FIG. 1. Sunflower plant on left was sprayed once with a solution of 0.1 mg/ml A-methopterin. It has not died, but its growth has been almost completely inhibited. Plant on right was sprayed with water only.

μg/litre of the active compounds. 4-Amino-P.G.A., (Aminopterin), 4-aminopteroylasparatic acid (Aminoanfol), and 4-amino-α-glutamyl-α-glutamyl glutamic acid (Amino-teropterin) exerted an inhibitory action about one-tenth as powerful as that of the first 3 compounds. No reversal of the inhibition could be obtained by the addition of pteroylglutamic acid to the medium containing A-methopterin. Pteroylglutamic acid itself inhibited the growth of these tissues at concentrations above 10 mg/litre.

A solution of A-methopterin (0.1 mg/ml) applied locally to young crown-gall tumors on sunflower plants completely inhibited growth of the tumors without damaging the plant, provided the tumors were located on mature tissue. Serious damage resulted when this substance was applied at this concentration to tumors on young, growing stems. Sunflower plants sprayed with this concentration of A-methopterin grew at a greatly diminished rate (Fig. 1) but did not die. Onion roots grown in 0.1 mg/litre A-methopterin were devoid of mitoses during the first 24 hr after exposure to this compound. After 48 hr mitoses were again observed. It seemed probable that these compounds acted by interfering with cell division.

The nitrogen mustard, bis-ß-chloroethylmethyl amine, totally inhibited the growth of excised tomato roots exposed for 1 hr to a concentration of 1 mg/litre. Tumor and embryo tissue of sunflower was less affected by this agent. The growth-inhibitory action of guanazolo was detectable at concentrations of 1 mg/litre in all 3 tissues. Cortisone strongly inhibited the growth of bacteria-free crown-gall tissue of sunflower at a concentration of 1 mg/ml. The tissue fragments recovered and grew at their normal rate as soon as the cortisone was removed.

None of the substances tested exerted a specific inhibitory effect on tumor as opposed to healthy tissue. Tissue of excised tomato roots proved more sensitive to the inhibitory action of these agents than did the other tissues tested. It would appear that all these materials, with the possible exception of cortisone, act by interfering with the process of cell division, but that they affect tumor and healthy cells equally.

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An Antigenically Distinct Subtype of Influenza Virus A Which is Virulent for Mice in Primary Passage of Allantoic Fluid[1]

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Influenza virus, when introduced nasally in mice, following primary growth in the chick embryo, usually produces no clinical disease or histological change. The virus has been shown to multiply in the lungs of these apparently normal mice (*2*, *6*). After several passages in mice, they are killed by lung suspensions containing virus in high dilution.

Kalter (*3*) recovered one strain of influenza virus in 1947 which was strikingly different from another influenza virus isolated at the same time, in that it was invariably fatal for white mice in 3–5 days. The lungs showed complete consolidation, typical of influenza virus infection. There was no explanation for this behavior, but Kalter stated that the possibility of a toxic substance had not been eliminated.

Payzin and Okkan (*5*) recovered 3 influenza virus strains directly from cases in mice which occurred during an epidemic in Ankara in 1949. The strains attained high mouse virulence after 5 serial passages in mice. The

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TABLE 1

ANTIGENIC RELATIONSHIP BETWEEN THE 5 NEW STRAINS AND OTHER A OR A′ STRAINS AS DETERMINED BY SERUM-VIRUS NEUTRALIZATION TESTS IN MICE

	Influenza Viruses										
	Baltimore strains						Other A or A′ strains				
Sera	Thompson	Icenroad	Morton	Hume	Vincentes	Hyde	FM1	WS	15 Swine	Weiss	PR8
Thompson	625–3,125	3,125	625	625	3,125	3,125	< 5	< 5	< 5	25–125	< 5–25*
Icenroad	625	3,125	3,125	625	3,125	3,125	< 5	< 5	< 5	125	5–25*
Morton	625	3,125	625	625	3,125	3,125	< 5	< 5	< 5	25–625*	5–25*
Hume	625–3,125	3.125	625	625–3,125	3,125	3,125	< 5–5	< 5	< 5	125–625*	5–25*
Vincentes	3,125	3,125	3,125	3,125	3,125	3,125	< 5	< 5	< 5	125–625*	5–25*
Hyde	625	3,125	625	625	3,125	3,125	< 5	5	< 5	25–125*	25
FM1	< 5	< 5	< 5	< 5	< 5	< 5	125–625	< 5	< 5		< 5
WS	< 5		< 5	< 5		< 5	5–25*	3,125	< 5	3,125	3,125
15 Swine	< 5	< 5	< 5	< 5	< 5	< 5	< 25	< 5	3,125		25
Weiss	25	25	< 5	5	5–625*	5–625*	5–125*	25–625*	< 5	3,125	625
PR8	< 5	25	< 5	5	5–125	5–125	< 5	625	< 5	625	3,125

* The highest dilution of antigen used was 1 : 3,125. End points not clear-cut.

titers were not given, but a report from Dr. Andrewes in London is quoted to the effect that they differed serologically from all the "classical strains."

The present study indicates that there are influenza virus strains which behave as laboratory mouse-adapted strains on first passage from eggs to mice. Five strains from among 35 influenza virus strains routinely isolated in the Baltimore area during the years 1946, 1947, and 1948 differed from the currently prevalent A′ strains in that they were highly pneumotropic for mice. They constituted an antigenically distinct group and differed from other A and A′ strains, with the exception of one isolated in 1935.

Two of the 5 strains were isolated from the lungs of fatal cases of pneumonia in which pneumococci were also present (*4*). One case occurred in May, 1946, and the other in February, 1947. The remaining 3 strains were recovered in March, 1947, from throat washings of influenza patients. An influenza epidemic was occurring in Baltimore at this time, and the predominating strains recovered were A′.

The 35 influenza virus strains were isolated by amniotic inoculation of embryonated eggs. Subsequently, they were maintained in the laboratory by allantoic passage. Eleven-day-old embryonated eggs were inoculated into the allantoic sac with 0.1–0.2 ml of 10^{-2}–10^{-5} dilutions of infected allantoic fluid in broth. The eggs were incubated 48 hr, and the fluid was harvested in the usual way. The fluid from each egg was tested for hemagglutination, using equal vol of 3 serial tenfold virus dilutions and 0.25% washed chicken erythrocytes. Pools were made of fluids which showed partial or complete hemagglutination in the last and highest virus dilutions. The pooled virus was cultured on blood agar and a semisolid meat infusion medium to insure freedom from bacterial contamination.

The pooled bacteria-free allantoic fluid containing virus was used to infect and also to immunize mice. Mice were infected nasally with 0.05 ml of each of several dilutions of virus-infected allantoic fluid. After 3 days some of the living mice were sacrificed. The lungs were removed aseptically, weighed, ground with alundum, and diluted with *M*/10 phosphate buffer pH 7.0 to make a 10^{-1} dilution. Further tenfold dilutions were made in meat infusion broth of pH 7.4–7.6. Titrations were then carried out by inoculating groups of mice nasally with 0.05 ml of the different dilutions of mouse lung virus. These mice were observed for 10 days, and deaths were recorded.

One egg-adapted strain in allantoic fluid killed mice in a 10^{-4} dilution, 2 strains in a 10^{-3} dilution, and 1 in a 10^{-1} dilution, the highest dilution used. Titrations of the first and second mouse passage virus material killed mice in dilutions ranging from $10^{-4.78}$–$10^{-5.62}$. One strain, Icenroad, was not tested until it had been passed through several mice. This gave a titer of $10^{-5.68}$ when tested.

Mice were immunized intraperitoneally with graded doses of live virus, and neutralization tests with their sera were carried out according to a method previously described (*1*). The results are given in Table 1. The figures express the highest dilution of antigen which produced serum capable of neutralizing the virus in a final dilution of 1: 4.

The 5 strains studied were similar and resembled the Hyde strain. This strain was isolated from nasal washings from a case of influenza in Alaska in 1935. Little or no immunological reactions were given with the FM1, 15 Swine, or WS strains, while varying cross-immunological reactions were found with the PR8 and Weiss strains when live antigens were used.

Lee influenza virus B did not cross immunologically with Thompson, Icenroad, Hume, or the Hyde strains by the methods employed.

Commercial influenza virus vaccines prepared by different methods and showing satisfactory potency with respect to the PR8 and Weiss or PR8 and FM1 strains afforded little or no protection against either the Hyde strain or the 5 new strains.

A more complete presentation of the data in this paper is in preparation. Further studies are necessary to determine the full meaning of the discovery of these antigenically different influenza virus strains at a time when the predominating influenza virus strains were A′.

The report is intended to emphasize the importance of

studying new influenza virus strains in mice, as well as in embryonated eggs. The observation that influenza virus strains may be pathogenic for mice without "blind" passage suggests that pathogenicity or lack of pathogenicity may be a characteristic of specific groups of influenza viruses with other biological properties in common.

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On the Validity of an Assumption of Resonance Theory[1]

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This discussion is concerned with an assumption of resonance theory which validates its application to the consideration of organic molecules. For the present purpose the resonance theory approach to organic molecules may be formulated as follows: There are some organic molecules whose properties cannot be satisfactorily described in terms of the structural formulas in current use. In such cases the properties of the molecules may be understood by considering the actual structure of the molecule to be derived in a dynamic manner from the several structures which may be represented in terms of the classical symbols. By the structural formulas in current use is meant the representation of carbon-to-carbon bonds as single, double, and triple bonds.

If this formulation of the resonance approach be accepted, then the following assumption is necessary for the application of resonance theory. Carbon-to-carbon bond distances not corresponding to the values represented conventionally by single, double, and triple bonds are distributed continuously throughout the remainder of the range of possible bond distances. The necessity of this assumption may be justified by the following argument. If the distribution of the bond distances is grouped rather than continuous in the intervening regions, then the resonance method must be considered to be logically unsatisfactory since (1) it then ignores an inherent order in the physical data it considered, and (2) it logically tends to the consideration of each particular molecule as a unique case.

The validity of the assumption as to the continuous distribution of bond distance values can be determined by an examination of existing data. Two questions must be considered:

1. Do the measured values of carbon-to-carbon bond distances appear to form a continuous or a grouped distribution?

2. If the distribution of these values appears to be grouped, do the members of each group bear a generic or analogous relation to each other with respect to other known properties?

Table 1 shows the frequency distribution of nominal carbon-to-carbon bond distances. The values were taken from the collection of Wheland (*2*). The areas corresponding to single, double, and triple bonds are quite well defined. In the region between the single and double bond there are apparently two additional groups of values which are designated along with the groups corresponding to the single, double, and triple bonds by Roman numerals. This readily apparent grouping provides the answer to the first question. Carbon-to-carbon bond distances in organic molecules are apparently not continuously distributed.

TABLE 1

DISTRIBUTION OF CARBON-TO-CARBON BOND DISTANCE

Distance in A	No. of cases	Bond type	Group
1.60	x		
.59	xx		
.58	xx		
.57	xx		
.56	x		
1.55	x		
.54	xxxxx xxxxx xxxxx xxx	Single	I
.53	xxxxx		
.52	xxxxx x		
.51	xx		
1.50	xxxx		
.49	xx		
.48	xxxx		
.47	xxxxx xxxx		II
.46	xxxx		
1.45	x		
.44	xxx		
.43			
.42	xxxxx xxxxx		
.41	xxxxx		
1.40	xxxxx		III
.39	xxxxx xxxxx xxxxx		
.38	x		
.37	x		
.36	x		
1.35	xxx		
.34	xxx	Double	IV
.33	xxx		
.32	x		
.31			
1.30			
.29	x		
.21	x		
1.20	xxx	Triple	V
.19	xx		
.18	x		

It is now necessary to consider the second question as to the interrelationship of the members of groups II and III. Group III may be readily recognized as being made up primarily of the carbon-to-carbon bond distances associated with aromatic ring systems and may thus be said to rep-

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