DBH in the pinealectomized group as compared with that in the unoperated control, the difference between pinealectomized and sham groups was not statistically significant in this second experiment with the lights left on.

*Discussion.* LEW and QUAY[8] have shown that adrenal norepinephrine (NE) content, in the same rat strain ($S_1$), is highest at middark, which is consistent with our early night time tise in DBH activity. However our results for midday DBH activity level do not parallel the high midday adrenal NE content observed by the above authors. Nor do they explain the findings of DUNN and LIN[9] who reported that adrenomedullary NE content is lowest during night when epinephrine (E) store is very high[9,10]. It might be noted, however, that there are at least several reasons why one should not expect necessarily close correlations in time between levels of adrenomedullary DBH activity and of NE and E: 1. Adrenomedullary NE, in addition to being the precursor of E, is also secreted as an independent hormone. 2. NE and E in the medulla are available interchangeably in storage-vesicles in bound form and in the cytoplasmic sap in a free pool. 3. They undergo a continuous and concomitant catabolic process of oxidative deamination (intra-vesicularly) and *O*-methylation (free cytoplasmic)[11].

Our results suggest that the nocturnal rise in adrenal DBH activity is both pineal- and darkness-dependent (Figure). However, we can not yet rule out the possibility that in the pinealectomized animal a phase shift in DBH rise occurred rather than necessarily an abolishing of the DBH periodicity. Although an effect of pinealectomy on an adrenomedullary activity or function has not been presented previously, physiological interrelations of the rat pineal gland and catecholaminergic and stress-related systems have long been suggested by other kinds of evidence[12,13].

[8] G. M. LEW and W. B. QUAY, Comp. gen. Pharmac. *4*, 375 (1973).
[9] J. D. DUNN and F. LIN, Experientia *30*, 348 (1974).
[10] L. E. SCHEVING, W. H. HARRISON and J. E. PAULY, Am. J. Physiol. *215*, 799 (1968).
[11] R. YAMADA, in *Neuroendocrine Controls* (Eds. K. YAGI and S. YOSHIDA; John Wiley and Sons, New York 1973), p. 137.
[12] W. B. QUAY, Am. J. Physiol. *196*, 951 (1959).
[13] W. B. QUAY, *Pineal Chemistry in Cellular and Physiological Mechanisms* (Charles C. Thomas Publisher, Springfield, Illinois 1974).

## THEORIA

# Molecular Superdelocalizability. A Correlation with Diamagnetic Susceptibility

K. S. ROGERS and A. CAMMARATA[1]

*Department of Biochemistry, Medical College of Virginia, Virginia Commonwealth University, Richmond (Virginia 23298, USA), and Laboratory of Physical Medicinal Chemistry, School of Pharmacy, Temple University, Philadelphia (Pennsylvania 19140, USA), 28 August 1975.*

*Summary.* Diamagnetic susceptibilities of 44 divers aromatic molecules were successfully predicted from molecular superdelocalizabilities calculated from Hückel molecular orbital theory.

In the past, diamagnetic susceptibilities for organic compounds were estimated through a set of PASCAL constants[2–5] which permitted the calculation of a molar susceptibility on an additive basis (a given contribution for each species of atom) provided that appropriate constitutive corrections were made. A more recent approach assumed that a molar susceptibility may be written as a sum of bond contributions and of correction terms that represented interactions between adjacent bonds[6–10]. Efforts to prove the validity of each of these methods from molecular orbital theory have been made[6–8] and on this basis an argument has been presented in favor of the bond over the atom contribution approach[6,9,10]. These additive procedure are fundamentally empirical despite their theoretical rationales. Thus it should not matter in a practical application which scheme is followed so long as the empirically derived contributions and associated corrections are used in a consistent manner.

We prefer an alternative correlative approach in relating diamagnetic susceptibilities to values calculated from indices obtained from Hückel molecular orbital theory[11]. Attempts to correlate experimentally determined diamagnetic susceptibilities by linear multiple regression analysis with molecular orbital indices: energy of highest

Table I. HMO parameters found suitable for correlations with diamagnetic susceptibility

| Atom | $h$ | Bond | $k$ |
|---|---|---|---|
| C | 0.0 | $C_{ar}$-$C_{ar}$ | 1.0 |
| C(≡N) | 0.0 | $C_{ar}$-$C_{al}$ | 0.9 |
| $\dot{C}H_3$ | 2.0 | $C_{ar}$-C(≡N) | 0.9 |
| $\dot{O}$ | 1.0 | $C_{ar}$-$CH_3$ | 0.7 |
| $\ddot{O}$ | 3.0 | C≡N | 1.2 |
| O(nitro) | 2.0 | C—$\dot{O}$ | 1.0 |
| $\dot{N}$ | 0.4 | C—$\ddot{O}$ | 1.0 |
| $\ddot{N}$ | 2.0 | C—$\dot{N}$ | 1.0 |
| N(≡C) | 0.4 | C—$\ddot{N}$ | 0.7 |
| N(nitro) | 1.0 | C—N(nitro) | 3.0 |
| F | 3.0 | C—$\ddot{S}$ | 1.0 |
| Cl | 1.1 | C—F | 0.7 |
| Br | 0.8 | C—Cl | 0.4 |
| I | 0.5 | C—Br | 0.4 |
| $\ddot{S}$ | 1.5 | C—I | 0.3 |
| | | N—O(nitro) | 0.2 |

[1] Laboratory of Physical Medicinal Chemistry, School of Pharmacy, Temple University, Philadelphia, Pennsylvania 19140, USA.
[2] P. PASCAL, Annls Chim. Phys. *19*, 5 (1910).
[3] P. PASCAL, Annls Chim. Phys. *25*, 289 (1912).
[4] P. PASCAL, Annls Chim. Phys. *29*, 218 (1913).
[5] A. PACAULT, Rev. Sci. *86*, 38 (1948).
[6] P. S. O'SULLIVAN and H. F. HAMEKA, J. Am. chem. Soc. *92*, 25 (1970).
[7] J. A. POPLE, J. chem. Phys. *37*, 53 and 60 (1962).
[8] P. S. O'SULLIVAN and H. F. HAMEKA, J. Am. chem. Soc. *92*, 1821 (1970).
[9] H. F. HAMEKA, J. chem. Phys. *34*, 1996 (1961).
[10] H. F. HAMEKA, J. chem. Phys. *37*, 3008 (1962).
[11] A. STREITWIESER, JR., *Molecular Orbital Theory for Organic Chemists* (John Wiley and Sons, Inc., New York 1961), p. 52.

occupied molecular orbital (HOMO); energy of lowest empty molecular orbital (LEMO); total $\pi$ energy; atom charge density; atom electrophilic or nucleophilic superdelocalizability; indicated that only electrophilic superdelocalizability may be related to diamagnetic suceptibility.

*Methods.* Experimental diamagnetic susceptibilities for 44 divers aromatic compounds, hydrocarbons, heterocyclics, substituted benzenes, etc., were obtained from reference[12]. The matrix equations[11] for Hückel molecular orbital treatment of the aryl $\pi$-electron framework were solved with the use of an IBM 1130 computer (Courtesy of Department of Biometry, Med. Coll. Va.) Coulomb ($h$) and bond ($k$) integrals used in our calculations are presented in Table I. These values were slightly adjusted from those of STREITWEISER[11] because 'in more quantitative specific correlations the values should be adjusted for the best individual fit'. Methyl substituents were treated as heteroatoms. Electrophilic superdelocalizability ($S^E$)values for the aryl atoms were calculated from the appropriate equation also given by STREITWEISER[11].

*Results.* Table II shows the molar diamagnetic susceptibilities ($-X_M$) recorded for 44 aromatic compounds and the molecular electrophilic superdelocalizabilities ($\Sigma S_E$) calculated for the respective compounds from simple HMO theory. $\Sigma S^E$ represents the addition of individual $\pi$-electron atoms' superdelocalizabilities to give the molecular value, eg. $\Sigma S^E$ of benezene (Table II) = 0.833 + 0.833 + 0.833 + 0.833 + 0.833 + 0.833 = 4.988. Equation (1) describes the linear relationship between the molar diamagnetic susceptibility and the magnitude of molecular electrophilic superdelocalizability for the varied aromatic molecules in Table II.

$$-X_M\,(\times 10^6) = 10.00\,\Sigma S^E + 0.28 \qquad (1)$$
$$(r = 0.998\,;\, n = 44\,;\, \mathrm{SE} = 1.87)$$

Comparison of the statistical values[13], $r$ the correlation coefficient for fit of experimental points to the equation and SE, standard error of the diamagnetic susceptibility

Table II. Molecular superdelocalizabilities and molar diamagnetic susceptibilities

| Compound | $\Sigma S^E$ | $-X_M\,(\times 10^6)$obs. | $-X_M\,(\times 10^6)$calc. | Ratio: $\frac{-X_M \text{ calc.}}{-X_M \text{ obs.}}$ [a] |
|---|---|---|---|---|
| Aniline | 6.253 | 62.95 | 62.81 | 0.998 |
| Toluene | 6.253 | 66.11 | 62.81 | 0.951 |
| Phenol | 5.996 | 60.21 | 60.24 | 1.000 |
| Phenylmercaptan | 7.081 | 70.80 | 71.09 | 1.004 |
| Benzaldehyde | 6.136 | 60.78 | 61.64 | 1.014 |
| Bnezene | 4.998 | 49.00 | 50.26 | 1.025 |
| Benzamide | 7.160 | 72.30 | 71.88 | 0.994 |
| Acetophenone | 7.160 | 72.05 | 71.88 | 0.998 |
| Benzoic acid | 6.859 | 70.28 | 68.87 | 0.980 |
| Fluorobenzene | 5.825 | 58.40 | 58.48 | 1.001 |
| Chlorobenzene | 6.983 | 69.97 | 70.11 | 1.002 |
| Bromobenzene | 7.737 | 78.92 | 77.65 | 0.984 |
| Iodobenzene | 9.227 | 92.00 | 92.55 | 1.006 |
| Cyanobenzene | 6.495 | 65.19 | 65.23 | 1.001 |
| Nitrobenzene | 6.266 | 61.80 | 62.94 | 1.018 |
| Indole | 8.651 | 85.00 | 86.79 | 1.021 |
| Thiophene | 5.763 | 57.38 | 57.91 | 1.009 |
| Anisole | 7.182 | 72.79 | 72.10 | 0.990 |
| *m,m'*-Bitolyl | 12.800 | 127.40 | 128.28 | 1.007 |
| Biphenyl | 10.292 | 103.25 | 103.20 | 1.000 |
| *o*-Xylene | 7.560 | 77.78 | 75.88 | 0.975 |
| *m*-Xylene | 7.510 | 76.56 | 75.38 | 0.984 |
| *p*-Xylene | 7.564 | 76.78 | 75.92 | 0.989 |
| N,N-Dimethylaniline | 9.011 | 89.66 | 90.39 | 1.008 |
| Phenylacetate | 8.260 | 82.04 | 82.88 | 1.010 |
| N-Phenylurea | 8.489 | 82.10 | 85.17 | 1.036 |
| *o*-Toluidine | 7.560 | 76.00 | 75.88 | 0.998 |
| *m*-Toluidine | 7.510 | 74.60 | 75.38 | 1.010 |
| Naphthalene | 8.874 | 91.90 | 89.02 | 0.968 |
| Phenanthrene | 12.352 | 127.90 | 123.80 | 0.967 |
| Anthracene | 13.485 | 130.00 | 135.14 | 1.038 |
| Pyrene | 14.754 | 147.90 | 147.82 | 0.999 |
| Benzyprene | 18.905 | 194.00 | 189.33 | 0.975 |
| *m*-Cresol | 7.252 | 72.02 | 72.80 | 1.011 |
| *o*-Cresol | 7.315 | 72.90 | 73.43 | 1.007 |
| *p*-Cresol | 7.332 | 72.10 | 73.60 | 1.020 |
| Carbazole | 11.605 | 117.40 | 116.33 | 0.991 |
| Methylbenzoate | 7.997 | 81.59 | 80.25 | 0.983 |
| Quinoline | 8.432 | 86.00 | 84.60 | 0.983 |
| Acridine | 12.603 | 123.30 | 126.31 | 1.024 |
| Pyridine | 4.724 | 49.21 | 47.52 | 0.964 |
| Diphenylmethane | 11.520 | 115.70 | 115.48 | 0.998 |
| *p*-Hydroxy methylbenzoate | 8.989 | 88.70 | 90.17 | 1.016 |
| Carbanilide | 13.718 | 134.05 | 137.46 | 1.025 |

[a] Ratio of 1.000 is a perfect concurrence.

estimate by the equation (predictability) indicates that the equation is quite satisfactory as a statistical model. The correlation coefficient of 0.998 indicates a good fit; a value of $r$ of 1.000 would be a perfect fit of the data to the line described by the equation. Thus, the molar diamagnetic susceptibility for an aromatic molecule can be expressed to a good level of approximation by an order of magnitude increase in the molecular electrophilic superdelocalizability. A major advantage of this method over use of Pascal constants, etc. in estimating diamagnetic susceptibilities is that a constitutive correction appropriate to a given molecule is made 'automatically' as a consequence of the linear combination of atomic orbital – molecular orbital calculation.

Self polarizabilities[14], localization energies[15] or Z-numbers[16] might also replace superdelocalizabilities[17] in the correlation as these are known[18] to be highly intercorrelated. We find however that $S^E$ values are easier to obtain than the others. A word of caution is now necessary. The correlation we have obtained may not necessarily be of fundamental physical significance. Superdelocalizability was originally derived[19] from perturbation theory as a reactive index for favorable charge-transfer transition state for a reaction whereby a weak $\pi$ bond was formed between attacking reagent and specific atom of substrate. Diamagnetic susceptibility reflects interaction of organic molecule's electrons with a magnetic field. Statistically, $\Sigma S^E$ may vary in the same direction as a more physically meaningful quantity or it may be related in a more complex manner to physically significant parameters (cf. reference[20]). Nevertheless while the 'true' physical meaning of the correlation may be suspect at this time, the correlation should prove useful in estimating diamagnetic susceptibilities for aromatic compounds. A striking feature of the correlation is that a single expression is all that is required for complex molecules that include alternate hydrocarbons, heterocyclics, and substituted benzenes. $-X_M$ values have been used in nuclear magnetic resonance[21], to calculate London dispersion forces[22], and to describe molecular interactions in biological systems[23, 24].

*Conclusion.* The diamagnetic susceptibility of an aromatic molecule may be predicted from its molecular electrophilic superdelocalizability calculated from Hückel molecular orbital theory. Correlation between the two variables was given by the least squares expression $-X_M = 10.00\,\Sigma S^E + 0.28$ for 44 aromatic compounds consisting of substituted benzenes and nonalternate hydrocarbons and heteroatomic substances. Diamagnetic susceptibilities have been used in calculation of London forces and of molecular interactions in biological systems.


[12] R. C. Weast, *Handbook of Chemistry and Physics* (Chemical Rubber Co., Cleveland, Ohio 1967), p. F9.
[13] A. L. Edwards, *Statistical Analysis* (Rinehard and Co., New York 1958), p. 127.
[14] C. A. Coulson and H. C. Longnet-Higgins, Proc. R. Soc., Lond. A*191*, 39 (1947).
[15] G. W. Wheland, J. Am. chem. Soc., *64*, 900 (1942).
[16] R. D. Brown, J. chem. Soc. *1959*, 2224.
[17] K. Fukui, T. Yonezawa and C. Nagata, Bull. chem. Soc., Japan *27*, 423 (1954).
[18] J. Koutecky, R. Zaharadnik and J. Cizek, Trans. Faraday Soc. *57*, 169 (1961).
[19] K. Fukui, T. Yonezawa and C. Nagata, J. chem. Phys. *27*, 1247 (1957).
[20] K. D. Bartle and D. W. Jones, J. phys. Chem. Ithaca *73*, 293 (1969).
[21] J. A. Pople, W. G. Schneider and H. J. Bernstein, *High Resolution Nuclear Magnetic Resonance* (McGraw Hill, New York 1959) Chaps. 2 and 7.
[22] B. Linder, J. chem. Phys. *33*, 668 (1960).
[23] A. Cammarata and S. J. Yau, J. pharm. Sci. *61*, 723 (1972).
[24] A. Cammarata, S. J. Yau and K. S. Rogers, Pure appl. Chem. *35*, 495 (1973).


## PRO EXPERIMENTIS

## A Method for Automatic Recording of Serum Lysozyme Activity with the Fragiligraph


P. Resnitzky and D. Danon[1]

*Department of Internal Medicine 'B', Central Emek Hospital, Afula (Israel), and Section of Biological Ultrastructure, The Weizmann Institute of Science, Rehovot (Israel), 25 August 1975.*



*Summary.* A quick and simple method for the estimation of lysozyme activity using the Fragiligraph, was described. Diminution of turbidity in a suspension of *Micrococcus lysodeikticus* produced by the addition of standard lysozyme (hen egg white) or serum sample, was continuously recorded for 5 min by the Fragiligraph. The normal mean serum lysozyme activity value obtained by this method is 6,80 µg/ml ± 1.85.


Serum lysozyme level is considered to be a reflection of the turnover of neutrophilic granulocytes[2, 3]. High values were reported in conditions with increased turnover or destruction of neutrophilic granulocytes, such as neutropenia due to hypersplenism[4, 5], polycythemia vera[6] and megaloblastic anemia[7]. Low values were reported in neutropenia due to hypoplastic bone marrow[4, 5]. Serum and urine lysozyme activity has recently been introduced as a useful test in the differential diagnosis of acute leukemia[8, 9]. Since lysozyme is filtered by the glomerulus and almost completely reabsorbed by proxymal tubular cells[10], the serum and urinary lysozyme activity can be used for monitoring renal function.

Immunological or bacteriolytic methods are currently used for human lysozyme activity determination in


[1] We gratefully acknowledge the excellent technical assistence of Miss M. Touma.



[2] M. E. Finch and S. C. Finch, Proc. Soc. exp. Biol. Med. *127*, 365 (1968).
[3] N. B. Hansen, Series Haematologica *7*, 1 (1974).
[4] D. Catovsky, D. A. G. Galton, C. Griffin, A. V. Hoffbrand and L. Szur, Br. J. Haemat. *21*, 661 (1971).
[5] W. M. Vietzke, P. E. Perillie and S. C. Finch, Yale J. Biol. Med. *45*, 457 (1972).
[6] J. Malmquist, Scand. J. Haemat. *9*, 258 (1972).
[7] P. E. Perillie, S. S. Kaplan and S. C. Finch, New Engl. J. Med. *277*, 10 (1967).
[8] E. F. Osserman and D. P. Lawlor, J. exp. Med. *124*, 921 (1966).
[9] D. Catovsky, D. A. G. Galton and C. Griffin, Br. J. Haemat. *21*, 565 (1971).
[10] T. Maack, D. D. S. Mackenzie and W. B. Kinter, Am. J. Physiol. *221*, 1609 (1971).