well as by the hydroxyproline analysis of the hydrolyzates (*7*).

The precipitate obtained by the addition of 3 mg of tyrosylgelatin to 25 ml of homologous rabbit antiserum (*9*) (the washed precipitate contained 1.9 mg of antigen and 8 mg of antibody) was treated with collagenase (15 units) as in the case of the gelatin-antigelatin floccules. The suspension became almost completely clear during the enzymic reaction. The fraction that dialyzed out accounted for 90 percent of the hydroxyproline present in the precipitate. The dialysis bag contained 85 percent of the antibody in the original precipitate (as calculated from the extinction at 280 mμ), as well as the remaining 10 percent of hydroxyproline. When subjected to paper electrophoresis, the antibody solution showed one spot only, with the mobility of normal rabbit serum γ-globulin. On sedimentation (Fig. 1) the solution was shown to contain a principal component with S_{20} equal to 7.06 and small amounts (approximately 6 percent) of a component with S_{20} equal to 19.2 [a 19 *S* component has been reported to be present in small amounts in many γ-globulins (*1*)]. Upon addition of new tyrosylgelatin to the antibody solution there was almost no precipitation, even when care was taken to inhibit any collagenase activity still present by the addition of Versene (*5*).

In the absence of the respective antibodies, collagenase converts both gelatin and tyrosylgelatin quantitatively into dialyzable fragments. Nevertheless, the possibility could be envisaged where the digestion of the antigen-antibody complex might leave the active site of the antigen bound to the antibody. With gelatin this does not seem to be the case, as the isolated antibody contained no hydroxyproline and could be precipitated with gelatin. On the other hand, in the case of tyrosylgelatin, the purified antibody still contained some hydroxyproline (it would correspond to 3 percent gelatin impurity, if calculated on the base of the composition of the whole antigen molecule) and could not be precipitated by the homologous antigen. This is consistent with the assumption that peptides containing the active site of the antigen are still bound to the antibody molecules. The above argument is further supported by the results of inhibition experiments of the specific precipitin reaction by means of collagenase digests of the antigens. While the collagenase digest of gelatin does not inhibit the precipitation of antigelatin by gelatin (*11*), it was found that the collagenase digest of tyrosylgelatin (the solution that dialyzed out from a mixture of 2 mg of tyrosylgelatin and 6 units of collagenase, after 3 hours of incubation at 25°C) completely inhibited the precipitation of antityrosylgelatin from 0.5 ml of antiserum, by the homologous antigen. It may be thus concluded that the hydroxyproline in the purified antibody solution is derived from fragments of the antigen bound to the active sites of the antibody molecules.

The method used here for the antibody purification is limited to systems where an enzyme may digest the antigen (protein or polysaccharide) in the antigen-antibody complex without damaging the antibody molecules. The active site of the antigen may under these circumstances remain, in some cases, bound to the antibody. This might permit the isolation of the active fragment (*12*).

RUTH ARNON
MICHAEL SELA
Department of Biophysics, Weizmann Institute of Science, Rehovoth, Israel

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Electronic Structure and Nicotine-like Stimulant Activity in Choline Phenyl Ethers

Abstract. Frontier electron density at the ether oxygen position and superdelocalizability at the ortho position show good parallelism with biological activity—that is, the stimulant activity of phenyl ether choline molecules. The mechanism of the biological action is discussed in connection with this finding.

The nicotine-like stimulant activity of phenyl ether cholines varies greatly with the substituents (*1*), but the mechanism of this activity remains obscure. The idea that the electron density at some points in the molecule, rather than the presence of some particular group, is important for the pharmacological activity, has been recognized. However, no quantum-mechanical approach in understanding the mechanism has been made.

We have established the frontier electron theory as one of the quantum-mechanical theories of organic chemical reaction, and its prediction agrees with experimental results better than previous theories (*2–4*). Furthermore, the theory has been successfully applied to the problem of some biological actions of conjugated molecules, such as the carcinogenic activity of polycondensed aromatic hydrocarbons (*5*) and the plant-growth activity of benzoic acid derivatives (*6*).

In this report, the frontier electron theory is further extended to explain the nicotine-like stimulant activity of phenyl ethers of choline. The theoretical indices used in this report are (i) frontier electron density (*2*) and (ii) superdelocalizability (*3*). These were derived as the reactivity indices of two extreme cases of stabilization at the transition state, due to the charge transfer from the substrate molecule to the pseudo-π orbital (that is, a π-like orbital which, according to the theory, comes into being near to the transition state, and consists of the orbitals in the reagent and the atom to be attacked in the substrate molecule), or vice versa. Let α be the coulomb integral of a carbon atom in benzene, and let h be the energy of the pseudo-π orbital. Then the frontier electron density and the superdelocalizability correspond to the case in which h is equal to the frontier orbital energy and to the case in which h is equal to α, respectively. The larger these indices are, the more reactive the position in question is, not only in substitution or addition reaction, but also in molecular complex formation.

Superdelocalizability has the following explicit formulas according to the type of reaction:
electrophilic reaction:

$$S_r^{(E)} = 2 \sum_j^{\text{occ}} (C_r^j)^2/\lambda_j$$

radical reaction:

$$S_r^{(R)} = \sum_j^{\text{occ}} (C_r^j)^2/\lambda_j + \sum_j^{\text{unocc}} (C_r^j)^2/(-\lambda_j)$$

nucleophilic reaction:

$$S_r^{(N)} = 2 \sum_j^{\text{unocc}} (C_r^j)^2/(-\lambda_j)$$

where C_r^j is the coefficient of the rth atomic π orbital in the jth molecular orbital, λ_j is the coefficient in the equation $\epsilon_j = \alpha + \lambda_j \beta$, ϵ_j is the energy of the jth molecular orbital, and α and β are the coulomb and resonance in-

Table 1. Nicotine-like activity and the electronic structure of phenyl ether cholines.

$R-OCH_2CH_2N(CH_3)_3 \cdot Br$ R =	Frontier electron density at ether oxygen* ($f_{oxy}^{(E)}$)	$S_o^{(N)}$*	Positive charge at ether oxygen*	Relative activity on blood pressure of cats after atropine†	
				Suprarenals intact	Suprarenals ligated
3,5-Dibromophenyl	0.764	0.952	0.179	337	268
Meta-bromophenyl	0.766	0.938	0.179	370	258
Meta-chlorophenyl	0.764	0.931	0.180	220	192
Phenyl	0.768	0.911	0.180	100	100
Meta-tolyl	0.730	0.847	0.179	13.1	13.5
Para-chlorophenyl	0.723	0.911	0.186	10.4	10.1
3,5-Xylyl	0.710	0.811	0.179	5.2	5.2
Para-tolyl	0.664	0.915	0.165	0.4	0.47

* The parameters used in the calculation are the following: coulomb integral of ether oxygen, chloro, bromo, and methyl group are $\alpha + \beta$, $\alpha + 2\beta$, $\alpha + 1.8\beta$, and $\alpha + 3\beta$, respectively. The coulomb integral of carbon attached to the chloro, bromo, and methyl groups are $\alpha + 0.4\beta$, $\alpha + 0.4\beta$, and $\alpha - 0.1\beta$, respectively, and the resonance integral between carbon and chlorine, carbon and bromine atom, and carbon and methyl groups are 0.8β, 0.7β, and β, respectively. † Hey's experiment (*1*).

tegrals, respectively, in benzene. The signs $\sum^{occ}$ and $\sum^{unocc}$ denote the summation of the occupied and unoccupied orbitals, respectively. Frontier electron density is the density of the π electrons in the frontier orbital at a position in the molecule. The frontier orbitals are defined as (i) the highest molecular orbital in the ground state, in the case of an electrophilic attack; (ii) the lowest vacant orbital of the ground state, in the case of a nucleophilic attack; and (iii) both of these orbitals, in the case of a radical attack.

We examined the calculated indices at all positions in the phenyl ethers and found that the frontier electron density for electrophilic attack at the ether oxygen position ($f_{oxy}^{(E)}$), and the superdelocalizability for nucleophilic attack at the ortho (*o*) position ($S_o^{(N)}$), have very intimate correlations with the nicotine-like activity (Table 1). This suggests that the interaction of active sites of these compounds, that is, the ether oxygen and ortho positions, with an electrophilic and a nucleophilic center in the receptor, is of great significance in the pharmacological action of these compounds. Wilson and Quan (*7*), carrying out the experiment of inhibitory action of phenyltrimethylammonium derivatives with regard to the hydrolysis of acetylcholine by cholinesterase, concluded that the steric condition was a very important factor in determining the inhibitory activity. 3-Hydroxy derivative (Fig. 1*A*), and 3-dimethylcarbamoxy derivative (Fig. 1*B*) were reported to have strong inhibitory activity; hence, these compounds were assumed to have a high degree of molecular complementariness with the enzyme. These two compounds are indicated in Fig. 1, together with phenyl ether choline (Fig. 1*C*). As is clearly seen in the figure, these compounds have sterically analogous conditions (part of the heavy line). Wilson and Quan suggested the hydrogen bond for (*A*) and covalent bond for (*B*). If these bonds are correct, the reactive center in the enzyme might be nucleophilic in nature because the proton of (*A*) or the carbamate carbon atom of (*B*) could be considered to be electrophilic. The ortho position of (*C*) corresponds sterically to the proton of (*A*) or the carbamate carbon of (*B*), and it is of interest that the value of $S_o^{(N)}$, which is the measure of reactivity of the ortho position with a nucleophilic center, shows good parallelism with biological activity. At the present stage of knowledge, it is impossible to decide what groups in the nucleophilic center might be involved in the interaction with the ortho and ether oxygen positions. In this connection, the conclusion obtained by Wilson and Bergmann (*8*) that nucleophilic groups such as imidazole and tyrosyl might be present in the esteratic site is suggestive for the nature of the group in the nucleophilic center. Perhaps a carbamate carbon atom will interact with an imidazole-like group, and ether oxygen will become an acceptor of a proton of the tyrosyl group. This might relate to the reason the value of $f_{oxy}^{(E)}$ indicates a distinct parallelism with biological activity.

The values of $S_o^{(N)}$ of *p*-chloro and *p*-tolyl compounds are rather large compared with their biological activity. The introduction of a group at the para position is expected to make the compound unfavorable for the occurrence of activity as in the case of plant-growth regulating compounds (*6*). In the fourth column of Table 1, the positive charge at the ether oxygen position is listed. The values of 3,5-dibromo, meta-bromo, unsubstituted, meta-methyl and 3,5-dimethyl are nearly constant, whereas the biological activity varies remarkably in this series of compounds. Hence the charge at the ether oxygen position seems to have no intimate connection with the biological phenomena.

It is noteworthy that the existence of the heavy line part is favorable for the occurrence of activity. That is, inhibitors of acetylcholinesterase and a greater part of nicotine-like stimulant compounds possess a chain of five atoms attached to the nitrogen atom. This steric circumstance strongly resembles the "five-atom chain" rule, proposed by Ing, associated with muskaline-like activity (*9*). It is true that the many molecules having four-atom or six-atom chains exhibit nicotine-like activity. However, activity of these compounds is not so great as one in which the "five-atom chain" rule is satisfied. Furthermore, the activity of the compound having three-atom or seven-atom chains has scarcely been reported. In this connection, the spatial factor is a very important, although not the exclusively determining one (*10*).

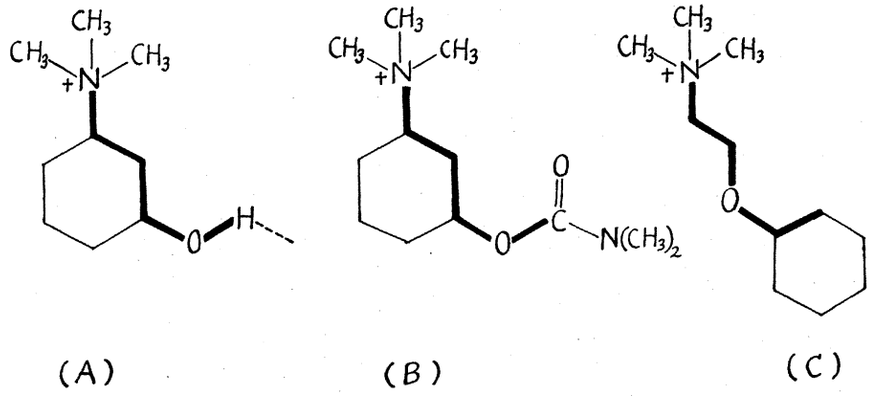

Fig. 1. Steric resemblance between the inhibitors (*A* and *B*) of acetylcholinesterase and phenyl ether choline (*C*).

KENICHI FUKUI
CHIKAYOSHI NAGATA
AKIRA IMAMURA
Faculty of Engineering, Kyoto University, Kyoto, Japan

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Influence of Currents on Form of Sponges

Abstract. Small reconstituted *Microciona prolifera* produced central oscular chimneys, perpendicular to the surface of attachment, in standing water; in a slow, steady current, the oscular chimneys were eccentrically placed and directed obliquely downstream. Gravity and directional illumination did not affect the orientation of the chimney.

In 1923, Bidder (*1*) pointed out that sponges of the same species differ in shape, apparently in adaptation to the water currents in which they grow; those exposed to currents of constant direction bear most of their oscula on the downstream side, while those in still water or variable currents bear oscula opening upward. Since, so far as I am aware, no attempt has ever been made to verify this suggestion experimentally, I examined the influence of certain environmental factors, including water currents, on the growth of the oscular chimney in small *Microciona prolifera* obtained by the method of H. V. Wilson (*2*), that is, by allowing cells dissociated by squeezing the sponge through bolting silk to settle on glass slides in dishes of sea water.

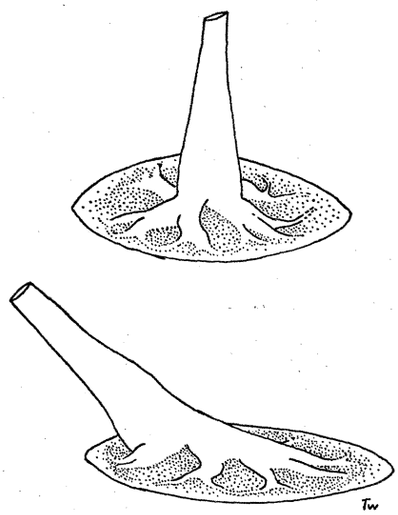

Fig. 1. Reconstituted sponges, *Microciona prolifera*, grown in standing water (top) and in slowly flowing water (bottom).

After 36 to 48 hours, before the reassociated masses of sponge cells had any detectable internal structure foreshadowing a canal system, they were well attached to the slides and could be transferred to other containers. Half the slides, with about 15 sponges, were placed in finger bowls of "standing" water—actually, kept gently turbulent by slowly dripping sea water. The other half of the slides were placed in a battery jar in which a constant, slow current was maintained by a stream of bubbles up one side.

Within a week each small sponge, 1 to 2 mm in diameter, had formed a system of excurrent canals radiating from the base of a tall oscular chimney. In standing water, every sponge produced a central chimney perpendicular to the slide on which it grew (Fig. 1, top). In flowing water, every sponge produced an eccentric chimney, pointing downstream at an angle of about 45° with the slide (Fig. 1, bottom). The same form was assumed whether the sponge grew on the upper surface of the slide, or on the lower surface, or on the vertical surface of a slide standing on edge, and whether illumination came from above, or from one side, or through the slide from below.

When a slide bearing such adapted sponges was transferred to the other kind of container, the sponges gradually transformed themselves to the form appropriate to the new conditions over a period of about 10 days. Except for a temporary shortening and constriction of the oscular chimney, the canal system seemed to remain functional throughout the transition.

These results agree well with Bidder's hypothesis that a growing sponge adopts a form which minimizes the quantity of exhaled water re-entering its incurrent pores (*3*).

FREDERICK E. WARBURTON
6171 Sherbrooke W.,
Montreal, Canada

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Uterine Vascular Clamping: New Procedure for the Study of Congenital Malformations

Abstract. Clamping the uterine blood vessels of the rat on the 9th day of gestation for ½ to 3 hours resulted in fetal death, growth retardation, and severe congenital malformations. This procedure has broad applications in the fields of teratology and cancer chemotherapy, and in the investigation of fetal-maternal physiology.

Mechanical or operative techniques in teratology have never been widely adopted, because, in general, they are not potent teratogenic agents, or they have only minimal application to the general field of congenital malformations. The following operative procedure, developed in our laboratory, is a potent teratogenic agent and should find wide use in all phases of experimental teratology, since one animal provides both the control and experimental fetuses.

An inbred strain of pathogen-free rats with an extremely low rate of random malformations was used in the study. The rats were subjected to a 12-hour mating period, and vaginal smears were used to determine pregnancy. The first day of a positive smear was used as day zero in calculations of gestational age.

On the 9th day of pregnancy laparotomy was performed with pentobarbital anesthesia. The number, location, and condition of the implantation sites were recorded. One horn of the uterus was then clamped at its cervical and ovarian ends, the clamps extending across the uterus and its mesentery. By this means, one horn was completely isolated from the maternal circulation. The other horn served as a control. There were five rats in each of the six time periods of ½, 1, 1½, 2, 2½, and 3 hours. After the specified clamping interval, the hemostats were removed, and the abdomen was closed. The rats were killed on the 21st day.

Complete isolation of the clamped uterus from the maternal circulation was an essential condition of this experimental procedure. Intravenous injections of trypan blue and fluorescent dyes qualitatively confirmed uterine hemostasis, since no dye was noted on the clamped side. A more quantitative method was obtained by injecting 12.48 μc of radioiodinated albumin intravenously, after clamping a (nonpregnant) uterus. The two horns were then excised and divided into three segments. The counts per minute per milligram of tissue showed negligible radioactivity in the clamped segments (Fig. 1*a*).

Either of two reactions followed clamping: (i) a sudden arteriolar spasm