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

Michael Lederer[1]

Science Department, Newcastle Technical College, Newcastle, New South Wales, Australia

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.

values decrease with an increase in the number of C atoms in the alcohol, but the R_F difference is by no means constant.

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Oxygen Uptake of Embryonated Eggs Infected with Western Equine Encephalitis Virus[1]

R. A. Siem, B. C. Smith, and Wm. F. McLimans[2]

Department of Bacteriology and Immunology, University of Minnesota Medical School, Minneapolis

During the course of an investigation designed to ascertain the effect of virus proliferation on the over-all gaseous exchange of the embryonate egg, it was observed that Newcastle's disease virus (NJ-KD) did not appear to stimulate or suppress the oxygen uptake until near the terminal stage of the experimental infection (*1*, *2*). At that time, approximately 40 hr in eggs inoculated with 1,000 MLD, a rapid reduction in the $Q^{e}_{O_2}$ (ml oxygen consumed/egg/hr) to the residual level indicative of infertile eggs occurred. Other investigators, using a group or pool technique, wherein the rates of oxygen consumption were determined not on individual eggs but rather on several in a pool, have reported a primary or initial stimulation in the oxygen uptake shortly after inoculation in the case of *Rickettsia prowazekii mooseri* (*4*) and influenza virus infections (*3*). Further experimental evidence, utilizing other viral agents, was deemed necessary in order to attempt to clarify this question; accordingly, this paper reports the results of a study of the oxygen uptake of individual embryonated eggs infected with Western equine encephalitis virus (WEE).

The reported experiments were conducted with the Olitsky strain of WEE.[3] The strain was initiated by inoculation into 10-day-old eggs and passed twice before use. It titered $10^{-7.5}$ on intracranial inoculation of 0.03 ml into Swiss mice. A 10^{-3} dilution of WEE-infected chick embryo tissue was chosen for the experiments reported here. Normal, uninfected chick embryos of the same age were likewise prepared in a saline suspension (10^{-3}). This embryo suspension served as a control on the possible effects of normal embryo proteins and metabolites on the $Q^{e}_{O_2}$ of the host system—embryonated egg.

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[2] Present address: Research Division, The Upjohn Company, Kalamazoo, Mich.

[3] Secured through the courtesy of Charles Armstrong, of the National Institutes of Health.

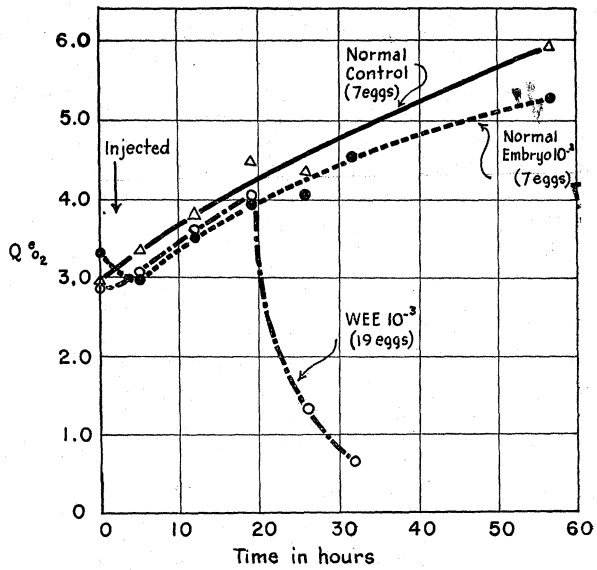

FIG. 1. Mean rates of oxygen consumption of embryonated eggs injected with WEE virus (○), normal chick embryo (●), and of normal control eggs (△).

A number of 10-day-old, trap-nested fertile eggs were divided into 3 lots. One group of 7 eggs served as a control and was not injected. This group provided a reference point against the normal mean $Q^{e}_{O_2}$ as previously established (*1*). A second control group consisted of 7 eggs injected with a 0.1 ml of a 10^{-3} suspension of normal embryo tissue. This control group, as indicated, was used to determine the effect of normal embryo constituents on the metabolic rate of the embryonated egg. A third test group was initiated by injecting 19 eggs with 0.1 ml (10^{-3}) of a chick embryo saline suspension of WEE. The eggs were injected at 4: 00 P.M. of the tenth day of incubation (37.8° C), and the rates of oxygen utilization determined at frequent intervals thereafter until all infected eggs had died, as indicated by their very low rates of respiration. All infected eggs died by the thirty-first hour. The rates of oxygen utilization of 6 eggs of the infected group were determined prior to injection. The postinoculation determinations were run at 3-, 10-, 17-, 24-, and 31-hr intervals. A final reference determination was made on both control groups 55 hr after inoculation.

It was observed that the rates of oxygen utilization of the eggs infected with WEE did not differ significantly from the normal or control series until after the seventeenth hour (Fig. 1). Thereafter, a rapid decline in the $Q^{e}_{O_2}$ of these eggs became apparent. There appeared to be no stimulation of the metabolism of the infected eggs prior to the terminal stage of the infection. This was our experience in every instance on utilizing the described virus. On the other hand, the injection of a normal embryo suspension appeared to suppress slightly the $Q^{e}_{O_2}$. Although this depressed $Q^{e}_{O_2}$ is not valid statistically, it does occur with remarkable regularity.

Data have been presented demonstrating the effect of growth and proliferation of WEE virus on the rates of