

some ten days before the disease became prevalent in the institution. The vaccine contained 8,000,000 bacilli per centimeter, and a total of 2,000,000,000, was given to each person. There were 461 people vaccinated, and 538 not vaccinated. Among the vaccinated, there was a morbidity of 35.4 per cent, with 17 per cent deaths, while among the unvaccinated there were 34.3 per cent cases with 13.5 per cent deaths. A similar experiment was done on naval personnel at the Pelham Bay Training Station (Notes on preventive medicine for medical officers, United States Naval Bulletin, nos. 50 and 51). Nine per cent of the 154 inoculated, and 5 per cent of the 800 uninoculated developed the disease. Similar results were obtained at a naval base in South Carolina. McCoy reports a few experiments carried out in various institutions by members of the United States Public Health Service in which a comparison between 484 vaccinated and 842 unvaccinated controls were made. Among the vaccinated there were 31.6 per cent cases, with no deaths, and among the unvaccinated there were 26.3 per cent cases with 1.8 per cent deaths. In this case the vaccine was a pure influenza bacillus suspension.

We may summarize this phase of the work, in complete agreement with McCoy, to the effect, that, in spite of the general impression favoring the value of vaccination in the prevention of influenza, gained from the study of poorly controlled experiments, the evidence furnished by experiments that have been controlled in every particular has so far failed to demonstrate any effects whatever upon either incidence of mortality.

#### *Inoculation experiments*

The most perfect proof of the etiological relationship of influenza bacilli with the disease could of course be obtained by the production of the typical disease in normal human beings by inoculation with pure cultures of influenza bacilli. During the earlier pandemic it seemed that certain laboratory accidents had definitely indicated that such transmission was possible, the one most frequently cited being the laboratory infection of Kretz whose nose touched a plate he was fishing, and who in consequence developed an acute inflammation of the respiratory passages with influenza bacilli persisting in his sputum for several months. In such cases, however, just appraisal of the

evidence is difficult because these accidents occurred during an epidemic or its early post-epidemic periods and happened to individuals the nature of whose work brought them into contact with infectious material. Also the symptoms described were usually those of localized catarrhal inflammations rather than of true influenza. Purposeful and well controlled experiments upon man would be more conclusive and extensive attempts in this direction have accordingly been made by a number of investigators during the last pandemic.

Since typical influenza cannot be produced in any of the lower animals, the only species which besides man are worth considering for such work are the monkeys and the higher apes. Within recent months Cecil and Blake working at the United States Army Medical School in Washington, have succeeded in producing typical lobar pneumonias by intratracheal inoculation of pneumococci in various species of monkeys. This encouraged them to experiment upon these animals with pure cultures of influenza bacilli. They used a Philippine monkey, *Macacus Syrichtus*, and a Central American species, *Cebus Capucinus*. The strains of Pfeiffer bacilli employed were isolated from an influenzal pneumonia in a child. The virulence of the strains, which has been on artificial media for six weeks, was raised by successive mouse passages and subsequent intraperitoneal passage through a series of 13 monkeys. They then inoculated a group of 22 monkeys. The material used for inoculation consisted of first or second subcultures of organisms isolated from animals dead of pneumonia or peritonitis, and of peritoneal exudates from such animals, used directly from the body.

In some monkeys the material was introduced into the nose by application with a sterile cotton swab or with a pipette. In another group the material was introduced low down into the trachea by injection with a syringe.

Twelve monkeys were inoculated by the nasal method and in every instance acute respiratory disease developed; three to five hours after inoculation there was prostration, in some cases with a temperature of from 103° to 106°F. in others there was very little or no fever. Sneezing, rubbing of the nose and other signs of catarrh became manifest. In most cases at the end of twenty-four to forty-eight hours the infection spread to the lower passages and a cough developed.

Five of the monkeys developed acute sinusitis from which the influenza bacillus could be obtained. Two animals developed pneumonia on the third and fourth days, and the influenza bacillus was obtained in pure culture from the lungs. Ten monkeys received intratracheal injections of 1 to 5 cc. Again prostration and temperature developed in most of them with respiratory symptoms. In one case general infection with septicemia and pericarditis ensued. None of these died. Seven developed pneumonia and were killed during the active stage, and from the lungs influenza bacilli in pure culture were recovered. The pneumonia which developed was widespread, and lobular in type, with extensive hemorrhage and edema appearing to Blake and Cecil similar to that occurring in man.

The experiments of these investigators show that the bacillus of influenza can produce a violent infection of the upper respiratory tract with catarrhal symptoms and other manifestations, common in man at the time of prevalence of influenza epidemics. There can be no doubt about the fact that these experiments add considerable weight to the assumption that the bacillus of influenza causes the disease in man. We will recur to this in our final discussion of this phase of the general evidence.

More directly pertinent are inoculation experiments on man. David J. Davis in a letter written to the Journal of the American Medical Association of May 3, 1919, writes that in 1906, having isolated influenza bacilli from a considerable number of cases of whooping cough, measles and varicella, he inoculated a young healthy man with pure cultures of the bacilli. Preliminary cultures showed no similar organisms in the subject's throat; he had had no serious illness of any kind within the immediately preceding period. The washings of 6 blood agar tubes were taken up in salt solution and the throat, tonsils, and nasal mucosa were smeared with the suspension. Forty-eight hours after the inoculation he complained of chilliness and great weakness. A temperature of 100.2° developed, but rapidly subsided, returning to normal on the third day. He complained of headache, general malaise, and coughed slightly. The throat was slightly congested, and the pharynx coated with thick, stringy mucus. The local condition persisted for about four weeks during which there was a very slight cough which did not in any sense resemble that of

whooping cough. During the first few days an almost pure culture of influenza-like bacilli was recovered. In the course of the next three weeks the influenza bacilli gradually disappeared from the throat. There were no complications. The description of this case, as given in Dr. Davis's letter has certain important points of similarity with early cases of epidemic influenza, especially as regards the mildness of the local symptoms with the sudden development of temperature, severity of the systemic symptoms and short duration of the fever.

In 1919, Rosenau, McCoy and collaborators, working under Government auspices, carried out a series of important experiments on man carefully controlled and elaborately planned. The group conducting the investigation were officers of the United States navy and of the Public Health Service; McCoy, Goldberger, Leake and Lake on the part of the Public Health Service, coöperating with Rosenau, Keegan and Richey, on the part of the United States navy. The experiments were carried out at Gallops Island, the quarantine station near Boston. The volunteers were all between eighteen and twenty-five years of age and in good physical condition. Of the 100 men used, none of them had had influenza or any febrile attack during the preceding winter. Preliminary experiments in which pure cultures of the influenza bacillus in moderate amounts were instilled into the nostrils of a few of the volunteers were entirely negative. In consequence, a more drastic experiment was decided upon. Nineteen volunteers were given a considerable quantity of a mixture of 13 different strains of Pfeiffer bacillis, some of them recently obtained from the lungs at autopsy, others representing subcultures of different culture-generations obtained from recent cases. Suspensions of the bacteria were sprayed into the nostrils, eyes, and throats with atomizers while the volunteers were inspiring. Several billions of the organisms were used on each one, but not a single one of them developed any kind of illness.

Following these negative experiments an attempt was made to infect directly with mucous secretions obtained from the mouths, noses, throats, and bronchi of active cases of influenza. The material was obtained from febrile cases by washing out nostrils and throats with 5 cc. of salt solution and allowing the patient to blow his nose

vigorously into a sputum dish. They were then made to gargle with some of the solution and this was added to the rest. Bronchial mucus was obtained after coughing, and the nares and throats were swabbed. The swabs with all the materials were then put into bottles with glass beads, and this (properly called "stuff" by Rosenau) was administered to the volunteers. Ten men were used and each of them received about 1 cc. sprayed into nose and throat while inspiring, and into the eyes. None of them became sick. Other experiments done at this time by the same Board will be recorded when we come to speak of filtrable virus. Most astonishing of all of the work done by this Board are the entirely negative attempts to infect such volunteers by bringing them into the closest possible direct respiratory contact with cases in the active stages of the disease.

McCoy and Richey carried out similar experiments at Goat Island in San Francisco Harbor, also with entirely negative results.

It is very difficult to comment upon these experiments. Their completely negative character would lead one to assume not only that influenza bacilli did not convey the disease, but the upper respiratory secretions of influenza patients were not the vehicle of infection. The latter conclusion can hardly be credited in view of the large volume of epidemiological evidence in favor of such transmission; and when we consider the experience and reliability of the investigators who carried out these experiments we must assume that some third factor, the most likely one being insusceptibility on the part of the volunteers must have played a part. Even this, however, would seem unlikely in view of the large number of men used and the careful scrutiny made before the experiment. As a matter of fact there is no satisfactory explanation for these failures at the present time.

Wahl, White and Lyall in 1919 also applied saline emulsions of fresh Pfeiffer strains from epidemic influenza cases to the nares and nasopharynges of 5 healthy men, absolutely without success; and these investigators did not succeed, except in a single case, in recovering the influenza bacilli from the nares forty-eight hours later. To this point we will refer in our summary since we consider it of considerable importance.

Bloomfield, investigating more particularly the length of time during which influenza bacilli would persist in the upper respiratory pas-

sages of healthy individuals, introduced 3 different strains of influenza bacilli in large amounts into the upper air passages, and in none of his 5 cases observed any local or general pathological effects. Moreover Bloomfield found that the organisms disappeared within from one to two days, and that a carrier state was produced in none of them.

Yamanouchi, in connection with his experiments on filtrable virus, has reported completely negative experiments with the Pfeiffer bacillus in man.

Lister and Taylor though unsuccessful with filtered material, inoculated 5 controls with unfiltered material from influenza lungs. Two of these had typical attacks of influenza, coming down with the disease thirty-six hours after the material had been instilled into the nasopharynx. One volunteer was sprayed with a pure culture of influenza bacilli and came down with a "mild attack."

More recently, Cecil reported to the Medical Section of the New York Academy of Medicine (May 19, 1920) a few experiments in which he introduced Pfeiffer bacilli (the virulence of which had been raised by methods analogous to those used in his previous monkey experiments) into 6 persons. He obtained moderate local and systemic symptoms which suggested very mild influenzal attacks, curiously enough there was absolutely no temperature in any of them.

Attempts to produce typical influenza with cultures of influenza bacilli have, therefore, been negative in most cases. With the exception of the few instances of apparent success by Lister and Taylor and the last suggestive experiments of Cecil no encouragement has been obtained along these lines. But it should be remembered that it has been shown that it is extremely difficult (as in Bloomfield's work) to induce the influenza bacillus to gain a foothold on the normal mucosa, and negative experiments cannot be taken as conclusive until a failure to obtain symptoms persists in spite of the establishment of the organisms in the inoculated throats for at least forty-eight to seventy-two hours.

#### *Filtrable virus*

Before we can attempt to summarize views on the etiological importance of the influenza bacillus, it becomes necessary to consider in some detail a series of investigations inspired by the suggestion that influenza may be due to a filter-passing virus.