successive crops continued to take up radiostrontium even though the soil was treated only once with Sr^{85}. The uptakes of crops 2, 3, 4, and 5 were comparable to one another, but they all show a statistically significant difference from crop 1. This is as would be expected since crop 1 resulted from a 60-day growth following germination, while each of the other crops was grown for only 28 days. The fact that there were no large differences in Sr^{85} uptake among the successive crops indicates that during the period covered by these experiments there was no extensive reduction in the availability of radiostrontium to the alfalfa. However, the difference in uptake for crops 2 and 5 was statistically significant, thus suggesting that there might have been a slight amount of fixation of the radiostrontium in the soil, just as Squire reported (*1*).

In the studies on possible influences of chemical nutrients on plant uptake of radiostrontium from soil, previous work in this laboratory has shown that with greenhouse experiments similar to those described here, monocalcium phosphate applied at a level of about 600 lb/acre of soil effected a statistically significant reduction of Sr^{85} uptake in the grain, chaff, stem, and leaf of Thatcher wheat (*4*). My results with alfalfa (Table 2) show that ammonium dihydrogen phosphate, a commonly used fertilizer, caused no reduction in strontium uptake. This behavior is the same as that found in Thatcher wheat. Of the other nutrient treatments studied (Table 2), only 1.0 meq of potassium per 100 g of soil resulted in a statistically significant reduction in Sr^{85} uptake.

It may also be noted from Table 2 that the addition of radiostrontium to the surface of the soil, without mixing, resulted in much less uptake than the control in which the Sr^{85} was mixed through the soil. This is not surprising since under the conditions of these greenhouse experiments, the roots of the alfalfa plant covered almost the entire bottom of the pot. Although ploughing is known to decrease the absorption of radiostrontium by shallow-rooted plants such as ryegrass (*3*), mixing surface-contaminated soil by ploughing probably will not be very effective in reducing strontium uptake by deep-rooted plants.

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3 August 1962

Serial Propagation of Influenza B (Lee) Virus in a Transmissible Line of Canine Kidney Cells

Abstract. The serial propagation of influenza B (Lee) virus has been demonstrated in a transmissible line of canine kidney cells, as evidenced by the presence of cytopathic effects and hemagglutinins in each of six consecutive tissue-culture passages, and by egg infectivity titers of $10^{-4.3}$ and $10^{-4.1}$ per 0.1 ml in 3rd and 5th tissue culture fluids, respectively.

The propagation of influenza A and B viruses has been demonstrated in a variety of primary tissue-culture systems including minced chick embryo, human embryo lung and kidney, monkey kidney and bovine embryo kidney (*1, 2*). The propagation of influenza A, B, or C viruses could not be demonstrated in transmissible cell cultures such as HeLa, human conjunctiva, KB or human fibroblast (*2*). More recently Solov'ev and Alekseeva (*3*) have shown that a strain of Asian influenza virus was capable of being serially propagated in primary monkey kidney cells but incapable of maintaining infectivity beyond two passages in SOTs (cynomolgus monkey heart) cells (*4*), a transmissible strain derived from cynomologus monkey heart.

I believe this is the first report of the serial propagation of influenza virus in a transmissible strain of mammalian cells, derived from dog kidney and designated MDCK (*5*). The canine kidney cells had been through more than 35 serial passages prior to their use for the propagation of Lee influenza virus. These cells were grown initially on a medium consisting of 5 percent calf serum and 0.5 percent lactalbumin hydrolyzate (LAH) in Earle's balanced salt solution (EBSS) with 0.002 percent phenol red, 200 units of penicillin per milliliter, and 200 μg of streptomycin per milliliter. After the formation of a continuous cell sheet, in 2 to 3 days, and before virus inoculation, the fluids were changed to a 3 percent horse serum, 0.5 percent LAH, 0.1 percent yeast extract, and 0.1 percent bovine albumin in Earle's medium containing phenol red and antibiotics as above. The *p*H of all mediums was adjusted to approximately 7.2 with 7.5-percent $NaHCO_3$ solution. Cultures of MDCK were inoculated initially with 0.1 ml of a 10^{-2} dilution of egg-adapted influenza B virus (Lee) having an EID_{50} titer of $10^{-7.8}$ per 0.1 ml. Two serial passages of the virus in MDCK cells were made using undiluted harvest fluids, while the remaining serial passages were made with a 10^{-1} dilution of the harvested fluids.

The medium was changed once, 3 days after inoculation, and this fluid or the 6- or 7-day-old fluids, or both, were tested for hemagglutination (HA) at 4°C using 0.5-percent fowl erythrocytes. Egg infectivity (EID_{50}) titrations were conducted on tissue culture fluids from the 3rd and 5th passages, using 10- to 11-day-old embryonated eggs, by amniotic inoculation with 0.1 ml of fluid. Fluid from the 4th serial passage of virus in MDCK cells was shown to be neutralized in MDCK cultures by

Table 1. Growth of type B (Lee) influenza virus in a transmissible strain of canine kidney (MDCK) cells.

Tissue culture passage	Time of harvest (days)	Egg infectivity titer* (EID^{50})	Hemagglutination titer† (days)		Cytopathic effect (CPE)	
					Response‡	Time, post-inoculation (days)
0		5.8§	3	6 or 7		
1	3	N.D.‖	80	N.D.	4+	3
2	7	N.D.	<5	5	1+	7
3	6	4.3	<5	160	3+	6
4	3	N.D.	80	N.D.	2+	3
5	6	4.1	<5	1280	2–3+	6
6	3	N.D.	160	20	3+	3

* Expressed as the logarithm (base 10) of the number of 50 percent infectious doses in 0.1 ml of inoculum. † Expressed as the reciprocal of the initial dilution; 0.5-percent fowl erythrocytes used. ‡ Cells affected: 4+, 100 percent; 3+, 75 percent; 2+, 50 percent; 1+, 25 percent. § Titer of the original, diluted amniotic fluid inoculum. ‖ N.D., not done.

rabbit anti-Lee serum (*6*) and not by anti-Asian influenza serum (*7*).

The results are presented in Table 1. In a total of six passages the original allantoic fluid inoculum was diluted cumulatively to $10^{-12.3}$. The EID_{50} titers of the 3rd and 5th tissue culture passage fluids were $10^{-4.3}$ and $10^{-4.1}$ per 0.1 ml, respectively. Previous work with transmissible cell cultures which did not support the growth of influenza virus showed that in all but one case no infectious virus could be detected after 6 days of incubation on the 1st tissue culture passage and that no increase in hemagglutinins occurred (*2*). Although the hemagglutination titers obtained from the fluids of infected MDCK cultures were not uniform, hemagglutinins were present in every passage, and in one case (5th passage) reached a titer of 1:1280. The irregularity in hemagglutination titers may be due to the use for passage of low dilutions of tissue culture fluid pools containing relatively large amounts of noninfectious virus. The cytopathic effect, though somewhat variable in degree, was definitely present in every passage.

A simultaneous attempt to propagate influenza B virus in transmissible cell lines of bovine (*8*), ovine (*8*), porcine (*9*), and caprine (*5*) kidney was unsuccessful, as evidenced by the lack of cytopathic effects, inability to demonstrate hemagglutinins in the tissue culture fluids, and the inability to detect viable virus in 3rd-passage tissue culture fluids by amniotic inoculation of embryonated eggs.

Andrewes (*10*) suggests a possible origin of the Asian influenza virus from an animal reservoir in China. My evidence of the ability of influenza B virus to propagate in cultures of canine kidney cells and the report by Ado and Titova (*11*) of the experimental infection of puppies with an Asian strain of type A influenza virus suggest that the dog may be a good prospect as an animal reservoir of human influenza virus (*12*).

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16 April 1962

Adjustment of Monkeys to Five Continuous Days of Work

Abstract. The efficiency of well-trained monkeys improved after repeated exposures to a prolonged task. This result suggests that resistance to fatigue may be increased by prior experience with the fatiguing situation.

Experimental studies of fatigue and sleep loss in humans have often revealed surprisingly small decrements in performance, even after several days without sleep (*1*). In our experiments with monkeys we have also observed the same small performance decrements during 5 days of prolonged vigilance. We found, however, that the extent of the decrement was a function of the amount of prior experience with the fatiguing situation.

Four mature male rhesus monkeys, seated in restraining chairs for the duration of the experiment, served as subjects. During 3- to 8-hour sessions these monkeys had been trained to press a lever in order to prevent electric shocks to their feet; any pause in responding longer than 5 seconds produced a 5 to 6 ma shock of approximately 0.5-second duration (*2*). The downward force required to depress the lever was approximately 50 g. All of the monkeys had become highly skilled in the lever-pressing task before they were tested in prolonged sessions; three of them had previously received more than 300 total hours of avoidance practice.

Two of the monkeys were studied in sessions of 48-, 72-, and 120-hour duration before the standard 120-hour test was selected. The other two monkeys were tested after experiments on the first two monkeys had been completed; only 120-hour tests were scheduled for these later animals. Subjects were fed their normal food ration in a 30-minute daily period (9:00 to 9:30 A.M.) during which they did not have to press the lever. Rest periods of varying length (2 to 10 days) alternated with periods on prolonged avoidance. The subjects ate well and appeared to be healthy throughout the course of the experiment.

A safety provision in the circuitry prevented danger to an animal from the frequent shocks that would occur if the subject stopped pressing the lever. The shock circuit was automatically disconnected and the test terminated whenever an animal received 250 shocks within one day.

Over the course of 4 to 6 months each monkey was tested in at least eight sessions of continuous lever-pressing (*3*), interrupted only by the 30-minute daily eating periods and terminated either by (i) the passage of 120 hours, or by (ii) the occurrence of 250 shocks within a single day—whichever occurred first. Each prolonged session was called a test. The total number of daily shocks was used as the measure of efficiency.

Figure 1 compares performance decrements after different amounts of prior experience with the prolonged tests; each curve illustrates the decreasing efficiency of the monkeys as the test continues for 5 days. Data of tests 1 and 2 represent group means for the first two 120-hour tests; data of tests 3 and 4, the next two tests; and so forth.

In the computation of results for the tests shortened by the occurrence of 250 daily shocks before the fifth day (which happened on only three tests during the experiment), monkeys were assigned 250 shocks for every scheduled test day that followed the day on which the 250-shock maximum occurred. This assigned value is probably a very conservative estimate of how many shocks the subject would have received if the test had actually lasted the entire 5 days.

The data show that decrements over the course of the tests are reduced by prior exposure to such tests. By parametric analysis of variance of the group data, differences among the