The Canadian
Medical Association Journal

VOL. XI. TORONTO, JULY, 1921 No. 7

THE ÆTIOLOGY OF EPIDEMIC INFLUENZA
A CRITICAL REVIEW

H. B. MAITLAND, M.B.
Assistant Professor of Pathology and Bacteriology

AND

GORDON C. CAMERON, M.B.
Fellow in Pathology and Bacteriology

From the Department of Pathology and Bacteriology in the University of Toronto.

THE recent and widespread outbreak of epidemic influenza has been the occasion for very extensive research regarding the cause of this disease. In many places the clinical material was only available during a short local epidemic and for this reason the preliminary experiments were often intensive and not fully developed until the epidemic had passed. The present time, therefore, would appear to be favourable for reviewing the more important results of these investigations.

Epidemic influenza is notably a pandemic disease and manifests itself by an unusual number of clinical conditions. In the recent literature there have been excellent descriptions, both clinical and pathological. The more severe form was the acutely fatal influenzal pneumonia. The lungs of these cases presented pathological findings that have only been seen during epidemics and were recognized as typical of epidemic influenza. There were, however, during these periods cases of all degrees of severity, the mildest showing only acute fever, headache, general pains and weakness for a few days, and having no lung involvement. These milder cases were considered to be epidemic influenza because they occurred during these periods. But until a causative agent has been found for both mild and severe forms it will be impossible to decide whether or not they are manifestations of the same disease. The clinical pictures of the milder types are seen in non-epidemic years and yet they are not diagnosed as epidemic influenza. No doubt sporadic cases continually occur—the virus must in some way be maintained between epidemics—but such cases can not at present be recognized with sufficient certainty to furnish experimental material. The ætiological agent must be sought in typical cases during epidemic periods. The sporadic cases can then be elucidated by demonstrating that they result from the same virus.

Two views regarding the nature of this virus have occupied the attention of most investigators. The first, originally advanced by Pfeiffer, regards *B. influenzæ* as the causative agent; the second, of more recent date, believes that the disease is due to a filterable virus. Rosenow, [1], [2] it may be added, has isolated a green producing *streptococcus* from cases of influenza and obtained experimental evidence which led him to believe that it is the cause of influenza. His work, however, has not been confirmed.

When considering these two views in detail

one has to keep in mind the conditions that must be fulfilled before one is justified in concluding any virus to be the cause of influenza. An admirable discussion of this point has been written by Fildes and McIntosh[3]. Briefly stated the conditions are those which Koch laid down for himself in his studies on the ætiology of tuberculosis. These are as follows:

1. The virus should be capable of being recognized in a large proportion of the cases of the disease, preferably in relation to the chief lesion.

This may be accomplished by staining the tissues, by cultivation on nutrient media, or by reproduction of the disease in animals.

2. The virus should be shown to be living. This may be demonstrated either by growing several generations of the virus, or by carrying out several animal passages.

3. The virus should be capable of reproducing the disease in other animals.

This condition offers several difficulties. The inoculation of the animal should be made with a pure virus. It is necessary to choose a susceptible species and a suitable route for the introduction of the virus. Should the animal become ill it is required to demonstrate that the disease in question has been reproduced and to recover the virus from the diseased animal.

The demonstration of immune bodies, particularly agglutinins in the serum of patients is not to be considered as indicating that the agglutinated organism is the cause of a disease. The agglutination of *B. proteus* by the sera of typhus patients is a case in point. The presence of immune bodies, either in patients or infected animals, is, however, of considerable confirmatory value.

Filterable Virus

Several workers have reported negative results from the inoculation of volunteers and animals with filtered materials from influenza cases.

Lister and Taylor[4] sprayed the nasal cavities of fifteen men with filtered nasal washings from early cases.

Wahl, White and Lyall[5] inoculated six men with filtered extract of influenzal lungs by introducing it into the nose and naso-pharynx.

McIntosh[3] inoculated monkeys, rabbits and guinea pigs with filtered nasal washings, sputum and lung juice.

Keegan[6] and Roseneau[7] reported similar results.

Nicolle and Lebailly[8] observed symptoms of influenza in two volunteers inoculated subcutaneously with filtered sputum; also in two monkeys inoculated under the conjunctiva and by nasal instillation with the same sputum unfiltered.

Dujarric de la Rivière[9] was himself inoculated subcutaneously with filtered influenzal blood and afterwards had symptoms of influenza.

Such negative or indefinite results can hardly be regarded as more than suggestive.

The first extensive experiments supporting a filterable virus as the cause of influenza were reported by Bradford, Bashford and Wilson[10]; Gibson, Bowman and Connor[11]. The former described a minute, filter-passing organism which they found constantly in cases of influenza and which they were able to grow by special cultural methods. Inoculation of monkeys and guinea pigs with these cultures resulted in symptoms and lesions that were noted as typical of influenza in man. The organisms were recovered in pure culture from the animals.

Gibson, Bowman and Connor independently isolated an organism which both groups of workers considered identical with that described by Wilson. They produced "typical experimental influenzal lesions" in monkeys, rabbits, guinea pigs and mice by inoculating them with the cultures, and recovered the organism from the animals. The lesion regarded as typical was a peculiar type of hæmorrhage into the lung. This they were able to transfer from one animal to another by reinoculating the Berkefeld filtrate of hæmorrhagic lungs. The same lesions were seen in animals inoculated with blood and sputum from influenzal patients. Therefore the organism was considered to be the virus of the disease.

At a later date Bradford and Wilson in a note appended to a criticism of their cultures by Arkwright[12], retracted their claim that a filter-passing organism had been grown in pure culture and stated that degeneration forms of bacteria and protein particles in their cultures had been mistaken for such an organism. This retraction may also be applied to the work of Gibson, Bowman and Connor, for they state that they were in agreement with Capt. Wilson that their "organism" was the same as he had found.

Further light has been shed on the nature of the lesions found in the animals through experiments carried out in our laboratories[13], [14]. During the epidemic of influenza in Toronto early in 1920, guinea-pigs and rabbits were

inoculated with filtered material from influenza cases. None became ill but when they were killed a few days later approximately half of them had lung changes. These were of two types. The most striking lesion was hæmorrhagic. Sometimes the lungs were spotted with small areas of hæmorrhage 2 or 3 mm. in diameter, seen on the pleural surface and on section. In other cases they coalesced to form irregular larger areas that extended into the lung substance. The most extensive change involved the larger part of one or more lobes. No fibrin was seen. Congestion and hæmorrhages were occasionally found in the tracheal mucous membrane and the trachea contained frothy fluid.

Microsopically the hæmorrhage varied in extent. The smallest lesion was extravasation of red cells into the alveolar walls. In places this broke through the walls into the alveoli. Where it was most extensive the air-holding tissue was almost completely obliterated, a few empty alveoli standing out prominently in the midst of an area of solid hæmorrhage. Various grades of the same lesion were to be found in the same section. In places there was also œdema. In some cases the lumen of the bronchi contained red blood cells. Two striking features of the hæmorrhage were the absence of leucocyte reaction about it and the absence of hæmosiderin in the cells of the lung tissue.

The second type of lesion was proliferative. In the gross it could not always be detected. Microscopically it consisted of a thickening of the alveolar walls due to prolifieration of endothelial cells. Where the change was least, the outline of the alveoli could be easily traced but the air space of the lung was considerably lessened. In places of greater change, single alveoli were completely obliterated by the proliferation; others nearby were only a small fraction of their original size and the outline of the alveolar walls was lost in the diffuse mass of cells. Where this was most marked, the tissue was solid and the air space entirely obliterated. The cell masses included capillaries. Giant cells were occasionally seen. A recent thrombosis of a small branch of pulmonary artery was associated with this type of lesion.

Although some lungs showed only proliferation it was common to find hæmorrhage as well. The picture presented by the combination did not suggest that the two processes had a common cause or that one resulted from the other. Both the hæmorrhage and the proliferation occurred alone so definitely and so frequently that their association appeared to be accidental and could not be construed as signifying a more fundamental relationship.

By passing an extract of a hæmorrhagic lung through a Berkefeld filter and reinoculating the filtrate into another guinea pig we found that the lesions were reproduced in the lungs of the second animal. In one instance they were carried through seven reinoculations, in another series through six, and in another th ough five. By employing a special technique an attempt was made to obtain cultures of a virus from human and animal material but without success. Some of the cultures became turbid and showed very fine regular bodies much smaller than bacteria and very difficult to stain, but the same features appeared in cultures of control material. Although none of these cultures contained ordinary bacteria, guinea pigs inoculated with them showed the typical lesions.

The hæmorrhage was undoubtedly the most singular and striking lesion in our animals and corresponded to that described in the articles already mentioned[10], [11], as typical of "experimental influenza" in animals. One of these authors[11], who examined our specimens in our laboratory, agreed with us that they were the same lesions as they had obtained.

After the Toronto epidemic had subsided we incolulated a series of guinea pigs with control material from healthy patients who had not had influenza. The animals were killed after a similar interval. In their lungs exactly the same lesions were found, and in the same percentage as after inoculation with influenzal material. We then killed some healthy guinea pigs from our stock pens,—pigs that had never been inoculated. They too showed the same pulmonary lesions; and in order to determine if some condition peculiar to our pens was responsible for their production, guinea pigs from another laboratory were killed, with the same result. In view of these controls it was quite apparent that the animal lesions had no connection with the infective agent of influenza.

Further experiments[14] were made in an endeavour to explain their causation and resulted in demonstrating that the hæmorrhage was an agonal phenomenon due to the method of killing. Our routine had been to give the animal a blow on the back of the head. It was shown that hæmorrhages did not occur when the guinea pigs were killed by a sudden incision with sharp

pointed scissors which opened the chest wall and the ventricles of the heart with one snip. But they did occur, in a small percentage of animals killed by bleeding from the vessels of the neck or by rapid chloroform anæsthesia.

The histology of the proliferative lesion showed it to be essentially a slow inflammatory proliferation of endothelial cells. The exact ætiological agent was not determined although there were some reasons for suggesting that it might be due to *B. bronchisepticus*.

The lesions described by Bashford, Bradford and Wilson; Gibson, Bowman and Connor, have thus been accounted for. They were evidently not the result of the virus of epidemic influenza and one is not able to deduce from their experiments that such a virus is a filter-passing organism.

Similar lesions have been obtained by others and advanced as evidence of the operation of a filterable virus. Major Milton W. Hall[15] inoculated filtered sputum from influenza cases into rabbits, guinea pigs and mice. The animals showed no uniformity of clinical symptons and many remained in perfect health. They were killed by a blow on the back of the neck. The lungs showed hæmorrhages, the description of which corresponds remarkably in its details with that which we have given above. The hæmorrhagic areas were regarded as undergoing organization resulting in irregular masses of large mononuclear cells, in some places retaining the outline of the alveolar walls. The photograph of this "carnification of thickened alveolar walls" is undoubtedly the proliferative lesion that we have described. The lesions were transferred through as many as nine animal passages by reinoculation of lung emulsion. However, when viewed in the light of the possibility that the lung changes were agonal phenomena or a spontaneous animal disease, there would not appear to be any evidence in them to indicate that a filterable virus is the cause of influenza.

Olitsky and Gates, in a series of papers[16],[17],[18],[19], have reported experiments made during two epidemics and the period between. They inoculated rabbits intratracheally with filtered and unfiltered nasal washings obtained from cases of influenza during the first thirty-six hours of symptoms. In the absence of bacterial infection no animals died. They were killed by a sharp blow which dislocated the cervical vertebræ. The respiratory organs only were affected. The typical pathological change consisted, in the gross, of hæmorrhage, œdema and emphysema. The appearance of the hæmorrhagic areas was like the typical lesion we have described. Microscopically the lungs showed foci of hæmorrhage, œdema and emphysema. The aveolar strands were infiltrated with large cells of a foreign nature. Although mononuclear cells were seen in the alveoli and interalveolar walls there does not seem to have been any prominent leucocytic reaction. The œdema appears to have been greater than in our lesions but on the whole there is a striking resemblance between these lungs and those we have described. A comparison of our sections with those figured by Olitsky and Gates[18] shows a picture almost identical with that which we have obtained in control animals and by special methods of killing, and we do not feel that these lesions are characteristically similar to those found post-mortem in epidemic influenza in man.

By reinoculating affected lungs the lesions were transferred through several animal passages, rabbits and guinea-pigs being used. They found that washings from patients later in the disease produced lobar consolidation, when any lung changes were found. Control animals inoculated with washings from non-influenzal cases did not show the "familiar clinical and pathological action". A few developed lobar pneumonia and others gave "inconstant effects".

Blood changes were also observed. The change corresponding to the typical hæmorrhagic lung condition was a leucopænia largely due to decrease in the mononuclears. The lobar infections were associated with a polymorphonuclear increase and the unaffected lungs were not accompanied by any great change.

In a later communication[20] small bodies were described which appeared in anærobic cultures of influenzal material and which passed through a Berkefeld filter. Animals inoculated with these bodies showed a leucopænia, a decrea e in mononuclear cells, and at autopsy the typical lung lesion.

To what extent this evidence warrants one in concluding that these bodies are the virus of influenza and that the typical clinical and pathological changes in the animals are due to the operation of this virus is a matter for discussion. The typical pulmonary lesions bear a very striking resemblance to those described in our experiments, so great in fact that it seems probable that the method of killing has been a factor in producing them. By itself the leucopænia has no very great significance, at best such

changes are only corroborative evidence of reproduced disease. The culture and recognition of filterable organisms is open to many pitfalls and in the absence of any more definite evidence it would seem justifiable to wait for further confirmatory work rather than to accept at once this virus as the cause of epidemic influenza. But, from the fact that with inoculation of normal material and a definite method of killing the same hæmorrhagic picture was obtained by us, we are not inclined to accept either the observations of Olitsky and Gates or the English observers.

At the time of going to press, a preliminary report of experiments by Loewe and Zeman[36] has appeared. They have cultivated a minute filter-passing organism which they state does not appear to differ from that isolated by Olitsky and Gates. The cultures were obtained from nasopharyngeal washings of influenza cases. Rabbits inoculated intratracheally were killed after one or two days and showed "characteristic" lesions. Cultures were obtained from the rabbit lungs, and emulsions or filtrates of these lungs produced similar changes in other rabbits, several animal passages having been made. The pulmonary lesions were of the same order as described by Olitsky and Gates and so far as we can determine from the preliminary report, their experiments are open to the same criticism as we have applied to the work of Olitsky and Gates and the English investigators.

B. Influenzæ (Pfeiffer)

This organism was discovered by Pfeiffer in 1892 who advanced it as the cause of epidemic influenza because he recovered it in pure culture from many cases during that outbreak. He did not succeed, however, in reproducing the disease in animals. Further study of hæmoglobinophilic bacilli, of which Pfeiffer's organism was the original type, revealed a large group of organisms having the common property of requiring blood pigment for their growth. *B. influenzæ* formed but one type.

Pfeiffer recognized another group which he called pseudo-influenza bacilli. Davis[21] considers this to be a heterogeneous group, morphologically the same as *B. influenzæ*, the organisms of which have little else in common with one another except their hæmoglobinophilic property. They have been found in a variety of diseased conditions and in normal tissues.

Stillman and Bourn[22] investigating sputum from cases of influenza and pneumonia and the pharyngeal secretions of healthy people found typical *B. influenzæ* and pseudo-influenza bacilli which differed by having the property of hæmolyzing blood agar, the typical group causing no hæmolysis.

It is the typical *B. influenzæ* of Pfeiffer that has been considered by some to be the cause of influenza.

During the recent pandemic it was present in a large percentage of cases, associated with other pyogenic organisms. The latter were regarded as secondary invaders and chiefly consisted of *streptococcus, staphylococcus, pneumococcus, micrococcus catarrhalis and B. mucosus capsulatis.*

The percentage of cases in which *B. influenzæ* was demonstrated varied considerably both in different localities and in the same place at different times. This was also true of the secondary invaders, and for this reason some have regarded *B. influenzæ* as having the same relation to the disease—viz., a secondary invader following in the path of some specific virus which had primarily affected the respiratory organs.

However, it must be admitted that *B. influenzæ* has been demonstrated in relation to the chief lesions of the disease in a sufficiently large percentage of cases to warrant one in considering it as having fulfilled the first requirement of Koch's postulates. The fact that it occurs in other diseases and may be present in normal throats during epidemic periods or between them, and that it is present in a varying proportion of influenza cases, do not in themselves rule out the possibility that it might be the cause of the epidemic disease.

The most important evidence, however, comes from inoculation experiments.

Probably in no other connection have so many volunteers offered themselves for the investigation of disease.

Roseneau[7] sprayed the nose, throat and eyes of nineteen men with an emulsion of living *B. influenzæ* containing thirteen strains, some of which had been recently isolated from lungs of fatal cases. None of the men became ill.

Lister and Taylor[4] introduced into the mouth and nose of nine men, living suspensions of three stock strains of *B. influenzæ.* All but one remained well and his illness could not be considered as an attack of influenza.

Wahl, White and Lyall[5] inoculated five volunteers with large doses of four strains, one being

the first subculture from a fatal case. None of the men became ill.

Yamanouchi, Sakakima and Iwashima[23] sprayed pure cultures of *B. influenzæ* and mixed cultures of the common cocci into the nose and throat of fourteen healthy people and did not cause any illness.

Sellards and Sturm, and Bloomfield have also reported negative results.

Cecil and Steffen[24], on the other hand, inoculated the nose and throat of six volunteers, who became ill. Two were given massive doses of saline suspensions from agar, two received peritoneal exudate from a monkey, and for the last two a blood broth culture was used. In each case an upper respiratory infection was produced. Considering the size of the doses the saline suspensions caused surprisingly mild infections; the growth from one chocolate agar slant was used for one patient and that from two slants for the other. Smaller doses of the fluid cultures gave better results. The local symptoms resembled an acute coryza and were more striking than the constitutional disturbance. "The systemic reaction in the experimental disease was not so profound as in true influenza and resembled more the prostration that accompanies a severe cold or bronchitis." The absence of fever in the cases was also unlike epidemic influenza. The authors conclude that "virulent bacilli, when injected into the nose and throat of healthy volunteers, may excite in them an acute respiratory disease similar in many respects to influenza, but falling short of the typical clinical picture."

Thus attempts to reproduce epidemic influenza in man with *B. influenzæ* have been markedly unsuccessful and the results suggest very strongly that this organism is not the primary cause of the disease. There is general agreement, however, that it played a part in producing the pneumonias, but in the light of these experiments it would appear to have been present as a secondary invader.

Blake and Cecil[25], [26] have worked extensively with animals. Two groups of monkeys were inoculated,—one group by way of the nose and throat, the other by intratracheal injection—with a strain of *B. influenzæ* from an empyema following influenzal pneumonia. Its virulence was increased by passage through eleven mice and the peritoneal cavity of thirteen monkeys before it was used for the experiments.

The question arises as to what findings one would require in the animals, as a result of inoculation, to support *B. influenzæ* as the cause of the disease in man. In animals clinical signs are suggestive but in themselves not conclusive. Decisive evidence would come from the character of the pathological changes in the respiratory organs and the association of the ætiological agent with them. As a standard of lung lesions to be expected from *B. influenzæ* there are on record [27], [28] the descriptions of human lungs from patients who have succumbed to a pure infection of this organism.

The first group of animals consisted of twelve monkeys which received as much as 3 c.c. of a blood broth culture or peritoneal exudate from another monkey. Signs of upper respiratory infection followed, in five complicated by acute sinusitis. None of the animals died. Nine were killed in from three to six days. At autopsy three showed bronchopneumonia, from two of which *B. influenzæ* was recovered. But the organism was also recovered from three without pneumonia, although two of these had tracheo-bronchitis.

The second group comprised eleven monkeys inoculated intratracheally. Five received massive doses, the growth from our four or five plates having been used for each animal. They were killed in from two to six days. Four had bronchopnuemonia, but only two yielded cultures of *B. influenzæ*. Cultures from the other two were sterile. The fifth monkey showed tracheobronchitis but no pneumonia. As a control a massive dose of killed organisms was given to the sixth monkey. It was killed on the sixth day and showed bronchopneumonia. The remaining five received the growth from one plate or the equivalent amount of peritoneal exudate from another monkey. They were allowed to live for from one to seven days. Three had bronchopneumonia, two of which yielded no *B. influenzæ*, the other giving a culture from the bronchus. The fourth had tracheo-bronchitis and sterile cultures, and the fifth did not become ill.

When these results are analyzed for evidence capable of being applied to the elucidation of the ætiology of influenza in accordance with the foregoing discussion, it is seen that only three animals had a bronchopneumonia associated with *B. influenzæ*. The monkeys inoculated with massive doses cannot be included in the evidence because a similar dose of dead organisms also produced bronchopneumonia. Six animals with lung lesions yielded no *B. influenzæ* and on this account cannot be considered as satisfactory evidence,

although it was possible that the organisms had died out.

The bronchopneumonia of the three animals, shown by culture to be due to *B. influenzæ* was compared with post-mortem lesions of human lungs in which a pure *B. influenzæ* infection had occurred. There was agreement in some particulars but the pictures were not the same. Because of this discrepancy and in view of the lung changes without associated organisms, and the production of bronchopneumonia by killed bacteria, the value of the experiments has been somewhat impaired. The experimental method has considerable merit, as shown by the clear out transference of measles to monkeys which has recently been reported[29], so that if *B. influenzæ* had been the virus of influenza one would have expected more decisive results than were obtained. A bronchopneumonia was initiated by *B. influenzæ* which in some respects resembled the human lesions but it is not possible to conclude from the experiments that this organism is the cause of epidemic influenza. In fact the authors themselves do not make this claim.

The theory has been advanced that a special strain of *B. influenzæ* with a markedly exalted virulence might have accounted for the epidemic. That variations exist has been demonstrated by several observers.

Cohen[30] found that strains from meningitis differed in agglutination and protection experiments and produced a fatal septicæmia in rabbits.

Stillman and Bourn[22] have made cultural distinctions in the production of indole and the fermentation of saccharose.

Recently in this laboratory two strains of *B. influenzæ* have been isolated from the same patient, one from the sputum the other from the lung at autopsy. The former was a coccoid bacillus, fairly uniform in size and shape, the latter a slender bacillus whose length was 1·5 to 4 times the width. The difference in morphology definitely indicated that the organisms wer not alike and this was further borne out by agglutination. An immune rabbit serum prepared from a third strain, having a titre of 1 in 2560 for the homologous organism, agglutinated the autopsy strain in a dilution of 1 in 1280 and failed to agglutinate the sputum strain in a dilution of 1 in 20.

Thus the finding of *B. influenzæ* in sputum should be controlled whenever possible by culture at autopsy since the strain in the sputum may not be identical with the one obtained from the diseased tissue. As the latter organism is the one likely to be taking part in the infection the culturing of sputum only, may lead to inaccurate conclusions.

Bell[31] has noted three main morphological types, the two we have just mentioned and a third, consisting of fairly long slender straight or curved bacilli with pointed ends, which we have also seen. These types did not correspond to agglutination groups. In one case two strains, isolated from one plate, differed in morphology but were identical by agglutination tests. In another case three strains isolated from the same plate differed in both morphology and agglutination. Most of his strains were not alike and he concluded that the group is heterogeneous but that identical strains do occur.

Valentine and Cooper[32] in Park's laboratory did agglutination and agglutinin absorption tests on 171 strains with 25 sera and could not detect any serological relationship by this means. Continuing this work in the same laboratory Povitsky and Denny[33] obtained similar results. They found that 185 strains from influenza cases were almost unrelated, five being the largest number to fall into any one group. Some meningitis strains had more in common, four out of seven belonging to the same type.

Cocoa and Kelly[34] have confirmed the r sults of Valentine and Cooper. The argument has been advanced by Park[35] and his assistants, supported by Cocoa and Kelly, that if *B. influenzæ* had been the primary ætiological agent a pandemic strain would hav existed, and therefore organisms obtained from cases in different places and at different times would have belonged to one type as revealed by agglutination tests. But the results did not reveal any such pandemic strain and therefore they belive the conclusion is warranted that *B. influenzæ* is not the primary infecting agent.

Summary

At present we believe there is not sufficient evidence to indicate what is the cause of epidemic influenza. There is no experimental certainty that the virus is a filter-passer. The experiments which favour this view have been criticized in the light of results obtained in this laboratory.

B. influenzæ i undoubtedly present in a large but varying percentage of cases, and is a factor in producing the pathological complex of the disease. However, the failures to inoculate man

form impressive evidence that it is not the primary infecting agent. The failure of animal inoculation and the absence of a pandemic strain are further facts in support of this view. The evidence favours the opinion that *B. influenzæ* is a secondary invader.

The difficulties of making any advance in the problem arise mainly from the want of exact knowledge as to the *essential* lesions of the milder forms of epidemic influenza, and therefore of the lesions in animals which would indicate a reproduction of the disease.

References:

1. ROSENOW, E. C.—Immunologic Experiments with Streptoccoci from Influenza, *J. Amer. Med. Assoc.*, 1919, 73, 318.
2. ROSENOW, E. C.—Studies in Influenza and Pneumonia, Studies V-XI., *J. Infect. Dis.*, 1920, 26, 469-622.
3. FILDES, PAUL and MCINTOSH, JAMES—The Ætiology of Influenza, *Brit. Jour. Exp. Pathology*, 1920, 1, 119, concluded p. 159.
4. LISTER, F. S. and TAYLOR, E.—Experimental Investigation of Epidemic Influenza at Durban, *Public, South African Inst. Med. Res.*, 1919, No. 12, p. 9.
5. WAHL, H. R., WHITE, G. B. and LYALL, H. W.—Some Experiments on the Transmission of Influenza, *J. Infect. Dis.*, 1919, 25, 419.
6. KEEGAN, J. J.—The Prevailing Pandemic of Influenza, *J. Amer. Med. Assoc.*, 1918, 71, 1051.
7. ROSENEAU, M. J.—Experiments to Determine the Mode of Spread of Influenza, *ibid.*, 1919, 73, 311.
8. NICOLLE and LEBAILLY.—Quelques notions experimentales sur la virus de la grippe, *Compt. rend. Acad. de Sci.*, 1918, 167, 607.
9. DUJARRIC DE LA RIVIÈRE.—La grippe est elle une maladie à virus filtrant? *ibid.*, 1918, 167, 606.
10. BRADFORD, J. R., BASHFORD, E. F. and WILSON, J. A.—The Filter-passing Virus of Influenza, *Quart. Journ. Med.*, 1919, 12, 259.
11. GIBSON, G. H., BOWMAN, F. B. and CONNOR, J. I.—The Ætiology Influenza; a Filterable Virus as the Cause, *Med. Res. Com., Spec., Report Series No.* 36, London, 1919, p. 19.
12. ARKWRIGHT, J. A.—A Criticism of Certain Recent Claims to Have Discovered and Cultivated the Filter-passing Virus of Trench Fever and Influenza, *Brit. Med. Journ.*, 1919, 2, 233.
13. MAITLAND, B. H., COWAN, MARY L. and DETWEILER, H. K.—The Ætiology of Epidemic Influenza; Experiments in Search of a Filter-passing Virus, *Brit. Journ. Exp. Pathology*, 1920, 1, 263.
14. MAITLAND, H. B., COWAN, MARY L. and DETWEILER, H. K.—Spontaneous and Artificial Pulmonary Lesions in Guinea Pigs, Rabbits and Mice, *ibid.*, 1921, 2, 8.
15. HALL, MILTON W.—A Study of the Lesions Produced by Filtrates of Influenza Sputum, *Arch. Int. Med.*, 1920, 26, 612.
16. OLITSKY, P. K. and GATES, F. L.—Experimental Studies of the Nasopharyngeal Secretions from Influenza Patients; Preliminary Report, *J. Amer. Med. Assoc.*, 1920, 74, 1497.
17. OLITSKY, P. K. and GATES, F. L.—Experimental Studies of the Nasopharyngeal Secretions from Influenza Patients; I. Transmission Experiments with Nasopharyngeal Washings, *Journ. Exp. Med.*, 1921, 33, 125.
18. OLITSKY, P. K. and GATES, F. L.—II. Filterability and Resistance to Glycerol, *ibid*, 1921. 33, 361.
19. OLITSKY, P. K. and GATES, F. L.—III. Studies of the Concurrent Infections, *ibid.*, 1921, 33, 373.
20. OLITSKY, P. K. and GATES, F. L.—Studies of the Nasopharyngeal Secretions from Influenza Patients; Preliminary Report on Cultivation Experiments, *J. Amer. Med. Assoc.*, 1921, 76, 640.
21. DAVIS, D. J.—Infections with Hæmoglobinophilic Bacteria, *J. Amer. Med. Assoc.*, 1915, 64, 1814.
22. STILLMAN, ERNEST G. and BOURN, JANET M.—Biological Study of Hæmoglobinophilic Bacilli, *Journ. Exp. Med.*, 1920, 32, 665.
23. YAMANOUCHI, T., SAKAKAMI, K. and IWASHIMA, S.—The Infecting Agent of Influenza, *Lancet*, 1919, 1, 971.
24. CECIL, RUSSELL L. and STEFFEN, GUSTAV I.—Acute Respiratory Infection in Man Following Inoculation with Virulent Bacillus Influenzæ, *Journ. Inf. Dis.*, 1921, 28, 201.
25. BLAKE, FRANCIS G. and CECIL, RUSSELL L.—Studies on Experimental Pneumonia. IX. Production in Monkeys of an Acute Respiratory Disease Resembling Influenza by Inoculation with *B. Influenzæ*, *Journ. Exp. Med.*, 1920, 32, 691.
26. BLAKE, FRANCIS G., and CECIL, RUSSELL, L.—X. Pathology of Experimental Influenza and of *B. influenzæ* Pneumonia in Monkeys, *ibid.*, 1920, 32, 719.
27. MACCALLUM, W. G.—Pathology of the Pneumonia Following Influenza, *Journ. Amer. Med. Assoc.*, 1919, 72, 720.
28. WOLBACH, S. B.—Comments on the Pathology and Bacteriology of Fatal Influenza Cases, *Bull. Johns Hopkins Hosp.*, 1919, 30, 104.
29. BLAKE, FRANCIS G. and TRASK, JAMES D., JR.—Studies on Measles, I. Susceptibility of Monkeys to the Virus of Measles, *Journ. Exp. Med.*, 1921, 33, 385.
30. COHEN.—La Méningite, cérébro-spinale septicémique, *Ann. de l'instut Pasteur*, 1909, 23, 273.
31. BELL, HOWARD H.—Relation of Different Strains of Influenza Bacilli as shown by Cross Agglutination and Absorption Tests, *Journ. Inf. Dis.*, 1920, 27, 464.
32. VALENTINE, EUGENIA and COOPER, GEORGIA M.—On the Existence of a Multiplicity of Races of *B. influenzæ* as Determined by Agglutination and Agglutination Absorption, *Journ. of Immunology*, 1919, 4, 359.
33. POVITZKY, OLGA R. and DENNY, HELEN T.—Further Studies on the Grouping of Influenza Bacilli with Special Reference to Permanence of Type in Carrier, *ibid.*, 1921, 6, 65.
34. COCOA, ARTHUR F. and KELLY, MARGARET F.—A Serological Study of the Bacillus of Pfeiffer, *ibid.*, 1921, 6, 87.
35. PARK, W. H.—Bacteriology of Recent Pandemic of Influenza and Complicating Infections. *Journ. Am. Med. Assoc.*, 1919, 73, 318.
36. LOEWE, LEO and ZEMAN, FREDERICK D.—Cultivation of a Filterable Organism from the Nasopharyngeal Washings in Influenza, *ibid.*, 1921, 76, 986.