second bird sang on the thirteenth day, the third bird on the sixteenth day, and the fourth bird on the twentieth day. From these data, it appears that the average length of time in administration of the hormone approximates fifteen days to produce song. Three of the treated birds gradually developed male-like tours¶ that were somewhat of the same pattern and quality, with limited variations. The fourth bird showed a song of a varied pattern that followed the same sequences when the songs were repeated. The songs developed exhibit a small repertoire with poor male quality so far, but with voice that was definitely male in character. Cessation of treatment resulted in a return to the ordinary female calls.

Conclusion. The administration of testosterone propionate to normal adult female roller canaries under conditions of complete song isolation brings forth male-like song in approximately 15 days after first administration, and thus substantiates previous theories.[3-7]

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Isolation of a Murine Neurotropic Virus by Passage of Monkey Poliomyelitis Virus to Cotton Rats and White Mice.*

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Armstrong[1,2,3] reported apparent transmission of poliomyelitis (Lansing strain) from the monkey to the Eastern cotton rat and to white mice. This report deals with attempts to adapt other strains of poliomyelitis virus to these rodents.

Cotton rats *(Sigmodon hispidus littoralis)* were infected intracerebrally with 5 recognized strains of monkey poliomyelitis virus (RMV, Aycock, Philadelphia, ST Los Angeles, SK New Haven). None of the animals injected with the first 4 strains showed any abnormal symptoms. However, of 2 cotton rats injected with the SK‡

¶ A tour is somewhat analogous to a syllable of language; there are thirteen recognized tours in roller canary song.

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1 Armstrong, C., *Public Health Reports*, 1939, **54**, 1719.

2 Lillie, R. D., and Armstrong, C., *Public Health Reports*, 1940, **55**, 115.

3 Armstrong, C., *Public Health Reports*, 1939, **54**, 2302.

‡ Received in its 11th monkey passage through the courtesy of Dr. John R. Paul.

strain 1 died the following day, evidently of trauma; the other one succumbed one week later without observed symptoms. No lesions were present except a markedly congested brain, sterile upon aerobic and anaerobic cultivation. Intracerebral transfer of this brain to another cotton rat resulted in mild nervous symptoms within 2 days, and death the next day. Further passage of the brain of the second cotton rat produced in a third cotton rat flaccid paralysis of both hind legs on the 6th day, followed by death 24 hr later. From the last 2 cotton rats intracerebral transfers of brain suspensions were made to groups of white mice. All injected mice developed complete flaccid paralysis of the hind legs, within 3 or 4 days, followed by generalized paralysis and death.

Subsequent attempts to reproduce passage from monkey to cotton rats and white mice with the original material were unsuccessful. Mouse virus, however, since its isolation, is transmissible from mouse to mouse in an unbroken series. At the time of this writing, *i. e.*, April 24th, 1940, the virus is in its 23rd passage. Over 2500 mice have been inoculated; excepting those injected with virus known to be inactivated or impotent all mice have developed the same characteristic symptoms, with only an occasional recovery, to wit: flaccid paralysis (unilateral or bilateral) of hind legs, seldom of forelegs, occasional encephalitic syndrome, death. The described symptomatology is somewhat similar to that of Theiler's[4] spontaneous mouse encephalitis, but the two viruses differ in important aspects (degree of virulence, incubation period, routes of infection, age factor, latent immunization, serological reactions). Moreover, mice from a Theiler-immune colony are not protected against infection with the mouse virus.

Stained brain suspensions from paralyzed mice, when examined microscopically, show no characteristic morphological unit. Seeding of blood agar, broth or 10% serum broth results in no visible growth after prolonged aerobic or anaerobic incubation. However, the infectious agent passes through V, N and W Berkefeld filters without appreciable diminution in virulence. It is completely destroyed by heating for ½ hr at 60°C and by exposure to ultraviolet light for 1 min, but resists phenol up to 1% concentration. In glycerin it has remained viable up to 1 month in the icebox.

White mice may be successfully infected by any one of the following routes: intracerebral, intranasal, intraperitoneal, intravenous, subcutaneous and by feeding. Upon intracerebral injection a constant potency of 1:1,000,000 is obtained and an occasional endpoint

[4] Theiler, M., *Science*, 1934, **80**, 122.

of 1:20,000,000. The maximum incubation period has not exceeded 1 week and may be as short as 48 hr with lower dilutions. Intraperitoneal injection of a dose of 1:1000 of a virus brain suspension uniformly produces paralysis within from 3 to 4 days. As early as 2 hr after introducing the virus into the peritoneal cavity it may be recovered from the brain and blood in both of which it persists until the terminal stage. Virus concentrations were highest in the brain and cord, with the adrenal next. Other organs (spleen and liver) carried smaller amounts of the infectious agent.

The virus fails to induce any symptoms in albino rats, guinea pigs and rabbits following repeated injections of large doses by a diversity of routes. It is easily transferable back to cotton rats, producing regularly paralysis and death in that species. Its pathogenicity for monkeys is questionable. Of 10 monkeys injected intracerebrally with either mouse or cotton rat virus 8 passed through a sharp fever cycle; only 5 animals developed weakness of the extremities and 2 others slight transitory facial paresis. In no case did the symptoms progress to typical spinal paralysis.

In the brain of paralyzed mice diffuse proliferation of glia cells and occasional foci of perivascular cuffing are observed. Severe damage occurs in the cord in both anterior horns, extending from loss of Nissl substance and irregular nuclear staining to a complete breakdown of the nerve cell with subsequent neuronophagia. Microglial proliferation is widespread at some levels as is perivascular infiltration.

Doses of virus ranging from 1:1,000,000 to 1:100 were tested for *in vitro* inactivation by the following sera: monovalent immune sera from monkeys convalescent from infection with RMV, Aycock, or SK virus, hyperimmune horse serum (anti-RMV), pooled human convalescent serum, normal serum from man, monkey and horse and antiviral immune sera against other neurotropic viruses (Theiler-mouse encephalitis, equine encephalomyelitis, rabies, St. Louis encephalitis, herpes). Normal animal and human sera, as well as the other antiviral immune sera, failed to bring about inactivation of the virus as its highest effective dilution (1:200,000); convalescent Aycock and SK monkey sera and convalescent human serum neutralized at slightly lower levels (1:100,000 to 1:50,000). Neutralization extending through a virus concentration of 1:1000 was obtained with the hyperimmune horse serum. RMV monkey convalescent serum failed to neutralize. The above data are based on results obtained in repeated tests. These immunological reactions are consistent with those of SK virus in monkeys, which is

neutralizable by SK and Aycock antiserum but not by RMV convalescent monkey serum.[5] A discrepancy exists regarding normal human serum which neutralizes SK virus in monkeys but not mouse virus in mice (3 sera tested).

Eight monkeys were immunized with a series of subcutaneous injections of live mouse virus and then tested for cross-immunity by intracerebral injection with 3 different strains of virulent monkey poliomyelitis virus. The results of this experiment were as follows: Three immunized monkeys, subsequently infected with the homologous virus, SK, remained free from paralysis of the extremities; none of the other 5 immunized monkeys, subsequently infected with the heterologous strains (Aycock and RMV), escaped the disease. An equal number of controls, infected with the same virus strains, developed typical poliomyelitis. Neutralization tests with monkey sera obtained at the end of immunization showed various titers of mouse virus neutralizing antibodies; in 6 instances neutralization was obtained in monkey tests against SK, Aycock, and RMV virus (3 with SK, 2 with Aycock, 1 with RMV).

In its 6th mouse passage the virus was cultivated in serum-ultrafiltrate tissue cultures (Sanders[6]) containing embryonic mouse or guinea pig brain or whole minced chick embryo by transferring every 3 days supernatant fluid or whole culture emulsion. The 6th serial passage of embryonic mouse brain cultures produced typical symptoms and death in mice following injection up to 1:10,000,000 dilution of the supernatant culture fluid. Similar passages of guinea pig brain cultures titrated up to 1:10,000. Only traces of virus were recovered from the chick embryo cultures.

[5] Trask, J. D., Paul, J. R., and Vignec, A. J., PROC. SOC. EXP. BIOL. AND MED., 1939, **41**, 241.

[6] Sanders, M., *J. Exp. Med.*, 1940, **71**, 113.