Phloroglucinol, 2-nitro- or 2-amino resorcinol and d,L-3,5-dihydroxyphenylalanine were inactive. Hydroquinone, m- and p-hydroxybenzoic acids yielded slight inhibition, while phenol, pyrogallol and m-methyl anisole had no activity. Catechol, a known substrate for the enzyme, produced dark gray spots of a melanin-like substance in concentrations as low as 1×10^{-5}.

The strongest inhibitor in a series of aromatic amines was m-aminophenol with an activity of 1×10^{-6}. p-Aminophenol was 20 times less active, whereas o-aminophenol was inactive. Of the 3 phenylenediamines only p-phenylenediamine proved to be a powerful enzyme inhibitor. Aniline, and several of its chlorinated derivatives, as well as toluidines showed little if any activity.

In order to compare quantitatively the activities of these new phenoloxidase poisons, a number of compounds previously known to be strong phenoloxidase inhibitors were examined. Mono substituted thioureas, such as the phenyl-, p-phenetyl-, and p-butoxy-phenyl-thioureas, showed activity ranges between 1×10^{-6} to 1×10^{-9}. Their efficacy was thus comparable to that of 4-chlororesorcinol. Definitely less potent was the most active representative of a group of 8-hydroxyquinolines. These compounds were effective in a dilution of 1×10^{-5}.

Discussion. Resorcinol derivatives probably substitute for the substrate or replace it to block the normal reaction. Support for this hypothesis is afforded 1) by the observation that the activity of 4-chlororesorcinol appears to be strongly dependent on the substrate concentration and 2) by the fact that 1,3-dihydroxybenzene derivatives are not likely to inactivate the enzyme by chelation with copper. Experiments are now in progress to analyze more specifically the possible competitive nature of the inhibitory mechanism of these compounds.

Summary. Using a previously described spot-spray technic, more than 400 simple, aromatic compounds were investigated as inhibitors of tyrosinase. 4-Chlororesorcinol inhibited melanin formation from tyrosine, DOPA or epinephrine at a concentration of 10^{-9}; it was thus 20,000 times more potent than resorcinol.

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Age and Susceptibility to Neurotropic Influenza Virus.* (21089)

SHEELAGH O'CONNOR AND ROBERT R. WAGNER.
From the Department of Internal Medicine, Yale University School of Medicine, New Haven, Conn.

Numerous reports have appeared in the literature on the relation between the age of a given host and its susceptibility to various infectious agents. Mice, guinea pigs, and rabbits have been among the experimental animals most commonly used in these investigations. In addition, chick embryos in various stages of development, newly hatched chicks and adult chickens have frequently been employed. Many viral agents have been studied in this connection, among them the viruses of rabies and pseudorabies, eastern

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TABLE I. Incidence of Encephalitis in Mice Following Intraperitoneal Injection of NWS Virus.

Age (days)*	1	2	3	4	5	6	7	8	9	10	11	15	16	
Mortality	8/8	10/10	6/6	8/8	7/7	9/9	10/10	8/8	23/23	8/8	9/9	6/6	5/5	
% survivors	0	0	0	0	0	0	0	0	0	0	0	0	0	
Mean day of death†	3.9	5.5	5.2	6.0	5.1	5.3	4.9	5.3	4.0	4.1	5.0	5.5	6.0	
Age (days)*	17	18	19	20	21	22	23	24	25	26	27	28	29	35
Mortality	7/7	6/7	1/7	3/6	55/94	4/7	0/6	0/6	1/6	3/7	1/7	3/5	0/12	3/10
% survivors	0	14	86	50	42	43	100	100	83	57	86	40	100	70
Mean day of death†	6.1	5.5	6.0	7.7	5.9	7.0	—	—	6.0	9.3	11.0	9.0	—	11.0

* At time of inoculation. † Excluding survivors.

and western equine encephalomyelitis, influenza A, lymphocytic choriomeningitis, vesicular stomatitis, poliomyelitis, rabbit pox, and yellow fever. The great majority of published reports indicates that resistance to experimental viral infection increases with increasing age of the host; the degree of susceptibility of the very young animal or extent of resistance of older animals varies somewhat with the type of host, the particular tropism of the virus and the route of inoculation. A few exceptions to this apparent pattern of decreasing susceptibility with increasing age are to be found in the literature. For example, Sabin(1) demonstrated that more Lansing poliomyelitis virus was required to produce infection in newborn mice than in adult mice, that the incubation period was prolonged in suckling mice, and the survival time after onset of paralysis was shorter in weaned mice than in suckling mice. The excellent review by Sigel(2) summarizes reports in the literature up to 1952 on the subject of age and susceptibility to infection.

The present experiments were designed to determine the relation between age and susceptibility of mice to a neurotropic strain of influenza virus (NWS) inoculated peripherally. Stuart-Harris(3) has found that this virus is pathogenic for mice when inoculated subcutaneously up to the 10th day of life, but that after this time the virus is no longer effective except after intracerebral injection.

Materials and methods. Virus. The Stuart-Harris neurotropic variant of WS influenza virus (NWS) was used in all experiments. This strain evolved from the non-neurotropic WS virus after passage in chick embryo allantois, chick embryo brain, and, finally, by serial intracerebral passage in weanling mice(4). It has been maintained in this laboratory by serial passage in 10-day-old embryonated eggs. The allantoic fluids were harvested 48 hours after inoculation and, after determination of hemagglutinin titers, were stored at −10°C until used. Allantoic fluids from egg passage numbers 8, 9, 12, 15, and 18, having hemagglutinin titers of 1:192 to 1:384, were used in these experiments. Undiluted fluid was used for all mouse inoculations, the dose being 0.05 cc for mice 11 days of age and younger, 0.1 cc for mice 15 days of age and older. *Mice.* Pregnant Swiss mice (Webster strain) were obtained from commercial breeders. Each mouse was kept in a separate box and the date and time of parturition were recorded. In a few instances postpartum mice were obtained and in these cases the date of birth of each litter had been noted by the breeder. *Determinations of virus content.* Brains of infected mice were removed aseptically, emulsified without abrasive and made up as a 10% suspension with sterile broth-saline solution containing 100 units of penicillin and 5 mg of streptomycin per cc. Three mice were killed and their brains pooled for each determination. Serial 10-fold dilutions were made and 4 eggs inoculated by the allantoic route with 0.1 cc of each dilution. After 48 hours incubation at 36.5°C the allantoic fluids were harvested and the presence of virus determined by the capacity of individual fluids to agglutinate an equal volume of 1% washed chicken erythrocytes. The egg-infective titers of the pooled brain emulsions were calculated according to the Reed-Muench method for determining 50% endpoints(5).

Two types of experiments were carried out. In the first, mice from one to 11, 15 to 29, and 35 days of age were inoculated intraperitoneally with NWS virus. These mice were observed for a period of 15 days and the onset

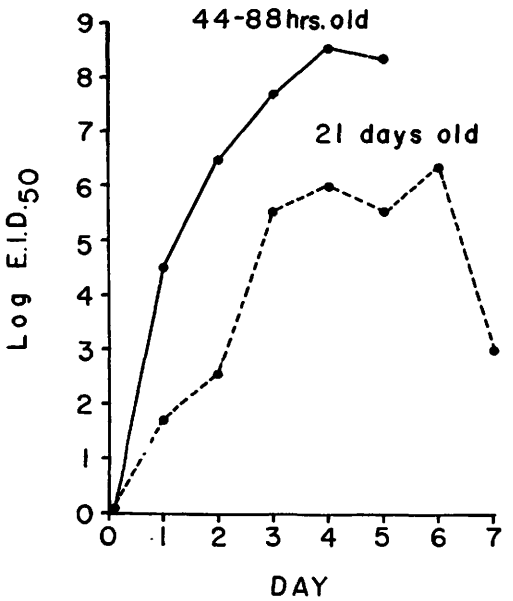

FIG. 1. Growth curves of NWS virus in infant and weanling mouse brain after intraperitoneal injection. Each point based on 3 animals; 0 day = 3½ hr after injection.

of paralysis and the day of death recorded. The other experiment was designed to compare the virus growth curves in the brains of infant and adult mice. Accordingly, 2 groups were inoculated with NWS virus (H.T. 1:256): in one group the mice were 44-88 hours old, and in the other 21 days old. Three mice from each group were killed 3½ hours after injection and an equal number at daily intervals thereafter. The experiment was continued for 7 days in the case of the 21-day-old animals; none of the infant mice survived beyond the 5th day.

Results. Table I shows the mean day of death at each age and the percentage of mice in each group that survived an intraperitoneal injection of virus. It can be seen that none of the 124 mice under 18 days of age at the time of inoculation survived the infection, whereas only 80 of the 180 mice between 18 and 35 days of age succumbed. Varying degrees of resistance were seen in the older age groups. There was no uniform increase in number of survivors with increasing age. Groups of animals between one and 17 days of age showed a mean day of death ranging between 3.9 and 6.1 days. In contrast to this, death occurred on an average of 5.5 to 11 days after injection of mice 18 days of age and older. All of the 24 mice in the 23-, 24-, and 29-day-old age groups survived.

A comparison of the daily virus titers in the brains of mice 44-88 hours and 21 days old is shown in Fig. 1. No virus was demonstrable in either group 3½ hours after injection (0 day). Subsequent to this, the virus titer of the brains of the younger age group rose more rapidly and to a higher level than that of the older animals. Each day the level of infectivity of infant mouse brain exceeded that of adult mouse brain by at least 10^2 EID_{50}; 48 hours after infection the difference was 10^4 EID_{50}.

Summary. In mice under 18 days of age, encephalitis and death invariably follow intraperitoneal injection of large doses of neurotropic-WS influenza virus. Older mice show varying degrees of resistance to infection. In general, decreased susceptibility is characterized by longer incubation periods and high proportion of survivors. The virus titer in infant mouse brain rises more rapidly and attains a higher level than in adult mouse brain. In addition, the degree of susceptibility may also be a function of the number of virus particles actually reaching the brain after intraperitoneal injection.

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