

**A FILTRABLE VIRUS AS THE CAUSE OF THE EARLY STAGE OF THE PRESENT EPIDEMIC OF INFLUENZA.**

(A PRELIMINARY NOTE.)

BY

MAJOR H. GRAEME GIBSON, R.A.M.C.,

MAJOR F. B. BOWMAN, C.A.M.C.,

AND

CAPTAIN J. I. CONNOR, A.A.M.C.

(Interim Report to the Medical Research Committee.)

A PRELIMINARY note by MM. Charles Nicolle and Charles Lebailly on investigations suggesting the etiological part played by a filtrable virus in influenza cases was communicated recently to the Académie des Sciences.<sup>1</sup> A similar claim for the importance of a filtrable organism in the production of common colds had previously been put forward by Major George B. Foster, Junr., Medical Corps, United States Army, in the *Journal of Infectious Diseases* of November, 1917 (Vol. xxi, No. 5, pp. 451-474).

These observations have raised the important question as to whether the micro organisms hitherto regarded as of etiological importance in influenza and certain other catarrhal conditions of the respiratory tract play a primary or a secondary part.

The following investigations, undertaken at the request of the Adviser in Pathology, British Expeditionary Force, France, appear, so far as they go, to confirm and amplify the work of MM. Nicolle and Lebailly, and it is thought desirable to publish a preliminary report without delay. Further experiments are in progress, and a detailed report will follow in due course.

Nicolle and Lebailly, in their communication to the Academy of Science, reported that the unfiltered bronchial secretion of patients suffering from "La Grippe" collected during the pyrexial period was virulent to Chinese bonnet and *Macacus cynomologus* monkeys when injected by the subconjunctival and nasal routes. They also reported that the inoculation of the filtered bronchial secretion caused the disease in two men inoculated by the subcutaneous route. In view of the serious nature of the present epidemic the repetition of the experiments on man with the filtered sputum was not thought to be warranted, and our experiments with both filtered and unfiltered secretions were carried out on monkeys.

A supply of rhesus monkeys having been provided through the kindness of the Medical Research Committee, we determined, first, to attempt the infection of these animals by means of the filtered sputum, as had been done, in the case of man, by Nicolle and Lebailly.

For the first experiments two monkeys were used and two kept as "controls." The method of preparation of the sputum for filtration used by Nicolle and Lebailly was followed, but the filter candle used by us was a Chamberland L. 1 bis, in place of the Chamberland L. 2 used by them, and, owing to our inability to obtain a large exhaust pump at the moment, a Potain's aspirating pump was used to obtain a vacuum during the process of filtration.

The two candles used were carefully tested two days before the experiment with suspensions of *B. coli* and *Staphylococcus aureus*, and the filtrates found to be sterile.

**EXPERIMENT 1.—Inoculation of Filtered Sputum taken from a Case of Influenza on the Fifth Day of the Disease.**

*Source of Infective Material.*—Case F., suffering from uncomplicated influenza. No chest symptoms developed in this case, and the patient made an uninterrupted recovery. The temperature was 101.2° F. when the sputum was taken.

*Infective Material.*—Sputum was collected on November 9th, 1918, the fifth day of the patient's illness. Smears and cultures were made from the carefully washed sputum, and Gram-positive diplo-streptococci and Gram-negative diplococci were found to be present. Pfeiffer's bacillus was not observed either in smears or in cultures.

*Method of Preparation of Inoculum.*—The sputum was placed in a sterile bottle with ten times its volume of normal saline, and shaken with glass beads for five minutes. It was then

centrifuged, at about 1,500 to 2,000 revolutions per minute, for a minute and a half. The supernatant fluid was passed through a Chamberland L.1 bis candle, the filtrate being collected in a sterile vessel. Cultures were made in serum glucose broth and on human blood agar plates, in the preparation of which the blood had been citrated and heated to 55° C. for ten minutes before mixing with the agar. This medium had given us excellent growths of all the organisms met with in the epidemic. These cultures were found to remain quite sterile in respect of non-filtrable organisms.

That afternoon (November 9th) 0.25 c.cm. of the filtrate was inoculated under the conjunctiva of the left eye of monkey No. 1, and 0.75 c.cm. was instilled up its nostrils.

*Subsequent History of Case (Monkey No. 1).*

On the evening of November 15th the monkey appeared to be somewhat off colour, and on the morning of November 16th it was distinctly ill, moping, and at times resting its head on its arms. It would not take its food, had some suffusion of the conjunctivae of both eyes, and its coat was "staring." On November 17th its coat was still "staring," but it was taking more interest in its surroundings and was eating its food again, and by November 18th, although its coat was still affected, it had apparently recovered.

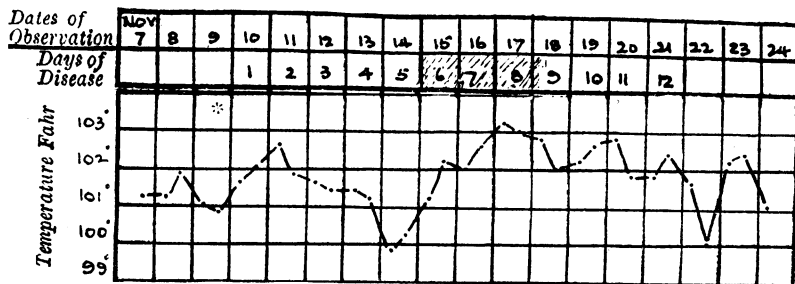


CHART 1.—The days on which the monkey was ill are "shaded in" in the days of disease line. \* = Inoculated.

The temperature chart, with records from the second day before inoculation up to the end of the experiment, is set forth in Chart 1. It will be observed that the temperature of this animal had fluctuated considerably while under observation previous to inoculation, a fact which makes it impossible to say how much the illness was associated with a true pyrexia. The incubation period in this case corresponded to that observed by Nicolle and Lebailly in their monkeys inoculated with unfiltered sputum.

The blood of the monkey was taken on the fifth day of his illness to determine its power of agglutinating Pfeiffer's bacillus. No agglutination was observed in dilutions of 1 in 2, 1 in 10, 1 in 40, 1 in 80, 1 in 100. The monkey was allowed to recover, and is being kept with a view to determining the possibility of reinfection.

**EXPERIMENT 2.—Inoculation of Filtered Sputum taken from a Case of Influenza on the Third Day of the Disease.**

*Source of Infective Material.*—This experiment was carried out also on November 9th, 1918. Sputum was taken from a man (Case W.) who had been suffering from influenza for three days and who died on the eleventh day of his illness as the result of secondary infection of the respiratory tract leading to bronchopneumonia. His temperature on the day the sputum was taken was 103.6° F.

*Infective Material.*—The examination of this sputum revealed the presence of pneumococci, a few Pfeiffer's bacilli, and many Gram-positive, non-haemolytic diplo-streptococci of a type which we have frequently observed in the bronchial secretions of men suffering from influenza in this area.

*Method of Preparation of Inoculum.*—The sputum was prepared for filtration in the same way as in Experiment 1. Before being filtered 0.5 c.cm. of the emulsion was inoculated subcutaneously into a white mouse. The mouse died eighteen hours later, and a pure culture of pneumococci (Type 1) was isolated from it. The sputum was filtered through a Chamberland L. 1 bis filter and the filtrate tested for sterility. The filtrate was then inoculated intraperitoneally into a mouse which showed no symptoms of any sort, and it also proved sterile for non-filtrable organisms on culture.

*Subsequent History of Case (Monkey No. 2).*

On the afternoon of November 9th monkey No. 2 was inoculated with 0.25 c.cm. under each conjunctiva, and 0.5 c.cm. was instilled up its nostrils. On November 16th—that is, on the seventh day after inoculation—the monkey appeared to be out of sorts, with slight diarrhoea. On the next morning it was very much worse, sitting with its head resting on its knees and moping. It was very depressed, off its food, and its coat was "staring." On the following morning it was much the same, and was apparently showing some loss of flesh and slight diarrhoea. It seemed to be slightly better in the afternoon, though still very depressed. Its temperature on this day was 103.2° F. The attack was more marked than in the first monkey.

The temperature chart is appended (Chart 2), but it is to be noted that the severity of the symptoms was much greater

than might be inferred from the pyrexia. That afternoon it was decided to kill the monkey in order to determine the exact condition of the respiratory tract at this stage. It was

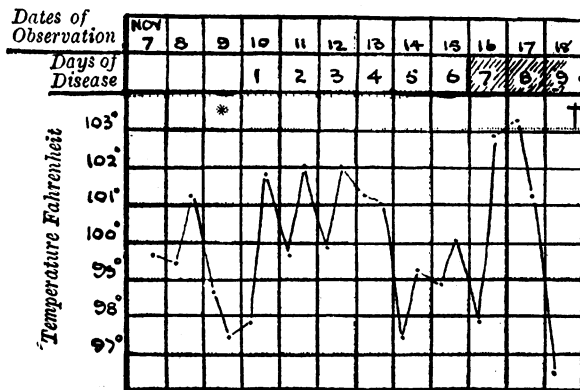


CHART 2.—The days on which the monkey was ill are "shaded in" in the days of disease line. \* = Inoculated. † = Killed.

chloroformed at 3.30 p.m., and a *post-mortem* examination was immediately performed.

#### POST-MORTEM FINDINGS.

The following condition was found *post-mortem* on November 18th:

**Larynx.**—Appears to be normal.

**Trachea.**—This shows a distinct reddening and apparent congestion of the mucous membrane of the trachea in the spaces between the tracheal rings. The lower portion of the trachea is filled with a frothy pinkish fluid which oozes up from the bronchi.

#### Lungs.

The pleurae are clear and glistening and apparently normal. The lungs are not collapsed.

**Left Lung (external appearance).**—**Upper Lobe.**—This lobe is salmon-pink in colour and air-containing. On the surface are a few deep red patches, the size of a pinhead; otherwise the lobe appears normal.

**Middle Lobe.**—The lower surface of this lobe shows a deep purple patch, triangular in shape, 2 cm. in size, across the base of the triangle. There is no evidence of pleurisy between the lobes.

**Lower Lobe.**—The upper margin is salmon-pink in colour. The lobe when placed on the table retains its shape and feels solid when compared with the upper lobe. On the posterior surface there is a large purple patch, extending from about 1 cm. from the upper margin of the lobe down to near the lower margin. It is about 3 cm. in width, and in the centre of this patch is another of a pinkish-yellow colour which projects from the surface of the lung, and is apparently a patch of emphysema.

**Right Lung:—Upper and Middle Lobes.**—These resemble the left upper lobe and are apparently normal except for the deep red points above described.

**Lower Lobe.**—The posterior surface shows a deep purplish patch similar to that seen in the left lower lobe, but not quite so large.

**Lower Lobes on Section.**—The lower lobes on section show the upper part pink and the lower part a deep purple. A viscid dark purple fluid, containing air-bubbles, drips from the cut surface of the lung.

Examination of the cut surface with a hand lens reveals no evidence of bronchopneumonia, and the lobe is so engorged that none of the smaller bronchi are visible to the naked eye in the purple area.

#### Abdominal Organs.

**Liver.**—This is of a deep purple colour and the surface shows a certain irregularly outlined mottling. A fine yellowish-grey mottling is also apparent when the liver is cut into. The cut surface bulges out from under the capsule. On scraping this surface a shiny gelatinous appearance is obtained. With a hand lens the small blood vessels are seen to be engorged and the lobules are outlined with irregular yellow lines.

**Spleen.**—This is somewhat engorged.

**Kidneys.**—There are no haemorrhages apparent on the surface of either kidney. The capsules strip easily, and on section the kidneys suggest the condition of cloudy swelling.

#### Microscopic Appearances.

Microscopical sections were cut of the lung, liver, and kidneys, and the following appearances were noted:

**Lung.**—Low power microscopic appearance shows an almost universal consolidation, the alveoli being filled with what appears to be an inflammatory and haemorrhagic exudate. In some areas the alveoli have escaped. The bronchi and larger vessels are filled with the above-described exudate. The mucous membrane of the bronchi is intact and some of the cells show a central mucoid accumulation. Leucocytic accumulation is scanty. There is nowhere any approach to grey hepatization. Large subpleural vessels are choked, but the pleura has escaped inflammatory change. Under the high power some capillary engorgement is to be seen and some leucocytic invasion of the plugged alveoli is present. The

inflammatory exudate is seen to be made up of a fibrinous reticulum which is studded with the remnants of degenerate red blood corpuscles, some still retaining their original shape.

**Liver.**—Low power shows a normal amount of stroma and capsule. The bile ducts are intact. There is present some engorgement of the central lobular vein, spreading in some cases to the capillaries. High power shows central fat accumulation in a few of the hepatic cells in the central lobular zone. No leucocytic reaction is apparent. No marked grade of cloudy swelling.

**Kidney.**—Low power shows normal stroma and capsule. The larger vessels are somewhat engorged and the secreting tubules apparently normal. The capillaries in the glomeruli are dilated with red blood corpuscles. The tubules in some areas show some blurring of the cellular outline with some granularity. The smaller tubules are apparently normal.

#### BACTERIOLOGY.

No *B. influenzae* (Pfeiffer) nor the diplo-streptococcus commonly found in the human respiratory tract in cases of influenza were isolated.

On blood agar plate cultures from the frothy fluid present in the bronchi and trachea very few colonies of any description appeared in twenty-four hours.

A staphylococcus was isolated and also a very small Gram-negative bacillus showing more or less pleomorphism, which on subculture grew profusely as a white slime in blood agar and showed a very delicate growth in plain agar. No other organisms were isolated.

From the affected area in the lung blood agar plate cultures were negative.

#### EXPERIMENT 3.—Inoculation of Unfiltered Sputum taken from a Case of Influenza on the Sixth Day of Illness.

Sputum from a case of influenza which had been ill for six days and had already developed chest symptoms was taken on November 16th. The patient's temperature on this day was 103° F. His sputum at this time contained a haemolytic streptococcus and a Gram-positive diplo-streptococcus, mentioned as seen in the sputum used in Experiment 2. The sputum was prepared in exactly the same way as in the two previous experiments, except that it was *not filtered*.

Monkey No. 3 was inoculated the same evening with 0.25 c.cm. under the conjunctiva of the lower lid of its left eye, and 0.75 c.cm. were instilled up its nostrils. Emulsion of sputum used was given without being filtered. The monkey developed no symptoms of any sort, and remained well.

#### EXPERIMENT 4.—Inoculation of Filtered Sputum taken from a Case of Influenza on the Sixth Day of Illness.

The sputum used in Experiment 3 was then filtered in the same manner as that used in Experiments 1 and 2—that is, through a Chamberland L. 1 *bis* filter. The filtrate, as before, was found to be sterile in respect of non-filtrable organisms. This monkey also showed no symptoms and remained well.

#### SUMMARY.

1. Two rhesus monkeys, inoculated subconjunctivally and intranasally with the filtered sputum from cases of human influenza, became ill on the sixth and seventh day respectively—that is, after a period corresponding closely with that noted in human cases under similar conditions by MM. Nicolle and Lebaillly.

2. Two control monkeys, kept in the same room under similar conditions, manifested no signs of illness during the same period.

3. Of the two inoculated monkeys, one rapidly regained normal health, all symptoms appearing to have subsided by the third day of the attack. The other seemed to be on the mend on the afternoon of the third day, when it was killed for further examination. In the animal the respiratory tract was found to show the presence of a haemorrhagic exudate affecting especially the lower lobes of both lungs. The condition found was in many respects comparable to that noted in certain human cases of influenza in which a fatal issue had supervened before the occurrence of marked secondary infection. In this connexion it is interesting to recall the tendency to haemorrhages which has so often been noted during the present influenza epidemic, and which is generally observed early in the disease.

4. Two rhesus monkeys, Nos. 3 and 4, at first used as controls and subsequently inoculated, the one with filtered, the other with unfiltered sputum, collected from a case of influenza on the sixth day of the disease, showed no symptoms and remained well.

5. Our observations, so far as they go, tend to confirm those of MM. C. Nicolle and C. Lebaillly above quoted.

The *post-mortem* appearances are undoubtedly suggestive, and further work in this direction may throw considerable light on the question.

#### REFERENCE.

*C. R. Acad. Sci.*, p. 607; *Presse medicale*, October 17th, 1918; *BRITISH MEDICAL JOURNAL*, November 2nd, p. 495.