experimentalism, is advancing rapidly with a keen analytical point of view freed from the sometimes misleading haze of words which has been the traditional nature writer's contact with the public. But what it builds is sometimes based on intensive rather than extensive knowledge, and is also at times, though thoroughly sophisticated, relatively meaningless from not resting on a broad enough foundation. Disregarding the matter therein not pertinent to the case, one can recommend this book of Professor Herrick's as an excellent background for bird behavior studies.

J. T. NICHOLS

AMERICAN MUSEUM OF NATURAL HISTORY

SPECIAL ARTICLES

CULTIVATION OF HUMAN INFLUENZA VIRUS IN AN ARTIFICIAL MEDIUM[1]

THE recovery of a filterable virus from cases of human influenza in Puerto Rico and Philadelphia[2,3] has been reported previously. These strains of virus were transmitted serially in ferrets and mice by the intranasal instillation of bacteria-free suspensions of infected animal lungs or of Berkefeld filtrates of such suspensions. In this manner the Puerto Rico strain (P. R. 8) has been carried through 43 passages in ferrets and 48 passages in Swiss mice without demonstrable alteration in its immunological characteristics.

After the virus had been found to be comparatively stable in its behavior in experimental animals, attempts were made to promote the multiplication of the agent in artificial media *in vitro*. In the media used for ordinary bacterial cultures no evidence of multiplication of the virus was obtained. Cultivation experiments were then undertaken in which living tissue cells were included in the culture medium. The technique devised by Li and Rivers[4] was employed, in which pieces of minced 10- to 14-day chick embryo suspended in Tyrode's solution in a Rivers flask are used.

Only the P. R. 8 strain of human influenza virus was used in this experiment. A titration of the virus content of the lungs of mice dying in the 42nd passage of this strain of virus was made with dilutions of a centrifugated but unfiltered suspension. All mice receiving the 1:1,000 dilution, based upon the weight of the original mouse lungs, showed advanced pulmonary lesions in 6 days; all that received the 1:10,000 dilution had mild lesions, while those receiving the 1:100,000 dilution presented no pulmonary lesions. The titer of the unfiltered virus as measured by the production of visible pulmonary lesions in white mice was considered to be 1:10,000.

The lungs of the mice of the 44th virus passage were then removed, ground and diluted to make a 10 per cent. suspension in Tyrode's solution. After centrifugation at 1,500 r.p.m. for 20 minutes, the supernatant fluid was filtered through a Berkefeld V candle. Cultures of this material in ordinary media revealed no bacteria. Flasks containing 4.5 cc of chick embryo Tyrode's medium were inoculated with 0.5 cc of the filtrate. Serial subcultures were made at 48-hour intervals by the transfer of 0.5 cc of the supernatant fluid of the culture to a flask containing 4.5 cc of fresh medium. Bacteria have not been demonstrated in the cultures, with aerobic and anaerobic methods of cultivation, or in stained microscopic films, except in a few instances in which gross bacterial contamination occurred.

Mice were inoculated intranasally with undiluted fluid of the first, third and of each subsequent subculture. In most instances the mice died in from 4 to 6 days with advanced pulmonary lesions. With the sixth generation culture, determinations of the concentration of the infectious agent in the culture fluid, taken after different intervals of incubation, were made by the inoculation of white mice with serial dilutions of the culture fluid. After 48 hours incubation, 0.05 cc of the culture diluted 1:1,000 produced distinct pulmonary lesions in mice, while 2 of the 3 mice receiving the 1:10,000 dilution showed suggestive lesions. After 72 hours' incubation of the culture, the mice which received a 1:1,000 dilution developed pulmonary lesions, but weaker dilutions were ineffective. After 5 days' incubation, the undiluted culture was still found to contain the infectious agent, but in diminished concentration. Titration of the tenth transfer of the culture yielded similar results. The mice which received culture diluted 1:1,000 developed pulmonary lesions, while those which received the 1:10,000 dilution did not.

From the lungs of the mice which were infected with the first generation culture fluid, a 10 per cent. suspension was made and filtered through a Berkefeld V candle. This filtrate, which contained active virus, was again introduced into the chick embryo medium, and through 20 successive subcultures the virus has retained its infectivity for mice. Thus the infectious

[1] From the Hospital of the Rockefeller Institute for Medical Research, New York, N. Y.

[2] T. Francis, Jr., SCIENCE, 80: 457, 1934.

[3] T. Francis, Jr., *Proc. Soc. Exp. Biol. and Med.*, 32: 1172, 1935.

[4] C. P. Li and T. M. Rivers, *Jour. Exp. Med.*, 52: 465, 1930.

agent has been transferred from mouse to culture, back through mice and to culture again. The same procedure was carried out with a culture in the tenth generation.

Furthermore, fluid from the culture of the seventh generation was inoculated intranasally into a susceptible ferret. The ferret developed marked fever and nasal symptoms, and mild pulmonary involvement was observed at autopsy. The disease picture produced was that of experimental influenza.

That the infectious agent in the cultures is the influenza virus is shown by the fact that the capacity of the culture fluid to produce lung lesions in mice is completely inhibited by the serum of ferrets that have recovered from infection with the P. R. 8, the Philadelphia or the Alaska[5] strains of ferret passage influenza virus which had never been passed through mice, while normal ferret serum does not exert this effect. Tests of this nature were performed with the fifth, eighth and fifteenth transfer cultures. Control cultures of the uninoculated medium have been entirely innocuous for mice.

The virus has been carried through 20 serial transfers in an artificial medium. Furthermore, the infectious titer of the culture virus after these repeated subcultures, while varying somewhat, remains essentially the same as that of the mouse passage virus from which the culture virus was derived. These facts indicate clearly that the virus has multiplied *in vitro*. The question of mere survival or of the carrying over of surviving virus in serial transfers appears to be excluded by the dilution factor.

Dochez, Mills and Kneeland[6,7] have reported observations concerning the cultivation *in vitro* of a filterable agent obtained from the throat washings of patients suffering from an upper respiratory disease resembling influenza. The inoculation of their culture into the nasal passages of human volunteers provoked upper respiratory disorders. The presence of the filterable agent in cultures was demonstrated only by the inoculation of human volunteers.

In the present experiments the culture virus has been transmitted readily to mice and ferrets, and it has been found that its capacity to infect experimental animals is inhibited by specific anti-influenza immune serum. Nevertheless, certain alterations in the immunological characteristics of the virus have been noted after repeated cultivation *in vitro*. Whether these differences in the behavior of the mouse passage virus and of the culture virus are merely quantitative or represent a definite change in the biological character of the agent is at present the subject of further investigation.

THOMAS FRANCIS, JR.
THOMAS P. MAGILL

[5] T. Francis, Jr. Unpublished experiments.

[6] A. R. Dochez, K. C. Mills and Y. Kneeland, Jr., *Proc. Soc. Exp. Biol. and Med.*, 30: 1017, 1933.

[7] *Idem.: ibid.*, 32: 406, 1934.

RELATIVE EFFICIENCY OF ROOTS AND TOPS OF PLANTS IN PROTECTING THE SOIL FROM EROSION

PLANT cover, soil and topography are the major factors concerned in the problem of erosion. During the earlier studies, conducted largely by engineers, chief emphasis was placed upon topography. Following extensive work by pedologists, it is now generally conceded that type of soil is an equally important factor. Intensive studies on the effect of plant cover reveal the extremely important rôle played by plants in holding the soil against the forces of erosion. In this preliminary account of the relative effectiveness of roots as compared to tops in holding the soil in place, the results are based on extensive experiments with cultivated crops and native vegetation.

Samples of soil 1 by 0.5 meter in area were obtained from fields, pastures and prairies by placing a wooden frame tightly about the sample, cutting away the core of soil and attaching a tight bottom to the frame at 10 cm depth without disturbing either soil structure or plant cover. The samples, taken in pairs, were transported to a rack which sloped at an angle of 10°, and the relative time required to wash away the soil was determined with plant cover intact and the cover clipped to the soil surface and removed. A stream of 13 gallons of water per minute from an open hose was played uniformly over the sample at a height of 2.5 feet and under a total force of approximately one pound per square inch.

Results with winter wheat are shown in Table 1.

TABLE 1
TURKEY RED WINTER WHEAT (TRITICUM AESTIVUM L.) GROWN IN WABASH SILT LOAM SOIL

Date samples taken	Height wheat inches	Stage of development	Erosion time, min.		Dry wt. gm.	
			Without tops	With tops	Underground parts	Tops
April 12 ...	3	"Rosettes" spread 6.5 inches	7	11	22	23
April 29 ...	7	Erect, heavily tillered	11	19	38	88
May 17 ...	20	Early "boot" stage	13	40	70	247
June 5 ...	36	Flowering	17	59	75	417
June 19 ...	45	Early dough	16	113	74	520
July 2 ...	45	Nearly ripe	15	99	65	521

Table 1 shows that with the growth of the roots the erosion time was somewhat more than doubled, but with the corresponding development of tops it increased 9 to 10 times. Decrease in efficiency in the ripening stage was due to the shriveling and drying