eosinophils determined. The calculated eosinopenic potencies were not quantitatively related to those reported in man. The possible implication of these data with respect to dosage selection of steroids for studies in dogs is briefly discussed.

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Inheritance of Resistance to Influenza Virus in Mice.* (29292)

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Natural resistance to certain bacterial infections in mice has usually been found to involve many factors and to have a complex mode of inheritance(1). In contrast, there are two viral infections the resistances to which depend on simple genetic mechanisms. Thus, a single dominant gene (or block of closely linked genes) is responsible for the resistance of PRI or BRVR mice to group B arboviruses(2,3). Similarly, resistance of C3H mice to mouse hepatitis virus is associated with one or two recessive genes(4).

The observation that inbred mice of the A2G strain were highly resistant to the lethal action of certain myxoviruses(5,6) prompted research into the genetic mechanism underlying this phenomenon. Crosses were arranged between resistant A2G and fully susceptible A or C3H mice. The results reported below suggest that a single dominant autosomal gene governs resistance to neurotropic influenza virus.

Materials and methods. Mice: A2G mice were obtained by inbreeding from a litter of A2G/EGA mice introduced in 1962 (see 6). A/Jax and C3H/HeJax mice were purchased from Jackson Memorial Laboratory, Bar Harbor, Maine; these mice will be designated A and C3H, respectively. ICR mice (noninbred) were purchased from Dublin Laboratories, Dublin, Va.

Virus: The Stuart-Harris strain of neurotropic influenza A virus, NWS, was used(7). A volume of 0.1×10^{-4} ml of mouse brain passage material was inoculated into the allantoic cavity of 10-day embryonated eggs. After 48 hours of incubation at 35°C a single allantoic fluid with an egg infectivity titer of $10^{7.5}$ 50% egg infective doses per ml was selected. Small aliquots of this virus stock were kept in sealed ampoules at —70°C and thawed only once for use. Virus dilutions

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were made in ice chilled phosphate-buffered saline. Titers of stock virus ampoules did not vary over 2 years.

Intracerebral inoculations: Mice were anesthetized with ether and 0.03 ml of appropriate virus dilutions were inoculated with a 27 gauge needle and tuberculin glass syringe through the skull into the left hemisphere. Mice were observed for at least 14 days after inoculations. Deaths occurring within the first 24 hours were disregarded.

Results. Previous observations had shown that inbred A and C3H mice were as susceptible to neurotropic influenza A virus, strain NWS, as non-inbred ICR Swiss mice, while A2G mice survived even the highest virus doses(5,6). A 10^{-2} dilution of stock NWS virus, representing 10^4 LD_{50} per intracerebral inoculation as measured with susceptible mice (A, C3H or ICR) was chosen as a suitable challenge dose for separating resistant from susceptible individuals. All challenge experiments described below were done with this amount of virus. Control groups of resistant A2G and susceptible ICR mice were run with each challenge experiment; A2G mice invariably survived, ICR mice always succumbed between the 4th and 7th day after inoculation.

Crosses between A2G and A mice: F_1 generation: Matings of 3 A2G males with 3 A females and of 3 A males with 3 A2G females yielded F_1 offspring of which 50 (24 males, 26 females) were challenged at the ages of 4 to 7 weeks. Only one F_1 mouse died, and this death occurred on the 9th day, 2 days after the last control ICR mouse had died. Deaths beyond the 8th day have so far never been observed in susceptible ICR or A mice. Since the F_1 mouse that died seemed particularly small, it was thought that maturation to resistance might be delayed in heterozygous mice. From previous experiments A2G mice were known to be susceptible to large virus doses when challenged during the first 24 hours of life; however, by the age of 7 days their resistance was fully established (unpublished). The speed at which resistance developed in F_1 mice from the cross A ♂ × A2G ♀ was studied in the experiment summarized in Table I.

TABLE I. Development of Resistance to Neurotropic Influenza A Virus in (A2G × A) F_1 Mice.

Type of mice	Age at challenge (days)	No. challenged*	No. dead	Avg time to death (days)
ICR	9	9	9	4.5
(A2G × A) F_1	9	12	10	12
"	14	15	6	9
"	19	10	1	9
A2G	9	11	0	—

* Intracerebral inoculation of a 10^{-2} dilution of stock NWS virus.

Thus at the age of 9 days, when A2G mice were already fully resistant, a majority of F_1 mice died, although considerably later than ICR mice of similar age. As a consequence, it was decided to test for resistance only after the 7th week of age. This was also thought to be a sufficiently long interval to avoid passive protection by maternal antiviral antibodies in those crosses where the mother had to be challenged prior to mating.

F_2 generation: Five unchallenged pairs of F_1 brothers and sisters were mated and their offspring were challenged at the age of 7 weeks. Both possible grandparental combinations were used. Of 176 mice tested, 53 proved susceptible (Table III). There were no significant differences between sexes or grandparental ancestries.

BC_1 generation: Male or female F_1 mice (challenged at 7 weeks) were mated with female or male A mice. Twelve mating couples were formed, 3 for each possible grandparental combination. The offspring, representing the first backcross generation (BC_1) were challenged at the age of 7 weeks. Of 132 mice tested, 65 proved susceptible (Table III). Again, sex or ancestry did not influence the result.

BC_2 generation: Male BC_1 mice which had survived viral challenge were mated with female A mice. Eight mating couples were formed, 2 for each great-grandparental combination. The offspring (BC_2) were challenged at the age of 7 weeks. Of 80 mice tested, 43 proved susceptible. Sex or ancestry had no effect.

BC_3 generation: Five BC_2 mice which had survived viral challenge were mated with 5 female A mice. The offspring (BC_2) were

TABLE II. Resistance to Neurotropic Influenza A Virus in (A2G × C3H) F_2 Mice.

Coat color	No. challenged	Resistant*	Susceptible
Wild	39	31	8
Cinnamon	9	6	3
White	20	15	5
Total	68	52	16

* Survived intracerebral challenge with 10^{-2} dilution of stock NWS virus for 14 days or longer.

challenged at the age of 7 weeks. Of 32 mice tested, 17 proved susceptible (Table III).

Crosses between A2G and C3H mice: F_1 generation: Five male A2G mice were mated with 10 C3H females. All 50 F_1 mice tested survived challenge.

F_2 generation: F_2 mice were obtained from crosses between unchallenged F_1. Segregation into 3 different coat colors was observed: Wild (like C3H or F_1), cinnamon and white. No black or chocolate-brown mice occurred, indicating that A2G mice carry the agouti allele. Table II summarizes the results. There were no significant differences between sexes.

BC_1 generation: Five unchallenged F_1 males were mated with 5 C3H females. The offspring (BC_1) were challenged at the age of 7 weeks. Of 37 mice tested, 21 proved susceptible (Table III). There were no significant differences between sexes.

Discussion. The results of all crosses are summarized in Table III. Assuming that one dominant allele (for which A2G mice are homozygous) governs resistance to neurotropic influenza A virus, the following percentages of susceptibility should be obtained in the various crosses: 0% in F_1, 25% in F_2 and 50% in each backcross. The numbers observed are close to those calculated under this simple hypothesis, as shown by reasonable χ^2 values (Table III). The character is not sex-linked. From the small numbers of (A2G × C3H)F_2 mice studied, the gene for virus resistance does not seem to be closely linked with either the gene for albinism (*c*, chromosome I) or coat color (*b*, chromosome VIII).

It is of course possible that resistance to influenza virus is just another expression of one of the many mouse alleles already known. Pending such identification, I wish to suggest the symbol *Mx* (myxovirus-resistant) to designate the dominant allele involved. In the experiments reported above neurotropic influenza A virus was used exclusively; challenge with other myxoviruses to which the A2G strain is resistant(6) has yet to be done.

Mice of strain A, from which strain A2G was originally derived(6), and mice of strain A2G differ in 2 out of 4 genes tested. Using the symbol suggested above, the genetic formulae could be written *aabbcc*++ for A and *AAbbccMxMx* for A2G.

Summary. F_1, F_2, first, second, and third

TABLE III. Resistance to Neurotropic Influenza A Virus in A2G, A and C3H Mice and Their Offspring.

Type of mice	No. challenged	Resistant*	Susceptible	Expected No. susceptibles†	χ^2‡	P
A2G	50§	50	0	0		
A	50§	0	50	50		
C3H	50§	0	50	50		
F_1 (A2G, A)	50	50‖	0‖	0		
F_1 (A2G, C3H)	50	50	0	0		
F_2 (A2G, A)	176	123	53	44	2.455	.1–.3
F_2 (A2G, C3H)	68	52	16	17	.078	.7–.9
BC_1 (A2G, A)	132	67	65	66	.030	.7–.9
BC_1 (A2G, C3H)	37	16	21	18.5	.676	.3–.5
BC_2 (A2G, A)	80	37	43	40	.450	.5
BC_3 (A2G, A)	32	15	17	16	.125	.7–.9

* Survived intracerebral challenge with 10^{-2} dilution of stock NWS virus for 14 days.
† Calculated on the assumption that one dominant allele is responsible for resistance.
‡ One degree of freedom.
§ Many more mice were challenged in other experiments with the same results.
‖ One mouse died on the 9th day after challenge. See text.

backcross generation mice resulting from crosses of A2G with A, and F_1, F_2 and first backcross generation mice resulting from crosses of A2G with C3H were challenged with neurotropic influenza A virus, strain NWS. The results were compatible with the hypothesis that a single dominant autosomal allele was responsible for resistance to the virus. The symbol *Mx* (myxovirus-resistant) is tentatively proposed for the allele involved.

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Photosensitization of Human Cell Cultures by Demethylchlortetracycline. (29293)

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Photosensitization following exposure to the sun when patients are being treated with demethylchlortetracycline (Declomycin) is well documented(1,2). Other tetracycline compounds produce this effect only rarely (3,4,5). No explanation for the differences in capacity of the different members of this group of antibiotics to produce this reaction is available, possibly because of lack of a laboratory method suitable for quantitative study of this problem. The present paper concerns a procedure applicable to the investigation of photosensitization and presents data which suggest a basis for the difference in activity of the various tetracyclines in inducing development of this phenomenon.

Materials and methods. Freshly-obtained human amnion was exposed to 0.5% trypsin dissolved in Hanks' balanced salt mixture for 2.5 hours. The cells were then washed and suspended in Eagle's medium to which horse serum (15%) was added. To aliquots containing 500,000 cells per ml, demethylchlortetracyline (DMCT), methacycline (MC), 6-methylene-5-hydroxytetracycline (supplied by Charles Pfizer & Co., Inc., New York) and tetracycline (TC) were added in concentrations ranging from 0.4 to 25 μg per ml. After incubation at room temperature for 18 hours, the treated cell suspensions were exposed to long and short UV light in the following manner: A 15-watt UV lamp (General Electric Co.) mounted in a box lined with aluminum foil was the source of the short wave light which was almost normochromic and had a peak wave length of 2537Å. A Woods light (Burton Mfg. Co., Los Angeles, Calif.) with a range of 3660Å to 3900Å was used to furnish the long wave UV. The cells being studied were placed in open Petri dishes at a distance of 20 inches from the light source and shaken mechanically at a rate of 140 per minute. The duration of exposure to short UV light was 10 seconds and to the long waves 30 and 120 seconds. At the end of these periods, the cells were placed in test tubes and plastic tissue culture flasks and incubated at 37° in a stationary position for 48 hours. At this point, the fluid was removed from all of the cultures and replaced with tetracycline-free Eagle's medium containing 100 units of penicillin and 50 μg of streytomycin per ml and 10% horse serum; these preparations were incubated at 37°C for 5 days when they were examined microscopically and photographed. Each experiment included a group of controls which were treated in a manner identical to the experimental cultures except that the cell suspensions were not exposed