be found present in such enormous numbers that material such as vaccine lymph, or fluid from a smallpox vesicle, even when diluted a thousandfold, is still found to be teeming with them.

The minuteness of these filter-passing micro-organisms—considerably smaller than the smallest of the pathogenic bacteria and only just within the boundary of microscopic vision—coupled with their strong resistance to ordinary stains go far to explain the success with which they have defied research. Add to this the extreme degree of parasitism that numerous unsuccessful attempts to cultivate them on ordinary media has served to illustrate, and the explanation of their immunity from investigation is complete. On the whole, however, it would seem probable that the chief reason why these filter-passing micro-organisms have escaped scrutiny is because the technique for their elucidation is far more difficult to acquire than in case of the ordinary bacteria.

So specialized are the pathogenic activities of the filtrable viruses that Lipschutz has suggested their classification according to the particular tissues upon which they severally operate. Thus, dermatropic viruses include the active principles of variola, vaccinia, varicella, and foot and mouth disease. Haemotropic viruses include those of pernicious anaemia of the horse and leucæmia of the fowl. Neurotropic viruses include those of rabies, distemper, and of poliomyelitis. Mumps on the other hand is an example of an organotropic virus. Finally, there is a group of filter-passing viruses that produce acute general disease such as measles in man, or rinderpest in cattle. Certain diseases of plants also are known to be due to virus that is filtrable in the same way as the active principles of these diseases of man or of animals.
Human experiments have already gone far towards solving the etiology of influenza. The introduction of Pfeiffer's bacillus into the nose and throat of healthy men has failed to produce this disease: thus Sellards and Sturm of Johns Hopkins injected five strains from measles cases without result; Bloomfield used three strains obtained from healthy men on fourteen volunteers without effect; and Davis succeeded in producing only a transient illness in a young man by injecting him with a thick suspension of Pfeiffer's bacillus isolated from a case of pertussis. Wahl, White and Lyall sprayed two volunteers with fresh cultures from cases of influenza; one was unaffected, the other only had a slight reaction in his nose. Lastly the United States Public Health Service in conjunction with the United States Navy Medical Department carried out experiments with Pfeiffer's bacillus isolated from cases of influenza: McCoy and Richey infected five volunteers with a heavy suspension composed of eight strains of Bacillus influenza; but although none of these men had previously had influenza all failed to show any symptoms. Blake and Cecil introduced a new note by raising the virulence of Pfeiffer's bacillus (obtained from an influenza case) by passing it through eleven white mice in succession, and next through thirteen monkeys. They then found that this bacillus freshly recovered from the peritoneal fluid and instilled into the nose and mouth of further monkeys gave rise to a respiratory infection which broadly resembled influenza in man: symptoms began three to five hours after inoculation and included prostration accompanied by sneezing and coughing. Leucopenia was present; and the illness lasted for three to five days. In addition to the very short incubation period, it is noteworthy that fever was small or absent. A striking point is that out of the 12 monkeys infected in this way 5 developed acute suppuration of the antrum from B. influenzae, and 2 monkeys got broncho-pneumonia on the third or fourth day. Blake and Cecil inferred that while these experiments established an etiological relationship between Pfeiffer's bacillus and acute sinusitis, tracheo-bronchitis, bronchiolitis, and broncho-pneumonia, a definite conclusion that this bacillus is the cause of influenza was not permissible owing to the impossibility of determining whether the respiratory disease thus produced in monkeys is identical with influenza in man or not. This question however was solved by Blake and Steffen who injected similar virulent cultures of Pfeiffer's bacillus into the nose and throat of two volunteers and found that while acute respiratory disease was produced broadly resembling influenza, it fell short of the typical clinical picture of that disease. Filtrates of cultures of Pfeiffer's bacillus tried in the same way by Cecil and Steffen on man gave rise to no symptoms of any kind.

In contrast to these negative or doubtful results with Pfeiffer's bacillus are the significant experiments of Yamanouchi, Sakakami and Iwashima who made excellent use of their fifty-two friends—doctors and nurses—
who volunteered for experiment. The sputa of forty-three cases of influenza were emulsified in Ringer's fluid. This emulsion was injected into the nose and throat of twelve healthy persons and the Berkefeld filtrate of it into twelve more. The results were very striking: among the subjects were six who had recently had influenza and these escaped, the remaining eighteen all developed influenza after an incubation period of two to three days. A filtrate of the blood of influenza cases was next injected into the nose and throat of six more healthy persons: the results were precisely the same as in the previous experiments. They next tried the subcutaneous route: four healthy persons received filtered sputum and four filtered blood: all with the exception of one who had previously had the disease developed influenza after an incubation period of two to three days. Lastly a pure culture of Pfeiffer's bacillus and a mixture of Pfeiffer with pneumococci, streptococci, staphylococci and diplococci from the sputum of influenza patients were sprayed into the nose and throat of fourteen healthy persons, but no symptoms and no illness followed these injections. Yamanouchi and his colleagues concluded that influenza is due to a filtrable virus which is present in the sputum and blood of patients and can infect either by implantation on to the mucous membrane or by inoculation, and that Pfeiffer's bacillus and other bacteria found in the sputum are not the cause of influenza. They point out that all the subjects who had influenza, or who received the sputum emulsion or its filtrate, became immune.

Taken with the failure of Pfeiffer's bacillus to reproduce the disease, and the positive results with filtered material on human subjects reported previously by Nicolle and Lebailly, de la Rivière, and others, these Japanese experiments would appear to establish the fact that the primary infective agent in influenza is a filtrable virus. A completeness at present lacking would have been given to their observations had the Japanese investigators shown that the filtered blood or sputum from influenza cases loses its power of reproducing the disease when heated for half an hour to 55° C.

SEARCH FOR A FILTER-PASSING MICRO-ORGANISM IN INFLUENZA.

Before the recent pandemic of influenza, it had been demonstrated by Kruse and confirmed by Colonel George Foster, of the U.S. Army Medical Corps, by means of human experiment that the virus of the common cold is a filter passer. Foster succeeded in cultivating in Noguchi medium a minute micro-organism from the filtered nasal secretion at the onset of acute coryza, and when a culture containing this micro-organism was instilled into the nostrils of volunteers coryza followed, and the filter-passing micro-organism was recovered from the nasal secretion. The incubation period was eight to thirty hours, and similar whether the diluted and filtered nasal secretion was used, or the culture of the filter passer. It should be added that Foster's observations were made on volunteers from a garrison of 250 soldiers, and that they were carefully controlled.
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It was to be expected a priori that attempts to define the primary infective agent of influenza by animal experiments would be far less conclusive than experiments on human beings. During the pandemic of 1918, the late Major H. Graeme Gibson, of the Royal Army Medical Corps, and his colleagues, Major F. B. Bowman, C.A.M.C., and Captain J. T. Connor, A.A.M.C., while attached to the British Expeditionary Force in France, showed that filtered or unfiltered sputum taken from cases of influenza at the earliest stage of the disease produced in the inoculated animals—monkeys, rabbits, mice, and guinea-pigs—pulmonary lesions closely resembling those found in the lungs of patients who succumb at an early stage of influenza. They were also able to transmit the disease from animal to animal. By employing Noguchi medium they succeeded in growing a filter-passing micro-organism (a) from the kidney of animals thus injected, (b) from the filtered extract of the lungs of these animals, (c) from the filtered sputum of human cases at the early stage of influenza. Cultures containing this micro-organism on inoculation into animals gave rise to pulmonary lesions similar to those produced by the sputum of patients, or by its filtrate; and the disease thus induced was transmitted from animal to animal. They concluded that the primary infective agent in influenza is a filtrable virus, and that the micro-organism cultivated by them was in all probability the primary cause. They were struck by the degree of pulmonary damage that might be present in these animals without any marked clinical evidence of disease. When the monkeys developed symptoms, they appeared on the fifth to seventh day after inoculation, which would seem to be an unusually long period of incubation.

The filter-passing micro-organism was described by them as follows:

"Numerous small coccoid bodies in size varying from about 0.1 to 0.2 of a micron, generally single, but often taking on a diplococcal arrangement and sometimes occurring in small agglomerations. Some showed a rather delicate halo the significance of which has not yet been determined. With Giemsa they usually stained a deep purple, but some which were apparently degenerate were paler in colour and of a pinkish tinge." On this first occasion on which they recovered this organism they found it to be Gram-negative, but they stated that since then in young cultures the organism might be Gram-positive. There is some reason, however, to doubt the correctness of the latter statement.

Independently of Gibson and his colleagues, Bradford, Bashford and Wilson recorded similar observations during the same outbreak. They found evidence of the presence of a filter-passing organism of similar morphology and succeeded in producing in experimental animals pulmonary lesions resembling those of influenza. Subsequent attempts, however, by Wilson to investigate the filter-passing micro-organism further were unsuccessful, and in a note appended to a criticism by Arkwright of this work Wilson and Bashford withdrew their claim that such a micro-organism had been cultivated.
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In two recent papers on the aetiology of epidemic influenza, Maitland, Cowan and Detweiler, of Toronto, report the result of their experiments in search of a filter-passing virus. Cultures in Noguchi medium were made from filtered nasal washings of one case, and from filtered plasma and filtered corpuscles of three cases, including the patient whose nasal secretion was filtered and cultivated. The actual stage of the attack when these materials were taken is not clear. Three samples of ascitic fluid were used, but no mention is made of their hydrogen ion concentration. A careful series of controls was set up. The results were quite negative. Maitland and his colleagues justly remark that these observations are too meagre to exclude a filter-passing organism as the aetiological factor in influenza. The observations of these investigators, however, served to bring out an important source of fallacy with regard to pulmonary lesions occurring in experimental animals—lesions corresponding in some respects with those described by the previous two groups of workers as occurring in animals after inoculation with influenzal material or cultures. They found in the first place that haemorrhages into the alveoli and sometimes into the bronchioles, but no leucocytic reaction in the lung tissue, occurred as an agonal phenomenon in guinea-pigs fairly frequently, and may be present in control animals. They also observed that a second kind of pulmonary lesion may occur spontaneously in these animals, namely, a slow proliferation of the cells of the alveolar epithelium. While these haemorrhagic and endothelial lesions may be found in the same area, they appeared to be independent. The explanation of the proliferative lesion is left open by them. These observations by Maitland and his colleagues are of much importance as indicating a possible source of error in experiments of this kind: but a comparison of their figures with those representing the microscopic appearances of the lungs of experimental animals and attached to the report of Gibson and his colleagues is reassuring, for prominent features in the latter are the presence of oedema and inflammatory exudate. Maitland and his colleagues are also entirely in error in assuming that the purely destructive if "wholesome" criticism of Arkwright necessarily invalidates the careful, laborious and constructive work of Gibson and his colleagues with regard to the presence of a filter-passing micro-organism in influenza, as the sequel has shown.

During the past three years Olitsky and Gates of the Rockefeller Institute have published a series of studies on the aetiology of influenza that have not only confirmed and added precision to those of Gibson and his colleagues, but also promise to place this matter of the filter-passing micro-organism on a new and firm experimental basis. Starting with the nasopharyngeal washings of cases within the first thirty-six hours of the disease they showed that this material unfiltered produces on intra-tracheal injection into rabbits a leucopenia falling especially on the lymphocytes, a lesion which appears to be particularly constant in influenza of the human subject. Accompanying this leucopenia they found pulmonary lesions
characterized by hæmorrhage, emphysema and œdema. The important point, however, is that Olitsky and Gates were able to transmit this syndrome in series, and to carry it on from rabbit to rabbit for as many as fifteen passages. Controls made in the same way with nasopharyngeal washings from sixteen non-influenzal cases including cases of coryza, also from cases of influenza after the second day proved negative. Foreign protein (e.g., human ascites fluid), B. influenza or its toxin prepared by Parker's method all gave negative results. Having thus proved that the diluted nasopharyngeal secretion at the onset of influenza has a peculiar pathogenic effect on rabbits, they proceeded to show that this action is still possessed by the secretion when the bacteria have been removed from it by Berkefeld filter. Still using the peculiar rabbit reaction as guide, they next showed that the pathogenic agent present in the nasopharyngeal secretion resists the addition of fifty per cent of glycerine for periods up to nine months, and finally that it consists of a minute “bacilloïd” body 0'15 to 0'3 micron in length, which they cultivated in Noguchi medium, using ascites fluid, having a p.H. of not over eight and preferably 7'8. In papers published during the present year, Olitsky and Gates report that they have succeeded in growing the filter-passing micro-organism anaerobically in fluid media containing reducing substances other than rabbit kidney—for instance suitable conditions can be established either by first growing B. coli in the medium for a few hours and then killing it by heat, or by adding unheated vegetable tissue to the medium as suggested by Avery and Morgan who recommend pieces of potato, carrot or banana for this purpose. Furthermore, Olitsky and Gates have succeeded in growing this filter-passing micro-organism of influenza under strict anaerobic conditions on agar plates enriched with nothing more than five per cent of rabbit's blood. On the latter medium fine colonies may appear within a week, and the filter-passing micro-organism may develop into a spindle-shaped rod as much as a micron in length; though it reverts to the smaller original form when transferred to the Noguchi medium. They have succeeded in proving that an immunity results in the rabbit from inoculation either with the nasopharyngeal secretion of influenza cases, or with the Berkefeld filtrate therefrom, or with the filter-passing micro-organism isolated from either of these materials; and by a series of cross-tests they have satisfied themselves that the immunity in all three instances is identical. This specific immunity appears to last in the rabbit for at least fourteen months. Lastly, they have established experimentally the formation of specific antibodies (complement-fixing substances, agglutinins, etc.), to this filter-passing micro-organism.

Personal Observations.

Early in the present year (1922) a sharp outbreak of influenzal illness occurred among the nursing staff of St. Bartholomew's Hospital. I am indebted to Dr. W. P. S. Branson for the following particulars of the
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epidemic. Between December 23, 1921, and January 23, 1922, fifty-seven nurses were attacked; the majority between December 25 and January 3. The disease was characterized by its sudden onset, and by the fact that for the time being it completely incapacitated the affected. The average duration was three days febrile illness necessitating a stay of altogether five days in bed, and an average absence from duty of seventeen days. Signs and symptoms at the onset were congested conjunctiva and facies, coryza, furred tongue, rapid pulse and pain in the back. The average highest temperature was 102°F. With regard to complications these fortunately were rare; one case developed bronchitis that did not pass into pneumonia, and one case of mastoiditis occurred.

Technique.—During the previous six months I had been practising the Noguchi method of culture on various occasions. It may be of interest to state that of three specimens of ascites fluid obtained for this purpose from cases in the hospital all were found to be too alkaline when their p.H. content was kindly examined by Mr. Archer. The two specimens of ascites fluid used in the following observations needed in the first case 0·5 cubic centimetre, and in the second 0·8 cubic centimetre of N/20 HCl per 10 cubic centimetres of ascites fluid to bring their reaction to 7·8—the figure specified by Olitsky and Gates. After their reaction had been corrected in the manner stated, the specimens of ascites fluid distributed in ten or twenty cubic centimetre amounts in test tubes were repeatedly heated to 55° C. for at least half an hour, and then stored for use when required. Their sterility was above suspicion. The manipulations to secure pieces of fresh kidney aseptically from the rabbit were essentially similar to those described by Foster and by Gibson and his colleagues with regard to the use of freshly sterilized instruments for each stage of the incision. I have preferred, however, to remove the kidneys through a lumbar incision rather than by the abdominal route, and after it has been anaesthetized, bled, and killed, the rabbit is skinned and soaked in strong zizal before incising the lumbar muscles in order to obtain the kidney. Each kidney should be removed as neatly and quickly as possible with freshly flamed scissors and forceps, and transferred to Petri dishes, where they may be cut up at leisure into pieces of the requisite size for transfer to the long tubes used in this work. A trained assistant is necessary in these manipulations; no visitors and no talking should be allowed. I have formed the opinion that the kidneys of young rabbits give better results than those of old animals in this work. Each piece of kidney transferred to a long test tube (20 centimetres long by 1·25 to 1·5 centimetres broad), has a little ascites fluid added to it, and is incubated for two days before use, so that any tubes showing contaminations or cloudiness can be rejected.

The diluted nasal or pharyngeal secretion from four nurses within twenty-four hours of onset was put through filter paper, then through the Berkefeld filter, by means of the arrangement shown in the diagram (which
was kindly drawn for me by Dr. T. G. M. Hine] a fresh funnel, filter, and holder being used for each. One cubic centimetre of each filtrate was inoculated into each of two Noguchi tubes, and one cubic centimetre into an ordinary broth tube. After filling up the Noguchi tubes till each contained ten cubic centimetres of ascites fluid, they were sealed with a layer of wax and vaseline. The first Noguchi tube was then heated for half an hour to 55° C., after which all three tubes were placed in the incubator. All the broth tubes remained sterile. In each case the unheated Noguchi tube showed between the fourth and sixth day a cloudiness near the piece of kidney at the foot of the tube precisely in the manner described by Olitsky and Gates. In each case also the heated Noguchi tube remained free of cloud over this period, as also did a control tube of the Noguchi medium left uninoculated and incubated with the rest. On examining the positive cultures with Gram's stain, no bacteria could be found, and subcultures on ordinary agar failed to grow.

When films of material taken from the foot of the positive cultures were fixed in methyl alcohol for twenty-four hours, and then stained in five per cent Giemsa for a similar period, and lightly differentiated in equal amounts of acetone and xylol swarms of minute round bodies which had for the most part a purple tint, but occasionally red, were found on careful examination. These bodies are very easily missed unless specially
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looked for, and would inevitably be dismissed as "ground" by an inexperienced observer. Having failed to find these minute bodies in the control tubes, I proceeded to look up the literature of filter-passing micro-organisms, and was much struck by the photographs of those found by Paschen in vaccine lymph which are included by Lipschutz in his article in Kolle and Wassermann's text-book. Having obtained some fresh vaccine lymph through the courtesy of Mr. Fremlin, I proceeded to examine films of it by the staining method recommended by Paschen, and after some difficulty succeeded in finding these bodies by using a modification of Loeffler's flagella stain. Having come to the conclusion that these bodies in vaccine lymph are of the same order as regards morphology as those present in the influenza cultures, I have since that time been endeavouring to acquire the requisite skill to enable me to investigate these minute micro-organisms, and I am very conscious of the magnitude of the technical difficulties at the present time and of the comparatively slow progress made. Results have been briefly as follows:—

Primary Cultures.—Through the kindness of colleagues and of the medical departments of the Ministry of Health and the London County Council, I succeeded in obtaining material during the late outbreak of influenza in London from sixteen further cases within thirty-six hours of the onset of symptoms, and cultivated the filtrate from each in Noguchi medium as before. Out of the total of twenty cases (including the nurses) in which the filtrate of the nasal or pharyngeal secretion was examined in this way, I obtained evidence of the presence of a filter-passing organism in fourteen. In addition, filtrates from the bronchial secretion were cultivated from three fatal cases of influenza, with positive results in two. The secretion that gave the best growth of the filter passer was literally swarming with Pfeiffer's bacillus; this bacillus, however, failed to pass the Berkefeld filter.

In addition to the above cultures, I have examined similar material from seven cases of measles at the onset of the rash. In three of these cases the filtrate produced some clouding of the Noguchi tube on the fourth to seventh day, and in two of these cultures I succeeded in satisfying myself of the presence of a filter passing micro-organism similar in morphology to that found in the influenza cultures. Both these positive cases, however, probably derived the infection from the same strain of virus, and there are clinical grounds (absence of Koplik spots, and enlargement of glands at back of the neck) for thinking that they were in reality cases of German measles.

Subcultures.—After three weeks' growth, three of the primary influenza cultures were diluted, filtered, and the filtrate sown in fresh Noguchi tubes. Two gave a positive growth; in the third the result was doubtful.

Demonstration of the Filter-passing Organism in situ in the Secretion.—As neither Foster nor Olitsky and Gates mention any observation on this point, I have investigated the matter, and in both of the last two cases of
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FIG. 1.—Anaerobic filter-passing micro-organism present in pure culture in kidney-ascites medium inoculated with the Berkefeld filtrate of diluted nasal secretion from a nurse within twenty-four hours of the onset of influenza. After incubation for a fortnight, material from the foot of this culture, which showed no bacteria by Gram's stain, was diluted 500 times in distilled water and then spread as thinly as possible over a coverslip which was allowed to dry in air. The film was fixed in methyl alcohol for one hour, and stained overnight in 5 per cent Giemsa. I am indebted to Mr. J. E. Barnard for the present microphotograph taken at a magnification of 1,000 diameters.

FIG. 2.—Nasal secretion at the onset of influenzal catarrh showing the minute filter-passing micro-organism in situ. The preparation was fixed for twenty-four hours in methyl alcohol, stained for a similar period in 5 per cent Giemsa, and differentiated in equal parts of acetone and xylol. A pure culture of the micro-organism was obtained from the Berkefeld filtrate of this material. Magnifying power 1,000 diameters.

To illustrate "The Filter Passer of Influenza," by Hon. Lieutenant-Colonel M. H. Gordon, C.M.G., C.B.E., M.D.
FIG. 3.—Nasal secretion from another case within the first twenty-four hours of onset. The relatively large diplococci are pneumococci. The much smaller filter-passing micro-organism is apparently being phagocyted by the polymorphonuclear cell in the middle of the photograph. In addition to the usual fixative, this specimen was fixed in warm acetic acid (1 per cent). Stain carbolfuchsin. Magnification 1,000 diameters.

FIG. 4.—Preparation of fresh calf-lymph diluted and mixed with staphylococcus. The filter-passing micro-organism described by Paschen is seen in large numbers, and towards the middle of the field is a single staphylococcus. Stain a modification of Loeffler's flagella stain. Magnifying power 1,000 diameters.

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influenza have succeeded in demonstrating what I believe to be the minute filter-passing organism in large numbers in the nasal secretion during the first twenty-four hours of the disease. In both cases the secretion was clear, and ordinary bacteria were scarce. The stain used successfully was Giemsa, the stained preparations being differentiated as before in xylool and acetone. Confirmatory results were obtained in both cases with methyl blue, and in the last one with azur I as well. Both secretions were filtered and found to yield a positive growth of the filter passer on the third to fourth day. The first case developed symptoms of gastric catarrh two days later, but was well again in a few days, and when his nasal secretion was re-examined eleven days from the onset, no certain filter passers were found either by film or by culture of his nasal secretion.

The morphology of the filter-passing micro-organism from the influenza cases has been accurately described by Gibson and his colleagues. Photographs of it, which I owe to the courtesy of Mr. J. E. Barnard of the National Institute for Medical Research, are attached to the present paper (figs. 1—3). The diameter of the organism shown in fig. 1 is 0'2 of a micron. I think that Olitsky and Gates are perhaps ill-advised in calling it "bacillloid," and the name they propose for it, Bacterium pneumosintes, seems to me premature. The general feeling I have is that we are dealing with a new field, so to speak, and that the individual filter passers had better not be given official names until we have agreed upon some family name for the whole group to which a distinctive name for each member may be added later.

It is, I think, self-evident that the relatively slow progress that has been made with the elucidation of filter passers is due to the fact that these micro-organisms require a technique far more difficult to acquire than that which is adequate for dealing with ordinary bacteria. The recent work of Olitsky and Gates, however, promises to simplify very considerably the technique of culture. We are badly in need at present of some simple and fairly quick-staining method that will tell us for certain whether these very minute "stain-fast" organisms are present or not in a given material.

For this purpose Loeffler's flagella stain is too complicated, uncertain and dirty. Giemsa is far cleaner and less liable to error, but the long time required for fixing and staining is a drawback, and the final differentiation needs the greatest caution or the finest preparation may be ruined. Furthermore, Giemsa fails occasionally to reveal filter passers; even when these are present in large numbers. Olitsky and Gates use a well-ripened methylene blue (either polychrome or Loeffler's), but the specimens of methylene blue I have been able to obtain, one and all have failed to stain filter passers. My best results so far have been obtained with a sample of methyl blue obtained in 1912 from Grubler. It is, of course, essential to fix these organisms very thoroughly before attempting to stain them. A mixture of equal parts of absolute alcohol, acetone, chloroform, and ether makes a good fixative, and seems to clear the ground—a great source
of error in this work. After air-dried coverslip preparations have been immersed in this mixture for half an hour, I place them in absolute alcohol for the same time, then dry them and place them in one per cent acetic acid for a few minutes, after which they are left in 1:200, or better, 1:400 methyl blue overnight. When other methods fail, I fix two preparations in the manner stated, and then warm one in one per cent acetic, the other in one per cent NaOH until vapour arises, then after a thorough wash in water and alcohol, place the films in watch glasses containing carbol-fuchsin, which are covered over and placed for half an hour in the 55°C. incubator. In studying the staining of filter passers, I have found it helpful to dilute the culture about 500 times in distilled water, and to mix some of this dilution with a fresh emulsion of staphylococcus. The staphylococcus is easy to focus, and enables one to find the field far more easily than when the minute filter passer alone is present. The very reluctance of filter passers to stain with ordinary bacterial dyes can be applied to differentiate them from accompanying bacteria. Thus a mixture containing 1:5,000 to 1:10,000 of ordinary basic fuchsin, and 1:200 to 1:400 methyl blue has been found to stain staphylococci a brownish red colour, and the accompanying filter passer a light blue. It is not every specimen of methyl blue, however, that is successful, and the value of this and other stains for demonstrating filter passers is a matter calling for further investigation.

Search for the primary infective agent in influenza, therefore, has led us into the realm of filter passers, and has revealed the presence of a micro-organism of this group. The further properties of this micro-organism and its precise relation to others including the similar filter passer cultivated by Foster from the nasal secretion during the acute stage of the common cold are matters awaiting research.

REFERENCES.


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