

THE ETIOLOGY OF INFLUENZA.

A FILTRABLE VIRUS AS THE CAUSE, WITH SOME NOTES ON THE CULTURE OF THE VIRUS BY NOGUCHI'S METHOD.

BY (THE LATE) 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.

(Report to the Medical Research Committee.)

SINCE the results of our preliminary experiments were reported in the BRITISH MEDICAL JOURNAL of December 14th, 1918,¹ in an article entitled "A Filtrable Virus as the Cause of the Early Stage of the Present Epidemic of Influenza," further work has been carried out which confirms that already reported. We have also attempted to cultivate the "filtrable virus" of influenza and have made experiments in the transmission of the disease to animals by inoculation with the cultures so obtained. These cultural experiments were suggested by one of us (H. G. G.) at an early stage of the work, and later, a paper by G. Foster,² to which we had access, clearly showed that this part of the work might prove to be a very important part of the investigation.

A full report of the work with which this article deals, with complete data and plates, will appear later. The present report is merely a summary of the work done to date and is framed in as concentrated a form as possible. It is forwarded at this time in order that other workers who may be engaged in the investigation of influenza may have the opportunity of knowing on what lines we have been working and the results that we have obtained.

The animals used by us for experimental purposes have been baboons, *Macacus rhesus* monkeys, rabbits, guinea-pigs, and mice. By the inoculation of sputum, both filtered and unfiltered, from cases of influenza, we have succeeded in producing, in all these animals, lesions that appear similar to those seen in human cases of the disease.

For experiments with blood transmission we used *M. rhesus* monkeys and mice. Although the results obtained in the only two cases in which monkeys were inoculated with blood were not wholly satisfactory, the experiments with mice gave a high percentage of positive results, and it seems to be possible to transmit the virus by means of infected blood to the latter animals. It should be noted that the mice received a very much larger dose per body weight than the monkeys, and this factor, no doubt, played a part in the results obtained.

With cultures it was found to be possible to infect monkeys, rabbits, guinea-pigs, and mice. Owing to the grave nature of the influenza epidemic in progress, it was not thought advisable to attempt any experiments in the transmission of the disease to human volunteers, both on account of the danger involved and owing to the fact that there would have been no guarantee, in the face of so widely spread an epidemic, that the individual to be experimented upon had not already been exposed to infection.

A general description of the clinical symptoms and the pathological lesions found *post mortem* is not included and will appear in a later report to the Medical Research Committee, as will also certain details regarding the preparation of material for inoculation.

ANIMAL INOCULATION AND CULTURAL EXPERIMENTS WITH FILTRABLE VIRUS.

The work may be divided into four parts:

- I. Inoculation of animals with sputum from cases of influenza.
- II. Inoculation of animals with blood from cases of influenza.
- III. Passage of the virus from animal to animal.
- IV. Cultural experiments and inoculation of cultures into animals.

I. INOCULATION OF ANIMALS WITH SPUTUM FROM CASES OF INFLUENZA.

Source of Infected Material.

The sputum used was as a rule collected as early as possible in the disease. As uncomplicated cases of

influenza as a rule present a pyrexial period of only a few days' duration, we considered that it would be during those few days that we should have the greatest chance of recovering the virus. As a rule, a certain amount of sputum was obtainable on the second or third day, and this, generally speaking, was frothy, of a greyish-yellow colour, tenacious, often markedly blood-stained, and did not present the nummular appearance seen in cases of bronchitis.

Method of Inoculation and Size of Dose Given.

Emulsions of unfiltered and filtered sputum were inoculated by the same routes and the size of the dose was the same in each case. When dealing with monkeys the method of administration of unfiltered sputum reported by Nicolle and Leballoy was followed. This consisted in injecting 0.25 c.cm. of the emulsion under the conjunctiva of each eye and instilling 0.5 c.cm. of the emulsion up the animal's nose. In one instance we inoculated one monkey by the conjunctival route alone, and gave another monkey 0.5 c.cm. of the same filtrate up its nostrils alone. The former gave a positive result, while the latter proved negative. Rabbits were given 1.2 c.cm. of the filtered sputum either intravenously or subcutaneously; positive results were obtained by both methods. Guinea-pigs and mice were always inoculated subcutaneously whether unfiltered or filtered sputum was used. Guinea-pigs were usually given 1 c.cm., and mice, when used, received 0.25 c.cm. of the unfiltered or filtered sputum.

Results.

In dealing with the results obtained it is convenient to separate the various animals employed into different groups.

Monkeys.—We may divide these into two main groups according to whether the sputum used as the infecting agent was taken from the patient during the first three days of the disease or at a later date. The results are shown in the subjoined table:

GROUP I.—Sputum Collected Early.

| Type of Monkey. | Material Used for Inoculation. | Day of Disease on which Sputum was Taken. | Result. |
|-------------------------|--------------------------------|---|----------|
| 1. <i>M. rhesus</i> ... | Unfiltered sputum | Third | Positive |
| 2. <i>M. rhesus</i> ... | Unfiltered sputum | Third | Doubtful |
| 3. <i>M. rhesus</i> ... | Filtered sputum | Third | Positive |
| 4. <i>M. rhesus</i> ... | Filtered sputum | Third | Positive |
| 5. <i>M. rhesus</i> ... | Filtered sputum | Second-third | Positive |
| 6. <i>M. rhesus</i> ... | Filtered sputum | Third | Positive |
| 7. <i>M. rhesus</i> ... | Filtered sputum | Third | Negative |

This gives 80 per cent. positive results among the *M. rhesus* monkeys inoculated with *filtered* sputum taken from cases of influenza before the end of the third day of the disease. Out of the two monkeys inoculated with *unfiltered* sputum taken during the same period, one positive result was obtained, and in the other instance, although the macroscopic examination revealed little, the microscope showed patches of acute inflammation with the typical inflammatory exudate. Thus 85.7 per cent. of all monkeys inoculated with sputum, filtered or otherwise, gave positive results, if the doubtful case is included; if excluded, 71.4 per cent. were positive. Where sputum taken at a later date was inoculated the results obtained were less satisfactory. One monkey was inoculated with *unfiltered* sputum taken on the sixth day from a case of influenza. A negative result was obtained. A monkey inoculated with the *filtered* sputum from the same case also showed no signs of disease *post mortem*. A monkey inoculated with filtered sputum from a case of influenza taken on the fifth day gave a positive result.

GROUP II.—Sputum Collected Later.

| Type of Monkey. | Material Used for Inoculation. | Day of Disease on which Sputum was Taken. | Result. |
|-------------------------|--------------------------------|---|----------|
| 1. <i>M. rhesus</i> ... | Unfiltered sputum | Sixth | Negative |
| 2. <i>M. rhesus</i> ... | Filtered sputum | Sixth | Negative |
| 3. <i>M. rhesus</i> ... | Filtered sputum | Fifth | Positive |

In this instance two out of three attempts failed, or only 33.3 per cent. were positive. These figures are very small and insufficient to justify any final opinion as to the period in which

it may be impossible to obtain the virus from a case of influenza, but with the number of monkeys which we had it was thought more profitable to concentrate our attention on sputum obtained at an earlier date. The time up to which the virus can be recovered must form the basis for further work on this subject. An attempt to infect a monkey with filtered sputum instilled up its nostrils, without any subconjunctival injection, failed, although subconjunctival injection, without nasal instillation, of the same filtrate, gave a positive result in another monkey.

Controls.—Two monkeys were inoculated with filtered sputum obtained from two cases of acute bronchitis on the third day of the disease. The sputum was prepared and filtered in the same way, and the monkeys were inoculated subconjunctivally and by nasal instillation in each case. They were given the same dose, but were both negative as regards symptoms, and the macroscopic and microscopic examination of their lungs. Another monkey died two days after arrival with a lobar pneumonia associated with M'Gowan's bacillus of distemper. The *post-mortem* findings were totally different from those seen in our experimental animals. Microscopically there was no great engorgement of capillaries, and no haemorrhagic exudate in the alveoli.

The obtaining or not of a positive result by means of the injection of monkeys with filtrable viruses depends very much more on the dose given than in the case of experimental infections with non-filtrable germs. At present it is not possible to determine the dose that is being given. It is known that while these viruses, as a rule, reproduce the picture of the human disease, as, for instance, in the case of poliomyelitis, the certainty of producing a positive result is not nearly so great as when dealing with the non-filtrable bacteria which are pathogenic for animals. In view of these facts the animal experiments with monkeys appear to us to be quite significant as far as they go.

Rabbits.—Four rabbits were inoculated. Of these, two were inoculated with filtered sputum taken on the third day from a case of influenza. One of these rabbits was given 1 c.cm. of the filtrate intravenously and the other 1 c.cm. of the filtrate subcutaneously. Both inoculations were followed by a positive result. One lung, taken from the rabbit that had been inoculated subcutaneously, was ground up in a mortar with normal saline and the extract filtered. The filtrate was inoculated into a second rabbit. (See passage experiments.) The kidney of the rabbit receiving the intravenous dose was placed in a Noguchi tube with ascites fluid. (See cultural experiments.) One rabbit was inoculated subcutaneously with 2 c.cm. of a filtrate of bronchial pus taken *post mortem* from a man who died from influenza on the twelfth day of the disease. The result was completely negative, and tends to confirm our opinion that it is likely that the virus is not recoverable after the first few days of the disease.

Control.—A rabbit was given 2 c.cm. of a filtered sputum taken on the third day of the disease in a case of acute bronchitis. Result negative.

Guinea-pigs.—Four guinea-pigs were inoculated. Two of these were inoculated with sputum taken on the night of the second day and morning of the third day from a case of influenza. One guinea-pig was given 1 c.cm. of the diluted (1 in 11) but unfiltered sputum, and the other received 1 c.cm. of the same dilution of this sputum, filtered. In each case a positive result was obtained. A guinea-pig inoculated subcutaneously with 1 c.cm. of the filtered bronchial pus mentioned above gave a negative result.

Control.—A guinea-pig inoculated subcutaneously with 1 c.cm. of the filtrate of sputum from a case of acute bronchitis also gave a negative result.

Mice.—Altogether fourteen mice were inoculated with sputum, unfiltered or filtered, from various sources. These may be divided into those inoculated with:

- Unfiltered sputum from cases of influenza.
- Filtered sputum taken during the first three days of the disease in cases of influenza.
- Heated filtered sputum taken during the first three days of the disease in cases of influenza (heated to 71° for half an hour).
- Filtered sputum taken on the sixth day of the disease and later, from influenza cases.
- Filtered sputum taken on the third day of the disease from a case of acute bronchitis.

Under Group (a) 3 mice were inoculated: 1 gave a negative result, 2 died of pneumococcal infection, 1 died of streptococcal infection.

Under Group (b) 5 mice were inoculated: 4 gave positive results, 1 was killed twenty-four hours after inoculation—result negative.

Under Group (c) 2 mice were inoculated: Both gave negative results. (Mice inoculated with the same filtrate unheated all died.)

Under Group (d) 2 mice were inoculated: Both gave negative results.

Under Group (e) *Control*, 1 mouse was inoculated: Result negative.

II. INOCULATION OF ANIMALS WITH BLOOD FROM CASES OF INFLUENZA.

The following are summaries of three experiments in which animals were inoculated with blood from cases of influenza:

Experiment 1.

A case of influenza of forty-eight hours' standing was selected; 25 c.cm. of blood was drawn aseptically into a flask containing 2 c.cm. of 10 per cent. sodium citrate solution. 10 c.cm. of the whole blood was diluted with 40 c.cm. of distilled water. This laked blood was then passed through a Chamberland L1 bis filter candle, under a negative pressure of 50 cm. of mercury. Both laked blood and filtrate proved to be sterile with regard to non-filtrable organisms.

Monkey No. 8 was inoculated with 5 c.cm. unfiltered whole blood subcutaneously. It was killed seven days later, and both macroscopically and microscopically gave a completely negative result.

Monkey No. 6 received 0.25 c.cm. of the filtrate subconjunctivally into each eye and 0.5 c.cm. was instilled into each nostril; five days later the animal was moping, and it was killed on the seventh day. At *post-mortem* examination the lungs appeared quite normal macroscopically, but microscopically some capillary congestion was seen with definite small patches of inflammatory change.

Three mice were inoculated. Mouse W 1 received 0.25 c.cm. of the filtrate subcutaneously. It died the same night, and the lungs were found to be markedly haemorrhagic. Mouse W 2 received 0.25 c.cm. of the filtrate subcutaneously. It was killed on the fourth day, and showed marked haemorrhages in both lungs. Mouse W 3 received 0.25 c.cm. of the filtrate, which had been heated to 55° for one hour. It died on the third day after inoculation, and also showed a marked haemorrhagic condition of the lungs. It might be noted that both mouse W 1 and mouse W 3 were infected with coccidia.

Experiment 2.

Three mice were inoculated subcutaneously with 1 c.cm. of blood from influenza cases. One mouse was inoculated with blood from a first-day case of influenza, and the lungs were typically haemorrhagic. Two of the mice were inoculated with blood from second-day cases. One was killed on the sixth day and one on the eighth day, and both were definitely positive.

As controls one mouse was inoculated with 1.25 c.cm. of normal human blood and another with 1 c.cm., and both gave entirely negative results.

Experiment 3.

This experiment was undertaken to attempt to obtain information as to the earliest time after inoculation into mice of blood from influenza cases that one may expect to find any pathological lesions in the lungs of those animals. Six mice were inoculated each with 1 c.cm. of blood taken from a case of influenza during the first twenty-four hours of the disease. These mice were each given 1 c.cm. of blood subcutaneously, and it was intended to kill these animals at varying intervals up to eight days after inoculation. However, this intention was frustrated by the fact that five out of the six mice died during the first forty-eight hours after they received the injection.

The results are best shown in tabular form:

| No. of Mouse. | Inoculated. | Died. | Appearance of Lung <i>post mortem</i> . |
|---------------|--------------|-----------------|--|
| U 1 | Dec. 2, 1918 | Same night | Lungs showed nothing abnormal (inoculated intraperitoneally). |
| U 2 | " " | Next morning | Both lungs showed haemorrhagic-like patches. |
| U 3 | " " | Same night | Nothing abnormal seen in lungs (inoculated intraperitoneally). |
| U 4 | " " | Next morning | Left lung showed haemorrhagic-like patches. |
| U 5 | " " | Night of Dec. 3 | Haemorrhagic-like patches on both lower lobes. |
| U 6 | " " | Killed Dec. 3 | Lungs apparently normal. |

Of the six lungs examined microscopically, two showed acute inflammatory reaction, with congestion in one instance. Of the remainder, three showed acute congestion only and one some leucocytic infiltration.

III. PASSAGE OF THE VIRUS FROM ANIMAL TO ANIMAL.

Filtered sputum from an early human case of influenza was administered to a group of two monkeys (Nos. 11 and 7), two rabbits (Nos. 1 and 2), and two mice (Nos. Z 1 and Z 2), on December 9th, 1918. Of this group, monkey No. 7 received no injection, but the filtered sputum was merely instilled into the nose. This animal remained negative. Mouse Z 2 was given an injection of heated filtrate, and, as was to be expected, remained negative. All the other animals were inoculated with the unheated filtrate and all gave positive results. Of these animals,

rabbit No. 2 was selected to provide material for passage experiments.

Filtered Sputum from Case.

Rabbit No. 2 +.

Rabbit No. 6 +.

Guinea-pig No. 5 +.

Rabbit No. 8 +.

Guinea-pig No. 8 -.

RABBIT No. 2.

This animal was inoculated subcutaneously with 1 c.cm. of filtrate from sputum on December 9th, 1918. No symptoms of illness were noted after inoculation. It was killed and examined on December 15th.

Post-mortem Findings.

The lower lobes of both lungs showed purplish discoloration and haemorrhagic areas. Microscopically, irregular acute inflammatory changes, with early leucocytic reaction, were noted in sections of the lungs, together with engorgement of the capillaries. Bacteriological examination of the lungs was negative so far as concerns non-filtrable organisms.

The lower lobe of the left lung of this rabbit was now removed, crushed, extracted with normal saline, and the extract passed through a Chamberland F filter and used as the inoculum for further passage experiments, being injected into rabbit No. 6 and guinea-pig No. 5.

RABBIT No. 6.

On December 15th, 1918, this rabbit was inoculated subcutaneously with 2 c.cm. of filtered lung extract from rabbit No. 2. It was killed and examined on December 23rd.

Post-mortem Findings.

Trachea.—There is found some tracheitis in the lower part of the trachea, which is filled with a frothy, blood-stained fluid, which oozes up from the bronchi.

Pleura.—Some fluid present in each pleural sac.

Lungs.—Retain shape on being placed on the table. The anterior surface of the right lung shows a patch of haemorrhage at the root of the upper lobe. The anterior surface of the left lung presents very little abnormality. The posterior surfaces of both lungs is uniformly of a dark red colour. On section, the lung substance is seen to be reddish-brown in patches, and some frothy, blood-stained fluid drips from the cut surface. Cultures on blood-agar and glucose broth were negative. Lung substance from this animal was emulsified and inoculated into rabbit No. 8 and guinea-pig No. 8.

GUINEA-PIG No. 5.

This animal was inoculated subcutaneously, on December 15th, 1918, with 2 c.cm. of filtered lung extract from rabbit No. 2. It was killed on December 23rd. At the *post-mortem* examination both lungs were found to show large dark red patches over both anterior and posterior surfaces.

RABBIT No. 8.

Received an intravenous injection of 2 c.cm. of lung extract from rabbit No. 6 on December 23rd, 1918, and died about one hour after inoculation.

Post-mortem Examination (December 24th).

Peritoneal cavity clear and glistening. Bowel generally pale and yellowish.

Trachea.—The upper part shows the blood vessels between rings outlined in deep red and an extreme degree of congestion seems to be present between all the cartilaginous rings. Frothy fluid wells up in the trachea from the bronchi.

Pleura.—No evidence of pleuritis on either side. No fluid present.

Lungs.—Both lungs appear much the same. Generally they are pink, with an overlying crimson and deep red coloration which looks haemorrhagic. It seems, in the gross, to be an extreme grade of what has previously been seen in infected rabbits' lungs. On section, the surface drips bloody fluid and shows numerous deep red small haemorrhagic areas on the cut surface.

Thymus.—Enlarged, soft; vessels deep red and engorged. Large numbers of deep purple haemorrhages seen on surface.

Kidneys.—Deep red. Drips blood on section. Capsule strips easily.

Liver.—Deep red. On section, blood drips from surface and lobules appear outlined in deep red.

GUINEA-PIG No. 8.

Was inoculated subcutaneously with 2 c.cm. of lung extract from rabbit No. 6 on December 23rd, 1918. It was killed on December 31st, and at the *post-mortem* examination the lungs and other organs appeared to be normal.

REMARKS.

In the above series of experiments a tendency to increase of virulence was noted in passage through rabbits. The lesions found in rabbit No. 2 were of only moderate severity. Those produced in rabbit No. 6 by inoculation of material from rabbit No. 2 were much more severe. The inoculation of lung extract from rabbit No. 6 into

rabbit No. 8 led to the rapid death of the latter, the symptoms and *post-mortem* appearances being very striking, and suggesting that the inoculum may have contained a toxin of high virulence in addition to the living virus. It should be noted that this animal received an intravenous inoculation, so that any toxin introduced would have been rapidly distributed throughout the organism. It is a remarkable thing, however, that an equivalent amount of the same lung extract proved harmless for guinea-pig No. 8, inoculated the same time as rabbit No. 8. This result may, perhaps, be explained by assuming that this guinea-pig was unusually resistant to infection. It is to be noted, too, that in this instance the injection was subcutaneous, not intravenous.

IV. CULTURAL EXPERIMENTS AND INOCULATION OF CULTURES INTO ANIMALS.

In view of apparently positive results we had obtained in the transmission of influenza to animals by means of filtered materials, blood, sputum, etc., from cases of influenza, it was decided to attempt to obtain cultures from the virus by means of the method used by Noguchi. We had always obtained uniformly negative results in attempting to culture different filtrates, using the ordinary media employed by us in investigating the bacteriology of sputum, *post-mortem* material, etc., from cases of influenza—that is, serum broth, blood agar, heated blood agar, etc.

Although the work is incomplete, we consider that the results are definite enough to warrant publication.

In November, 1917, Foster reported³ that in studying the etiology of common colds he had been able to isolate an organism by means of Noguchi's methods, and that he had reproduced the disease in human beings by means of nasal instillation of his culture. His method and technique are described in great detail in his monograph, and we have followed them as closely as was possible in a laboratory in the field.

Preparation of Noguchi Culture Tubes.

At first we had great difficulty in obtaining a supply of ascites fluid, and our earlier experiments were made with human blood serum with negative results. This is not surprising, as it is recognized that blood serum tends to inhibit the growth of filtrable organisms, that of poliomyelitis being an example.

Finally, a case of general polyserositis occurred and a litre of clear amber-coloured ascites fluid was obtained and proved to be sterile.

Special tubes were obtained measuring 1 cm. by 20 cm. These were sterilized by hot air and filled with ascites fluid to within 6 cm. of the top. These tubes were then allowed to stand five days at 37° to eliminate the possibility of contamination.

To obtain sterile rabbit kidney Foster's technique was followed as closely as possible. After removal from the rabbit a small portion of kidney was placed in each tube and allowed to settle to the bottom. Sterile liquid paraffin was poured into each to within 2 cm. of the top. The tube was then plugged again with cotton and a rubber cap put on and they were allowed to stand for one week before being used.

When cultures were made, the material to be cultured was drawn up into a long teated capillary pipette, and then expelled until it had formed a drop on the tip when the pipette was quickly plunged through the paraffin and ascites fluid to the kidney, and the material all expelled to within 3 or 4 cm. of the tip. The pipette was now withdrawn quickly, and the tube plugged again.

Staining Methods.

When it was desired to examine microscopically material from the bottom of the tube, this was obtained with a capillary pipette and the pipette broken off above any oil that might have clung to it. A few drops were then placed on a slide, spread slightly, and allowed to dry in the open air upside down. They were fixed for one hour in pure methyl alcohol, and stained for twenty-four hours in 5 per cent. Giemsa, always having the smear downward to prevent any stain from depositing.

Foster stated that one should see in uninoculated tubes in twenty-four to forty-eight hours only a clear zone of haemolysis above the kidney, and possibly a very faint opalescent zone, but nothing more, and we have confirmed

this. Even, the haemolytic zone may be very faint if the animal from which the kidney was removed had been thoroughly exsanguinated.

When we first commenced the cultural work, in December, 1918, we were temporarily somewhat short of animals. This fact led to our obtaining our first culture from the kidney of an infected animal instead of directly from the material from human cases. This came about in the following way:

Wishing to prepare some Noguchi tubes, and having no normal rabbits from which to obtain the tissues, we used the kidney from a rabbit that had been inoculated with a filtrate of influenza sputum, and which had reproduced the characteristic signs in its lungs. The Noguchi tubes thus prepared were placed in the incubator to ensure their sterility. In twenty-four hours a clear pale-red zone had developed above the tissue, and in forty-eight hours it was noticed that a faint cloud was appearing. In seventy-two hours this had increased, and a week later the cloud was about 3 cm. high, and a fine deposit had begun to settle on the bottom of the tube. The cloud showed no tendency to blend with the clear ascites fluid above and was rather more dense immediately above the kidney. The culture was examined by subculturing for the presence of any non-filtrable contamination, and this was found to be absent.

On the thirteenth day a smear was made and stained with 5 per cent. Giemsa for twenty-four hours after fixation in methyl alcohol for one hour. In this smear the following appearances were noted:

Numerous small coccoid bodies, in size varying from about 1μ to 2μ , and 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 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 particular occasion the cocci were Gram-negative, but it has since been shown that very young cultures may be Gram-positive.

The cloudy material was transferred to other Noguchi tubes and the same phenomena occurred. Smears made from the resulting subcultures, stained with Giemsa, showed the same picture as described above.

ANIMAL EXPERIMENTS WITH CULTURES.

Experiment No. 1.

Original cultures were put up on December 15th, 1918. On December 28th, when the cultures were thirteen days old, a mouse was inoculated subcutaneously with 0.25 c.cm. of the original culture. It died six days later. Its lungs showed some haemorrhagic areas.

Post mortem its kidneys were removed with aseptic precautions, and implanted in a Noguchi ascites fluid tube. A subculture was obtained, but not proceeded with further.

A rabbit was also inoculated intravenously with 1 c.cm. of the culture. It was killed seven days later, and, although the lungs of the animal did not show any typical signs of a marked infection, a culture was obtained from the rabbit's kidney.

In addition to these animal inoculations, subcultures of this virus were made on December 28th, and a definite cloud was obtained. Fifteen days later, on January 12th, 1919, these cultures were mixed together, and the following animals were inoculated as shown in the table below.

At the same time as the animals were inoculated the subculture R.2 was inoculated into another Noguchi tube. This culture produced the usual cloud, and the smear showed the presence of the typical coccoid bodies. Thus the third generation by direct subculture has been reached, and at the time of writing it has not yet been taken further.

Besides these experiments we have also succeeded in growing the organism: From a filtered sputum from a case of influenza—this also has been carried through to the second generation; from the filtered lung extract of Baboon 12; from the kidney of another infected rabbit.

Controls.—The kidneys from normal rabbits when cultured in Noguchi tubes have failed to produce these organisms on culture, as also the kidney from a monkey inoculated with sputum from a case of simple acute bronchitis.

CONCLUSIONS.

The number of experiments carried out by us is too small to justify the drawing of final conclusions. These experiments were brought to an end by the cessation of the epidemic, and the loss of laboratory attendants consequent on demobilization. We feel, however, that we are in a position to make the following deductions from our work:

I. The apparent immunity of some animals to filter-passing viruses and the occasional difficulty of the transmission of these viruses by means of blood is well known. When this is taken into account the number of positive results obtained by us would seem to be significant.

II. The pathological lesions in what may be called experimental influenza in animals closely resemble those seen in the lungs of men.

III. There is some evidence in favour of the view that the passage of the virus from one animal to another may raise its virulence.

IV. Inoculation of the filtered and unfiltered sputum taken from cases of influenza, especially at an early stage of the disease, has been found to produce lesions in the lungs in a high proportion of inoculated animals. The inoculation of blood may not always produce such striking results.

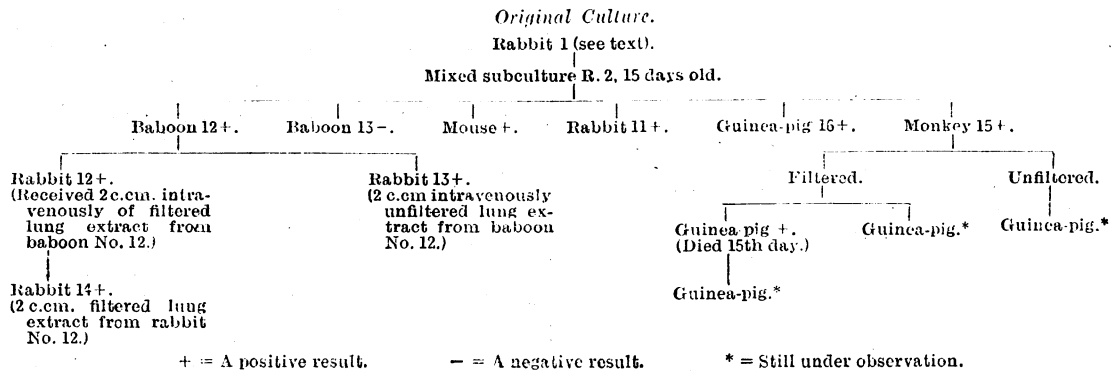
V. A minute micro-organism of a coccoid shape may be grown by Noguchi's cultural methods from: (a) The kidney of infected animals; (b) the filtrates of lung tissue, and (c) the filtered sputum from cases of influenza. The cultures have been carried by us to the third generation by direct culture. The cultures when inoculated into animals produce typical "experimental influenzal" lesions, and cultures can be recovered again from the animals so inoculated.

VI. In view of these findings we consider that there are very strong grounds for considering that—

- The organism isolated by us is capable of passing through a filter.
- That it is in all probability the cause of influenza as seen to-day.

A similar organism, having the same properties, has also been described by Captain J. A. Wilson, R.A.M.C., working quite independently at No. 20 General Hospital.⁴ Since the publication of his work on this subject we have shown him our preparations, and he considers the organism isolated by us to be the same as his own, in which opinion, after examination of his slides, we are in agreement.

We are indebted to the Medical Research Committee for providing the material for the work; to Colonel S. L. Cummins, C.M.G., A.M.S., Adviser in Pathology, British Armies in France, for valuable advice; to the Officers Commanding No. 2 Stationary Hospital and No. 3 Australian General Hospital for allowing us the use of the laboratories attached to their hospitals; and to the Officer Commanding No. 2 Stationary Hospital for the use of his wards.



The help of Private Webster, A.I.F., and Private Whally, R.A.M.C., has been invaluable in the care of the animals, and our thanks are also due to Private Urquhart, R.A.M.C., our laboratory attendant, for much hard work in the preparation of media.

NOTE BY COLONEL S. L. CUMMINS, C.M.G., A.M.S.,
Advisor in Pathology, British Armies in France.

While Major H. G. Gibson, R.A.M.C., was actually engaged in preparing the above summary he was himself attacked by the disease in its severest form. He and his colleagues had been working for long hours in the laboratory at cultural and passage experiments with the organism believed to be the "filtrable virus" of influenza, and the attack found him overdone and weary from his self-forgetting efforts to solve a problem of pressing military and general importance. Major Gibson had no opportunity of revising or even finishing the summary, which has had to be completed from his notes and records.

His death, a grievous loss to medical science and to the Royal Army Medical Corps, was still such an end as a soldier would have chosen. Laborious tasks cheerfully undertaken and dangers resolutely faced are no less glorious in the laboratory than in the trenches.

REFERENCES.

¹ A Filtrable Virus as the Cause of the Early Stage of the Present Epidemic of Influenza, 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., BRITISH MEDICAL JOURNAL, December 14th, 1918. ² The Etiology of Common Colds, George B. Foster, jun., Major, Medical Corps, U.S. Army, *Journal of Infectious Diseases*, vol. ii, No. 5, November, 1917, pp. 451-474. ³ *Journal of Infectious Diseases*, vol. xxi, No. 5. ⁴ Preliminary Report on the Presence of a "Filter-passing" Virus in Certain Diseases, Major-General Sir J. Rose Bradford, A.M.S., Captain G. F. Bashford, R.A.M.C., Captain J. A. Wilson, R.A.M.C., BRITISH MEDICAL JOURNAL, February 1st, 1919.

SYMPTOMS OF HYPERTHYROIDISM OBSERVED
IN EXHAUSTED SOLDIERS.

BY
W. JOHNSON, MAJOR R.A.M.C.(S.R.).

THE close association between emotional states and Graves's disease has long been pointed out. During the course of the present war reference to mild conditions of the disease occurring in soldiers has been fairly frequent, and warrant a consideration of the question of the relation between emotion and hyperthyroidism.

In the later part of the year 1917, in a certain forward area, I was able (largely with the help of Captain F. S. Walker, R.A.M.C.T.) to observe and take brief notes on fifty cases in which the condition was clinically demonstrable. These cases were admitted into a centre for psychoneuroses and formed a small percentage of the total admissions.

EMOTIONS AND THE INTERNAL SECRETIONS.

The observations of Cannon have thrown considerable light on the intimate relation existing between emotions and the internal secretions.

Beginning by directing attention to the fact that the well known manifestations of emotional states (for example, pallor, blushing, palpitation, increased cardiac and respiratory action, etc.) are all evidence of sympathetic nervous activity, he proceeded to investigate the question of how an emotional upset produces such results. By experiments on animals kept in a state of fear, anxiety, and anger, he was able to demonstrate that suprarenal activity was stimulated; and that an increase in the adrenin content of the blood in the suprarenal vein could be demonstrated. Adrenin acts by direct stimulation of that portion of the sympathetic nervous system which is styled the vertebral sympathetic or the sympathetic proper. It is therefore seen that it is the increased output of adrenin, by producing profound sympathetic activity, which is responsible for the physical manifestations of emotion.

Cannon also points out that the sympathetic activity produced by increased adrenin secretion is all directed towards toning up and sustaining the physical powers for battle—that is, the staying power. Without going into the question of the relative degree to which the various

organs of internal secretion may be involved in the production of this result, it seems certain that, at least, the suprarenal and thyroid glands are two of the most important.

Increased adrenin secretion causes:

1. An increase in the blood sugar;
2. An improvement in the contraction of a fatigued muscle;
3. An increase in the coagulability of the blood;
4. A rise in the blood pressure by constricting the splanchnic area.
5. An increase in force and frequency of the cardiac beat.

In view of the cases about to be described, I add here the generally accepted view of the function of thyroid secretion.

1. It is said to reinforce the action of adrenin.
2. When it is absent or diminished, conditions of mental inactivity—that is, myxoedema and cretinism, result.
3. Where it is present in excess there is a state of mental excitation.

In an individual who is exposed to emotional stress for weeks—sometimes months—without being able to perform the active bodily movements (fighting, running, etc.) which are the natural expression of the emotion, the increased internal secretions which have been produced in his body become, as it were, "a drug on the market." Their physiological circle is uncompleted, and where the emotional state has been excessive and prolonged—as was the case with soldiers under recent military conditions—it seems possible that the accumulation of the excessive products of internal secretion in the body may produce pathological conditions.

It would appear, then, that the various symptoms exhibited by men breaking down in the line have a definite basis. The patient presents those states which are recognized as being produced by sympathetic activity, but he presents them in a riotous way. He has palpitation and tachycardia, disordered activity of the alimentary canal, dilated pupils, protrusion of the eyeballs, sweating and vasomotor disturbance. Added to these are the various subjective complaints which are the results of these states—that is, headache, dizziness, dyspepsia, indigestion, loss of weight, weakness on exertion, and that general unhappy condition described by the patient as "nervousness." In this latter term he includes that feeling of irritability which he is distressed to find he is unable to control.

In a recent paper¹ it was stated that a series of cases, belonging to the group of irritable heart, psychoneuroses, etc., showed a hyper-susceptibility to injections of adrenin. The present paper supplies evidence that in the early days of severe exhaustion, symptoms attributed to hyperthyroidism may be observed. These symptoms were observed within a few hours of the patient leaving the battle line, and it was found that, after two or three weeks' rest, marked amelioration of the condition occurred. Details as to the particular nature of the fighting and the relation to the total number of psychoneuroses admitted during the time cannot be given, and systematic and scientific investigation was impossible under the circumstances.

Clinical Description.

In the first two cases that drew attention to the condition exophthalmos was pronounced and there was definite enlargement of the thyroid gland. In five other cases only was any increase in size of the thyroid gland noted, and in these the enlargement was limited to one or other lobe. The clinical picture of Graves's disease was, in all these cases, complete.

In the remaining forty-three cases it could not be said that the thyroid gland showed any alteration in size, although in many there was a slight fullness about the neck. The degree of exophthalmos, however, sufficed to occasion remark and to call for special observation and examination. It then became apparent that the whole fifty cases formed one clinical group and varied only in the degree with which the symptoms were exhibited.

The ages of the patients varied from 21 to 43 years—the majority being between the ages of 23 and 35. In only one