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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resent a homogeneous group of compounds possessing not only similar bond distances but similar chemical properties as well. Group II is made up predominantly of carbon-to-carbon bonds usually represented as single bonds which occur as part of a conjugated nonaromatic system; for example, the central bond in butadiene, the central carbon-carbon bond in biacetyl, and so forth. In addition, the "single bond" adjacent to an acetylenic linkage in compounds such as methyl acetylene appears in this group. Thus the bonds in Group II form a reasonably homogeneous class with respect to chemical properties as well as to bond distance. The several values falling above 1.56 A are principally derived from measurements of oxalic acid and its salts and may be considered an anomalous group corresponding to a unique chemical composition.

Thus the assumption that has been formulated is not justified by existing data, and the resonance treatment of organic molecules is to this extent logically unsatisfactory. This statement, of course, does not maintain that resonance theory cannot furnish an extremely valuable method for the understanding of organic chemistry, but it raises the possibility of an alternative approach to organic chemical phenomena based on a symbolic system more in accord with physical data. One proposal in this direction has been made by the author (*1*).

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The Paper Chromatography of pH Indicators

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Many types of organic and inorganic substances have already been separated and identified in mixtures by paper chromatography since the separation of amino acids was first described by Consden, Gordon, and Martin (*1*).

During the development of the paper chromatography of the lower fatty acids in the rumen content and blood of ruminants (this will be described separately), it was found of importance to measure the R_F values of the usual pH indicators so as to be able to select a suitable indicator for the determination of the total acids present.

Later, also, the spots of the acids on the chromatogram were shown up by spraying with indicators (*2*), and for quantitative determination by the measurement of spot size it was found best to include the indicator in the solvent so as to get an even concentration on the paper. For this, too, the R_F value of the indicator is of importance, since the indicator should go ahead of the acids to be determined.

There appears to be no reference in the literature that the pH indicators were previously examined by paper chromatography.

The indicators were chromatographed in isopropyl alcohol, butyl alcohol, and amyl alcohol, all containing ammonia, and it was found that many usual mixtures, such as universal indicator, can be readily separated. Also, with only minute quantities of the indicator, it is possible in most cases to determine the identity of an unknown indicator. As additional criterion for an unknown indicator, the color of the spot with acids or alkalies can be observed. Phenolphthalein and thymolphthalein both stay in the colorless form and have to be shown up by spraying the paper with aqueous NaOH solution.

The observation of the colored substances during development may be of theoretical interest, since spot area changes can be studied visually or photographically.

The solvents used were prepared as follows:

Ninety ml of isopropyl alcohol was mixed with 10 ml of 5N NH_4OH; 100 ml of butyl alcohol was shaken with 100 ml of aqueous 1·5N NH_4OH, and the top layer used as solvent; 100 ml of amyl alcohol was shaken with 100 ml 1·5N NH_4OH, and again the clear top layer was used as the solvent.

The development technique as previously (*3*) is that of Williams and Kirby (*4*), but instead of 5-gal crocks, glass battery jars with fitted glass lids were employed. The lower layers in the case of butyl acid amyl alcohols were placed in beakers and stood on the bottom of the jar, and the top layer was poured on the floor of the jar.

The paper used was Whatman's No. 2, and the indicators were dissolved in ethyl alcohol; spots were placed on the paper and dried before development.

Table 1 gives the R_F values of 16 indicators examined. Isopropyl alcohol is not a suitable solvent, since most indicators travel too fast.

The general trend there appears to be that the R_F

TABLE 1

Indicator	R_F values		
	Isopropyl alcohol	Butyl alcohol	Amyl alcohol
Congo red		0.0	0.0
Indigo carmine	0.0	.0	.0
Chlorphenol red		.17	.01
Phenol red		.18	.01
Cresol red		.41	.12
Brom cresol purple	.68	.43	.10
Brom cresol green	.84	.47	.24
Brom phenol blue		.55	.19
Methyl orange	.77	.55	.26
Methyl red	.73	.59	.33
Neutral red		.66	.53
Brom thymol blue	0.93	.79	.63
Methyl violet	.95	.88	.86
Thymol blue	1.0	.90	.75
Phenol phthalein	1.0	.92	.89
Thymolphthalein	1.0	0.92	0.92

[1] Thanks are due to A. Bryson, of the Sydney Technical College, for gifts of indicators, and to R. L. Reid for criticism and advice.