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.

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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.

old tuberculin (Schroeder uses P.P.D.) into the loose tissue about the eye where the erythema and edema of a positive reaction may readily be seen. At first, inoculations were made below the eye; later in the upper lid, since it was found that positive reactions were more conspicuous in the latter region, especially in the sooty mangabeys and Java monkeys in which the upper lids alone are unpigmented. The old tuberculin was diluted 100-fold in sterile physiological saline and 0.1 cc was used as the inoculum, which thus contained 1.0 mgm of old tuberculin. Positive reactions consisted in local redness and swelling. These varied in degree: in severe reaction the eyelids might become closed and necrosis and ulceration of skin might appear, as the reaction subsided. Usually the reaction was visible in 24 hours, increased for two or three days and then subsided gradually. Occasionally positive reactions were not apparent until the third or fourth day. If no reaction was seen by the fourth day the test was considered negative. In the colony of the Department of Pediatrics direct smear examination of suspected material for tubercle bacilli was also made at autopsy.

In reading the tables (I, II and III) attention should

TABLE I

PRIMATE TUBERCULIN TESTS (APRIL–OCTOBER, 1938)

Department of	Tested	Positive	Negative	Equivocal
Medicine and Pediatrics	136	44	90	2
Physiology	99	8	91	
Gynecology and Obstetrics	64	3	57	4*
Anatomy	59	15	44	
Primate Biology	24	0	24	
Total	**382**	**70**	**306**	**6**

* Previous immunization with B. C. G.

TABLE II

PRIMATE AUTOPSIES (APRIL–OCTOBER, 1938)

Department of	Total	Positive	Negative	Equivocal	Insufficient data
Medicine and Pediatrics	86	33	51	2	
Physiology	23	8	12	2	1
Gynecology and Obstetrics	3	3			
Anatomy	20	15	2		
Primate Biology	0				
Total	**132**	**59**	**65**	**4**	**4**

be directed to the fact that statistics from the various colonies differ because of the rate of turnover. Thus, the colonies of the Departments of Anatomy, Obstetrics and Gynecology and the Laboratory of Primate Biology are kept for long-term experiments, and new

TABLE III

CORRELATION OF TUBERCULIN TESTS AND AUTOPSIES

Department of	Test and autopsy positive	Test and autopsy negative	Test negative, autopsy positive	Test positive, autopsy negative
Medicine and Pediatrics	23	48	5	3
Physiology	7	12	1	1
Gynecology and Obstetrics.	3	0	0	0
Anatomy	12	2	3	0
Total	**45**	**62**	**9**	**4**

(potentially infectious) animals are rarely introduced. The animals in the Departments of Medicine and Pediatrics are used rapidly in contrast and those of the Laboratory of Physiology somewhat less rapidly.

RESULTS

The results are presented below: Table I shows that 70 monkeys, roughly 20 per cent. of the total, furnished positive tests. Table II shows that among 132 autopsies 59, or 45 per cent., revealed the presence of tuberculosis. Not shown in the table is the fact that this was confirmed by finding acid-fast bacteria on direct smear in 31 of 33 trials. The correlation between tests and autopsies is presented in Table III, which shows that 49 positive tests were confirmed by autopsies 45 times, and 71 negative tests were contradicted at autopsy 9 times. In other words, a positive test correctly indicated gross tuberculosis in 92 per cent. of 49 trials, and a negative test incorrectly indicated the absence of tuberculosis in 13 per cent. of 71 trials.

DISCUSSION

Some of the failures in correlation might have been avoided by repetition of the test or by using larger doses. However, even in man the test fails at times, and this test, which is approximately 90 per cent. accurate, is of great service in detecting tuberculous monkeys.

CONCLUSION

The periorbital subcutaneous inoculation of 1.0 mgm of old tuberculin is of practical value as a test for the presence or absence of tuberculosis in several varieties of monkeys.

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