canyon development. The explanation at present most popular, that the canyons are normal river valleys deeply submerged, calls for very great vertical oscillations of land level or sea level. Evidence corroborative of such great changes is lacking. Erosion by submarine currents is widely questioned on the ground that the potency of such currents seems small in comparison with the results achieved. Various other interpretations meet difficulties equally formidable. It is believed that the hypothesis of spring sapping under conditions similar to, but not identical with, those now existing on land has advantages which make it worthy of serious consideration.

Biographical memoir of Wallace Hume Carothers: ROGER ADAMS. (Read by title).

Biographical memoir of Edwin Herbert Hall: P. W. BRIDGMAN. (Read by title).

SPECIAL ARTICLES

AN INTERMEDIATE HOST FOR THE SWINE INFLUENZA VIRUS

SWINE influenza is a disease in which two infectious agents, one a virus and the other a bacterium, are etiologically essential. The disease, either as it occurs naturally in the field or as it is transmitted experimentally in the laboratory, is highly contagious, and no intermediate host is required to explain its epidemiology, once an epizootic has started. However, no satisfactory explanation of how or where the disease persists during the 8 or 9 months elapsing between the yearly epizootics has yet been advanced. During such interepizootic periods the swine population in the middle western hog-raising states is, so far as can be told, free of swine influenza. The bacterial component of the etiological complex, *H. influenzae suis,* can persist apparently indefinitely in the upper respiratory tracts of some recovered swine, but similar persistence of the virus can not be demonstrated. The origin or source of swine influenza virus responsible for the fresh epizootics each autumn thus has remained obscure. It is with the epidemiology of these "first cases" of swine influenza that the experiments to be briefly outlined in the present paper are concerned.

Because the swine lungworm, a nematode parasitic in the bronchioles of the bases of the lungs of swine, enters prominently into the experiments to be described, a short account of its life cycle, as determined by the Hobmaiers[1] and by Schwartz and Alicata,[2] will be given. The cycle in brief is as follows. The embryonated lungworm ovum passed in the swine feces is swallowed by an earthworm, in which it hatches as a first-stage larva. After undergoing development within the earthworm the larva eventually reaches its third stage in which it is capable of infesting swine. It remains in this stage until its earthworm intermediate host is ingested by a swine, when it is liberated, penetrates the swine intestinal mucosa and migrates to the respiratory tract by way of the lymphatics and blood stream. The whole of this cycle can occupy a span of several years for its completion or, under the most favorable conditions, can be completed in slightly less than one month. Lungworms constitute a very common parasite in swine reared under the usual farm conditions.

In the present experiments feces and bronchial exudate, containing embryonated lungworm ova, and adult lungworms were obtained from swine that had been ill of swine influenza for from three to five days. This material, after mincing the adult lungworms with scissors to free their ova, was mixed with loamy topsoil and fed to earthworms. Beginning one month later, earthworms were removed from time to time, from the barrels of earth in which they were kept, for use in experiments. They were fed to swine intact but usually mixed with a small amount of dry grain feed. Two swine fed in this way in the first experiment remained apparently normal, and it seemed that the experiment must be interpreted as negative. These two particular swine had, prior to their earthworm feeding, been used in another experiment during the course of which they had received three intramuscular injections of a suspension of live *H. influenzae suis* at eight-day intervals. They had developed no illness. After the apparently negative result of the earthworm feeding there was occasion to inject them again intramuscularly with a suspension of live *H. influenzae suis.* On the third day following this injection they developed clinically typical swine influenza. With this fortuitous suggestion that a provocative stimulus was required to elicit infection, other similar experiments were conducted. Swine were fed the lungworm-infested earthworms and developed neither illness nor virus-neutralizing antibodies. After a period of observation of from 11 to 30 days they were injected intramuscularly with a suspension of living *H. influenzae suis.* No illness resulted from the first injection. However, when the injection was repeated eight days later, characteristic swine influenza resulted after three days.

In other experiments the procedure was varied to coincide with that of the initial experiment, and in these the swine received two preliminary intramuscular injections of suspensions of live *H. influenzae suis* at

[1] A. Hobmaier and M. Hobmaier, *Münch. Tier. Woch.,* 80: 365 and 433, 1929.

[2] B. Schwartz and J. E. Alicata, *Jour. Parasit.,* 16: 105, 1929–30; 18: 21, 1931; and *U. S. Dept. Agric. Tech. Bull.* No. 456, 1934.

eight-day intervals before being fed lungworm-infested earthworms. After the earthworm feeding, sufficient time for the lungworms to complete their migration to the swine respiratory tract was allowed to elapse. Then the swine were again injected intramuscularly with a suspension of live *H. influenzae suis*. Swine influenza usually resulted on the third day after this injection, although in two instances its appearance was delayed until the fourth and fifth days.

In all experiments the clinical diagnosis of swine influenza was confirmed either by the direct demonstration of swine influenza virus by mouse inoculation or by the development of specific swine influenza virus-neutralizing antibodies in the sera of swine that were allowed to recover. Numerous control swine have been given series of from three to twelve intramuscular injections of suspensions of living *H. influenzae suis* at eight-day intervals with entirely negative results. In like manner no swine fed the lungworm-infested earthworms has developed swine influenza without a provocative stimulus having been first applied. Earthworms that had been fed lungworm ova as long as three months earlier have been used successfully. The results of experiments over longer periods of time are not yet complete.

Swine influenza virus has not yet been demonstrated directly by mouse inoculation, either in earthworms known to be carrying infective third-stage lungworm larvae or in lungworms obtained from swine thought to be ready for provocation. If this finding is duplicated in a larger series of tests it would appear that the virus in the intermediate host is present either in very minute quantities or in a masked form. However, in a single experiment containing two swine, the animals were given the provoking stimulus and one was killed the day before the expected onset of illness. Swine influenza virus was demonstrated in or about the lungworms obtained from the bronchi at the bases of the lung of this animal but not in the anterior lobes usually involved in swine influenza. The following day the second swine in the experiment came down with typical swine influenza. It was autopsied, showed pulmonary alterations characteristic of early swine influenza, and swine influenza virus was abundantly present not only about the lungworms at the bases but in the pneumonic anterior lobes as well.

The present experiments have been conducted with known species mixtures of both lungworms and earthworms. Dr. Norman R. Stoll has kindly identified the lungworms employed as *Metastrongylus elongatus* and *Choerostrongylus pudendotectus,* while Dr. Libbie Hyman has tentatively identified the earthworms being used as *Helodrilus foetidus, Helodrilus caliginosus* var. *trapezoides, Octolasium lacteum* and *Lumbricus terrestris.* Furthermore, all experimental swine thus far employed have been carrying lungworm infestations of various degrees prior to their use. Experiments with single pure species of either lungworms or earthworms or with lungworm-free swine have not yet been completed.

The findings described are tentatively interpreted in the following way. Lungworm larvae from pigs with swine influenza harbor swine influenza virus throughout their development both in their intermediate host, the earthworm, and in their definitive host, the swine. The virus apparently lies latent within the lungworm after the parasite has finally migrated to the swine respiratory tract and is only liberated or activated to cause infection when a provocative stimulus is applied. In the experiments just outlined multiple intramuscular injections of *H. influenzae suis* are believed to have supplied the provocative stimulus. *H. influenzae suis* does not, however, appear to be specific or requisite as the provocative agent because, in a preliminary experiment, a single intrapleural injection of calcium chloride solution has served equally well in provoking the swine influenza virus infection.

RICHARD E. SHOPE
THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH,
PRINCETON, N. J.

A CUTANEOUS TEST FOR TUBERCULOSIS IN PRIMATES

THE use of monkeys for experimental purposes has become so common during the past few years that many institutions now maintain permanent primate colonies. The chief single menace to the health of such colonies is tuberculosis.[1] Its incidence for 1936–1938 in the laboratories of the Yale University School of Medicine has been about 30 per cent., as determined by gross post-mortem examination. The desirability of reducing the spread of this disease by isolation of infected animals is obvious. The present report describes the results obtained by a simple diagnostic cutaneous test for tuberculosis.

MATERIAL AND METHODS

All departments in the Yale School of Medicine which maintain primate colonies cooperated in the study. Tests were made on 382 monkeys (353 *Macaca mulatta* [rhesus]; 17 mangabeys; 8 baboons; 4 Java monkeys); from the Departments of Anatomy, Obstetrics and Gynecology, Medicine, Pediatrics, Physiology and Primate Biology. Autopsies were done on 132 animals and the remainder were living (October 27, 1938) when this material was compiled.

The test[2] consists in the subcutaneous injection of

[1] C. R. Schroeder, *Am. Jour. Pub. Health,* 28: 469–475, 1938.

[2] C. R. Schroeder, *Zoologica,* 21: 397–400, 1938.