Quantum-biological Analysis of the Relation of Urea and its Related Compounds to Water in Renal Medulla

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SINCE the introduction of the countercurrent concept explaining renal concentration mechanism, it has been noticed that there exists a close relationship between movement of urea and that of water in renal medulla, although its detail has not been elucidated so far. Especially concerning the character of urea transport in the renal medulla, some investigators¹⁻³⁾ insist on the passive transport but others⁴⁻⁷⁾ suggest a possibility of active transport.

The authors have already reported results of retrograde infusion experiments in rabbits^{8,9)} and of urinary urea/nonurea ratio in human subjects and phenacetin fed rats^{10,11)}, which suggest the active transport of urea in the renal medulla. Moreover, it has been reported that urea related compounds also play the similar role to urea for water movement in the renal medulla and the toad bladder¹²⁻¹⁷⁾. Through these studies, our interest was attracted to further studies which could explain the relation of urea and its related compounds to water from the standpoints of quantum biology. It would be expected that urea or its related compounds plays some role to water movement, because of the physical interference between urea as well as its related compounds and water due to the asymmetrical electrolicity of these substances. In this paper, intramolecular electron density of urea and its related compounds and its related values were calculated by Simple-Linear-Combination of Atomic-Orbital and Molecular-Orbital Method.

CALCULATION PROCEDURES^{19,20)}

Simple-Linear-Combination of Atomic-Orbital and Molecular-Orbital Method was applied to the calculation of π electron density of urea, methylurea, acetamide 1-3-dimethylurea and thiourea. Distribution of π electrons of these molecules was illustrated in Fig. 1.

The fundamental in HÜCKEL's Orbital Method is represented as

$$\varphi = \sum_{r=1}^n C_r \chi_r \quad (1)$$

where n means the number of atom or atomic group which has π electron and $n=4$ in urea, acetamide and thiourea, $n=5$ in methylurea and $n=6$ in 1-3-dimethylurea. χ_r represents the wave equation of the molecular orbital of the atoms or atomic groups which are numbered in Fig. 1. C_r is to be determined so that it may satisfy the wave equation,

$$h\varphi = \varepsilon\varphi \quad (2)$$

In HÜCKEL's Molecular-Orbital Method, it is further assumed that resonance integrals are not to be zero only when the two atoms are bonded in a classical structural expression. Then we could obtain the following secular equation of urea molecule.

$$\begin{array}{ll} 1) & C_1(\alpha_1 - \varepsilon) + C_2\beta_{12} = 0 \\ 2) & C_1\beta_{21} + C_2(\alpha_2 - \varepsilon) + C_3\beta_{23} = 0 \\ 3) & C_2\beta_{32} + C_3(\alpha_3 - \varepsilon) = 0 \\ 4) & C_2\beta_{42} + C_4(\alpha_4 - \varepsilon) = 0 \end{array}$$

where α_r and β_{rs} represent the Coulomb and resonance integrals respectively, defined $\alpha_r =$

$$\int \chi_r^* h \chi_r d\tau, \quad \beta_{rs} = \int \chi_r^* h \chi_s d\tau. \quad \text{The procedure of}$$

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The dot in the molecule represented π electron. Electrondensity was shown in the parenthesis. Formal charge was described under the parenthesis. The total number of electron was checked and was shown in the parenthesis of the right side of the molecule.

Fig. 1. Distribution of π electron and calculated results of electrondensity and formal charge of urea and its related compounds

HÜCKEL's Molecular-Orbital Method always depends on the choice of the reasonable values for some parameters. Parameters α_r and β_{rs} were usually expressed in units of the standard α_0 and β_0 of the carbon atom of the benzene, as follows;

$$\alpha_r = \alpha_0 + h_r \beta_0, \quad \beta_{rs} = k_{rs} \beta_0 \quad (\beta_{rs} = \beta_{sr})$$

The values of h_r have frequently been taken as the differences of the empirical electronegativities between the atom r and carbon (c). These parameter were selected STREITWIESSER's Monograph²⁰⁾. Thus, $\alpha_1 = \alpha_0 + 1.0\beta_0$, $\alpha_2 = \alpha_0$, $\alpha_3 = \alpha_0 + 1.5\beta_0$, $\alpha_4 = \alpha_0 + 1.5\beta_0$

$$\beta_{12} = \beta_{21} = k_{12} \beta_0 = 1.0\alpha_0,$$

$$\alpha_{32} = \beta_{23} = \beta_{24} = \beta_{42} = 1.0\beta_0$$

The secular equation of thiourea is as follows,

$$1) \quad C_1(\alpha_1 + \varepsilon) + C_2\beta_{12} = 0$$

$$2) \quad C_1\beta_{21} + C_2(\alpha_2 - \varepsilon) + C_3\beta_{23} + C_4\beta_{24} = 0$$

$$3) \quad C_2\beta_{32} + C_3(\alpha_3 - \varepsilon) = 0$$

$$4) \quad C_2\beta_{42} + C_4(\alpha_4 - \varepsilon) = 0$$

$$\alpha_1 = \alpha_0, \quad \alpha_2 = \alpha_0, \quad \alpha_3 = \alpha_0 + 1.5\beta_0,$$

$$\alpha_4 = \alpha_0 + 1.5\beta_0 \quad (h_s \cong 0)$$

$$\beta_{12} = \beta_{21} = k_{12}\beta_0 = \beta_0 \quad (k_{c-s} \cong 1),$$

$$\beta_{23} = \beta_{32} = \beta_{24} = \beta_{42} = \beta_0$$

The secular equation of methylurea is as follows;

$$1) \quad C_1(\alpha_1 - \varepsilon) + C_2\beta_{12} = 0$$

$$2) \quad C_1\beta_{21} + C_2(\alpha_2 - \varepsilon) + C_3\beta_{23} + C_4\beta_{24} = 0$$

$$3) \quad C_2\beta_{32} + C_3(\alpha_3 - \varepsilon) = 0$$

$$4) \quad C_2\beta_{24} + C_4(\alpha_4 - \varepsilon) = 0$$

$$5) \quad C_3\beta_{35} + C_5(\alpha_5 - \varepsilon) = 0$$

$$\alpha_1 = \alpha_0 + 1.0\beta_0, \quad \alpha_2 = \alpha_0,$$

$$\alpha_3 = \alpha_0 + 1.5\beta_0, \quad \alpha_4 = \alpha_0 + 1.5\beta_0,$$

$$\alpha_5 = \alpha_0 + 2.0\beta_0 \quad (h-\text{CH}_3 \cong 2.0)$$

$$\beta_{12} = \beta_{21} = k_{12}\beta_0 = 1.0\beta_0,$$

$$\beta_{23} = \beta_{32} = \beta_{42} = k_{23}\beta_0 = \beta_0,$$

$$\beta_{35} = \beta_{53} = 0.7\beta_0 \quad (k_{C-\text{CH}_3} \cong 0.7)$$

The secular equation of acetamide is as follows;

$$1) \quad C_1(\alpha_1 - \varepsilon) + C_2\beta_{12} = 0$$

$$2) \quad C_1\beta_{21} + C_2(\alpha_2 - \varepsilon) + C_3\beta_{23} + C_4\beta_{24} = 0$$

$$3) \quad C_2\beta_{32} + C_3(\alpha_3 - \varepsilon) = 0$$

$$4) \quad C_2\beta_{24} + C_4(\alpha_4 - \varepsilon) = 0$$

$$\alpha_1 = \alpha_0 + 1.0\beta_0, \quad \alpha_2 = \alpha_0,$$

$$\alpha_3 = \alpha_0 + 2.0\beta_0, \quad \alpha_4 = \alpha_0 + 1.5\beta_0$$

$$\beta_{12} = \beta_{21} = k_{12}\beta_0,$$

$$\beta_{23} = \beta_{32} = k_{23}\beta_0 = 0.7\beta_0,$$

$$\beta_{24} = \beta_{42} = k_{24}\beta_0 = \beta_0$$

The secular equation of 1-3-dimethylurea is as follows;

$$1) \quad C_1(\alpha_1 - \varepsilon) + C_2\beta_{12} = 0$$

$$\begin{aligned}
 2) \quad & C_1\beta_{21} + C_2(\alpha_2 - \varepsilon) + C_3\beta_{23} + C_4\beta_{24} = 0 \\
 3) \quad & + C_2\beta_{32} + C_3(\alpha_3 - \varepsilon) + C_5\beta_{35} = 0 \\
 4) \quad & + C_2\beta_{42} + C_4(\alpha_4 - \varepsilon) + C_6\beta_{46} = 0 \\
 5) \quad & + C_3\beta_{53} + C_5(\alpha_5 - \varepsilon) = 0 \\
 6) \quad & + C_4\beta_{64} + C_6(\alpha_6 - \varepsilon) = 0
 \end{aligned}$$

$$\begin{aligned}
 \alpha_1 &= \alpha_0 + 1.0\beta_0, \quad \alpha_2 = \alpha_0, \\
 \alpha_3 &= \alpha_0 + 1.5\beta_0, \quad \alpha_4 = \alpha_0 + 1.5\beta_0, \\
 \alpha_5 &= \alpha_0 + 2.0\beta_0, \quad \alpha_6 = \alpha_0 + 2.0\beta_0, \\
 \beta_{12} &= \beta_{21} = k_{12}\beta_0 = 1.0\beta_0, \\
 \beta_{23} &= \beta_{32} = \beta_{24} = \beta_{42} = 1.0\beta_0, \\
 \beta_{35} &= \beta_{53} = \beta_{46} = \beta_{64} = 0.7\beta_0
 \end{aligned}$$

After substituting $\alpha_0 - \varepsilon/\beta_0$ as $-\lambda$, the solutions of each secular equations were gained by means of the computer. Thus, the wave equations of each molecule was obtained as follows;

$$\varphi_1 = 0.35896\chi_1 + 0.55638\chi_2 + 0.52990\chi_3 + 0.52990\chi_4$$

$$\varphi_2 = -0.37390\chi_1 + 0.82138\chi_2 - 0.30457\chi_3 - 0.30457\chi_4$$

$$\varphi_3 = -0.85519\chi_1 - 0.12558\chi_2 + 0.35559\chi_3 + 0.35559\chi_4$$

$$\varphi_4 = -0.70711\chi_3 + 0.7071\chi_4$$

(thiourea)

$$\varphi_1 = 0.81174\chi_1 + 0.35169\chi_2 - 0.32969\chi_3 - 0.32969\chi_4$$

$$\varphi_2 = 0.53970\chi_1 + 0.75679\chi_2 - 0.26076\chi_3 - 0.26076\chi_4$$

$$\varphi_3 = 0.22316\chi_1 + 0.55098\chi_2 + 0.56860\chi_3 + 0.56806\chi_4$$

$$\varphi_4 = -0.70711\chi_3 - 0.70711\chi_4$$

(methylurea)

$$\varphi_1 = 0.23263\chi_1 + 0.41788\chi_2 + 0.61357\chi_3 + 0.32235\chi_4 + 0.53933\chi_5$$

$$\varphi_2 = -0.36913\chi_1 + 0.81639\chi_2 - 0.31901\chi_3 - 0.30106\chi_4 + 0.06953\chi_5$$

$$\varphi_3 = -0.63781\chi_1 - 0.00255\chi_2 + 0.63012\chi_3 + 0.0513\chi_4 - 0.44285\chi_5$$

$$\varphi_4 = -0.54498\chi_1 - 0.15156\chi_2 - 0.33173\chi_3 + 0.68301\chi_4 + 0.32167\chi_5$$

$$\varphi_5 = -0.32530\chi_1 - 0.36864\chi_2 + 0.12107\chi_3 - 0.58217\chi_4 + 0.63616\chi_5$$

(acetamide)

$$\varphi_1 = 0.82071\chi_1 + 0.16101\chi_2 - 0.14021\chi_3 - 0.52996\chi_4$$

$$\varphi_2 = -0.40682\chi_1 + 0.83133\chi_2 - 0.19120\chi_3 + 0.32684\chi_4$$

$$\varphi_3 = 0.32288\chi_1 + 0.40550\chi_2 + 0.64875\chi_3 + 0.47891\chi_4$$

$$\varphi_4 = 0.23810\chi_1 + 0.19350\chi_2 - 0.72312\chi_3 + 0.61883\chi_4$$

(1-3-dimethylurea)

$$\varphi_1 = 0.21938\chi_1 + 0.41546\chi_2 + 0.49141\chi_3 + 0.49141\chi_4 + 0.38490\chi_5 + 0.38490\chi_6$$

$$\varphi_2 = -0.36462\chi_1 + 0.81169\chi_2 + 0.31531\chi_3 + 0.31531\chi_4 + 0.06842\chi_5 + 0.06842\chi_6$$

$$\varphi_3 = -0.75763\chi_1 - 0.00217\chi_2 + 0.37773\chi_3 + 0.37773\chi_4 - 0.26517\chi_5 + 0.26517\chi_6$$

$$\varphi_4 = -0.57800\chi_3 + 0.57800\chi_4 + 0.40733\chi_5 - 0.40733\chi_6$$

$$\varphi_5 = -0.49480\chi_1 - 0.41054\chi_2 - 0.12811\chi_3 - 0.12811\chi_4 + 0.52617\chi_5 + 0.52617\chi_6$$

$$\varphi_6 = -0.40733\chi_3 + 0.40733\chi_4 - 0.57800\chi_5 + 0.57800\chi_6$$

From these solutions, electrondensity of each atom of molecule, bond order (Prs) and bond length (Rrs) were calculated, generally accepted formula for the calculation of the above term are $2 \sum_j^{occ} \text{Cir}^2$, $2 \sum_j^{occ} \text{CirCis}$ and $1 - \{s - d / 1 + k(1 - \text{Prs})\}$ (by COULSON: s =single bond length, d =double bond length and $k=0.765$) respectively. And formal charge of each atom was calculated by the equation; $Q_r = n_r - q_r$. (n_r =the number of electrons now discussed, q_r =electrondensity of the atom now discussed) Molecular radius was estimated by consideration of VAN DER WAALS radius of the atom and Rrs above defined. As the radius of atom, VAN DER WAALS radius was adopted. The atom circles were depicted by VAN DER WAALS radius at the distance of calculated bond length far from the center of the nearest neighbor atom. Bond angle was assumed as 120° . Finally, the radius of a minimum circle which was depicted to contain every circles of the atoms of the molecule, was measured. The calculated results of bond length C=O, C=S, C=N and C-CH₃ and VAN DER WAALS radius of the atom of oxygen, nitrogen, sulphur and methyl group were also presented in Fig.-3.

The negative formal charge of the molecule which only locates in the oxygen or sulphur atom, was divided by VAN DER WAALS radius of the oxygen or sulphur, the (molecular radius)² or (molecular radius)³ and (MR-D)² or (MR-D)³, to induce a new index expressing the negative charge of a molecule as a whole.

Fig. 2. Molecular size of urea and its related compounds

(cited from Rabinowitz's article)

Fig. 3. Relation between MR-D and Molecular radius

These physical or physicochemical indexes were cited from PAULING's Monograph²¹⁾.

RESULT

Electrondensities and formal charges of these molecules calculated as above are illustrated in Fig.-1. These negative formal charges always located only at the site of oxygen or sulphur atom. In the order of the magnitude of these values, these are 1-3-dimethylurea, methylurea, urea, acetamide and thiourea. Molecular radii of urea and its related compounds estimated as above were illustrated in Fig.-2.

Molecular radii were as follows, urea; 2.8A, thiourea; 3.1A methylurea; 3.2A, acetamide; 3.1A and 1-3-dimethylurea; 3.8A.

These values were compared with MR-D (molar refraction-D by LORENZ which was cited from RABINOWITZ's article¹²⁾). The results were showed in Fig.-3.

A linear relation was observed between molecular radius and MR-D except thiourea. The negative formal charge of oxygen or sulphur of the compounds was corrected by various manners. These results were summarized Table I. The calculated negative formal charge was divided by $(MR-D)^{2-3}$ and $(\text{molecular radius})^{2-3}$. By this procedure, the degree of magnitude of the

TABLE I VALUE OF FORMAL CHARGE AND ITS CORRECTION BY MOLECULAR SIZE OF THE COMPOUNDS

	(1)	(2)	(3)	(4)	(5)	(6)
urea	0.7205	0.2625	5.000×10^{-3}	4.166×10^{-4}	9.190×10^{-2}	2.822×10^{-2}
methylurea	0.7275	0.2651	2.245×10^{-3}	1.247×10^{-4}	7.100×10^{-2}	2.221×10^{-2}
acetamide	0.6690	0.2438	3.413×10^{-3}	2.438×10^{-4}	6.857×10^{-2}	2.224×10^{-2}
1,3-dimethylurea	0.7340	0.2675	1.388×10^{-3}	0.532×10^{-4}	5.083×10^{-2}	1.341×10^{-2}
thiourea	0.4164	0.1249	1.041×10^{-3}	0.521×10^{-4}	4.332×10^{-2}	1.071×10^{-2}

(1) = negative formal charge of O or S of these compounds

(2) = (1)/(Van der Waals radius of O or S)³

(3) = (1)/MR_D² (4) = (1)/MR_D³

(5) = (1)/r² (6) = (1)/r³

In this table, r represented the molecular radius calculated by us.

index which was considered to be negative charge of a molecule as a whole, was the highest in urea, followed by acetamide=methylurea, 1-3-dimethylurea and thiourea in the decreasing order.

DISCUSSION

In the renal medulla, it has been considered that water is passively transported by the hyperosmolality of the renal medulla created by countercurrent system. As in the other tissues²³⁾, an active transport of water in the renal tubule was neglected by thermodynamics and bioenergetics²²⁾. The hyperosmolality of the renal medulla was mainly consisted of urea which increased markedly in antidiuretic stage. And it was reported by ROBINSON et al.²⁴⁾ that the production of urea in the renal medulla was negligible, so it seems to be very important to clarify the relation between urea and water. Recently, it was revealed that urea related compounds also showed almost the same characteristics as urea in the renal medulla and the toad bladder¹²⁻¹⁷⁾. There would be exist some molecular and structural regularity between these substances and water. RABINOWITZ et al.¹²⁾ showed that in their ordinary infusion experiment in dogs, the ability to enhance urinary osmolality of these substances was in the following order; urea, methylurea or acetamide, 1-3-dimethylurea and thiourea. They failed to find any molecular regularity about this sequence of these molecules.

TRUNIGER and SCHMIDT-NIELSEN⁷⁾ showed that urea, methylurea and acetamide were ac-

tively accumulated in the renal medulla of antidiuretic rats fed with low protein. Present studies aimed to obtain some molecular structural regularity which could explain the phenomena above described. The author's consideration was based on the basic analysis of relation between urea or its related compounds and water by quantum-statistics. It is believed that water molecules attach together by hydrogen bond. BERNAL and FOOWLER²⁵⁾ considered that 46 water molecules combined together by hydrogen bond at near the temperature of melting point. Though their attached numbers would be decreased in physiological temperature comparing with that of near melting point, the role of hydrogen bond is important in the bio-physics. And it was also reported that urea solution could denature enzyme protein by cutting its hydrogen bond between amino and carboxyl group²⁶⁾. Because of PAULING's monograph²¹⁾ that the lone pair electrons of the oxygen atom of urea had the nature of forming hydrogen bond with hydrogen nucleus, it may be expected that urea or its related compounds depolymerize water molecules which are attached together by hydrogen load. And then it was also expected that existence of urea or its related compounds in the solution accelerates water movement.

In this calculation, Self-Consistent-Field Method was omitted, but in these simple molecules, our calculation procedures seemed to be sufficient enough to support our opinion. For our consideration. Intramolecular electrondensity or negative formal charge seemed to be

most important. Intramolecular negative formal charge was divided by the (molecular radius)²⁻³ or (MR-D)²⁻³, because these charges are suspected to distribute on the whole surface or in the space of molecule and effect other molecules.

The molecular radius was depicted considering VAN DER WAALS radius of each atom and the bond length of the adjacent two atoms. Except thiourea the linear relationship was obtained between MR-D and molecular radius as shown Fig. 3. This discrepancy might be due to the values of resonance integral of 2p-3p electron combination of C=S and COULOMB's integral of 3p electron orbital of sulphur atom in thiourea. But this discrepancy did not influence for our interpretation, because the negative formal charge was markedly small in the sulphur of thiourea. Since the results of our calculation well agreed with the result of RABINOWITZ's experiment, it could be expected that the new index which was obtained to divide negative formal charge of oxygen or sulphur atom of these molecules by its (MR-D)²⁻³ or (molecular radius)²⁻³ has a close relation to the ability of urea and its related compounds which enhance water absorption in renal medulla in experimental animals. Through these studies, the availability of the basic quantum-statistical analysis for the relation between water and solutes would be greatly estimated. Water movement accompanied with solute transport was called as solvent drag²⁷⁾. These studies might afford some interpretation to this problem. The author's consideration seemed to be very speculative. Then further studies would be needed.

SUMMARY

The intramolecular negative formal charges of urea, methylurea, acetamide, 1-3-dimethylurea and thiourea were calculated by Simple-Linear-Combination of Atomic-Orbital and Molecular-Orbital Method. Then, these charges were divided by (molecular radius)²⁻³ or (MR-D)²⁻³. By this calculation, a new index showing the intensity of negative charge around the molecule was obtained.

The order of the magnitude of these indices agreed well with that of ability to enhance

urinary osmolality or water reabsorption in renal medulla of dogs shown by RABINOWITZ et al. and that of the accumulation strength of these substances into the renal medulla in anti-diuretic low-protein rats shown by TRUNIGER et al.

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REFERENCES

- 1) Pena, J. C., and Malvin, R. L., Transtubular diffusion of non-electrolytes across the renal tubular epithelium. *J. gen. Physiol.* **65**: 643-649, 1962.
- 2) Koike, T. I., and Kellogg, R. H., Effect of urea loading on urine osmolality during osmotic diuresis in hydropenic rats. *Am. J. Physiol.* **205**: 1053-1057, 1963.
- 3) Cooke, C. R., Andrew, D. J., and Paulino, A. B., Stop flow studies of renal concentrating mechanism. *Am. J. Physiol.* **203**: 331-338, 1962.
- 4) Schmidt-Nielsen, B., and O'dell, R., Effect of diet on distribution of urea and electrolytes in the kidney of sheep. *Am. J. Physiol.* **197**: 856-860, 1959.
- 5) Schmidt-Nielsen, B., Urea excretion in mammals. *Physiol. Rev.* **38**: 139-168, 1958.
- 6) Bray, G. A., and Preston, A. S., Effect of urea on concentration in the rat. *J. Clin. Invest.* **40**: 1952-1960, 1961.
- 7) Truniger, B., and Schmidt-Nielsen, B., Intrarenal distribution of urea and related compounds: effect of nitrogen intake. *Am. J. Physiol.* **207**: 961-978, 1964.
- 8) Urakabe, S., Orita, Y., Furukawa, T., and Abe, H., Evaluation of a new experimental method for observation of renal medullary function in rabbits. *Med. J. Osaka Univ.* **16**: 201-211, 1965.
- 9) Orita, Y., Urakabe, S., Furukawa, T., and Abe, H., Retrograde infusion studies on urea accumulation in renal medulla of rabbits. *Med. J. Osaka Univ.* **16**: 213-224, 1965.
- 10) Urakabe, S., Orita, Y., Furukawa, T., and Abe, H., Analysis of impaired concentrating ability of phenacetin fed rat. *Jap. Circ. J.* **29**: 841-845, 1965.
- 11) Orita, Y., Urakabe, S., Shirai, D., Furukawa, T., and Abe, H., Effect of Urinary urea/nonurea on urinary concentrating ability and renal water economy in human subjects. *Jap. Circ. J.* **30**: 225-235, 1966.
- 12) Rabinowitz, L., and Kellogg, R. H., Enhancement of renal concentrating ability in the dog by urea and related compounds. *Am. J. Physiol.* **205**: 112-116, 1963.
- 13) Leaf, A., and Hays, R. M., Permeability of the isolated toad bladder to solutes and its modification by vasopressin. *J. gen. Physiol.* **45**: 921-932, 1962.
- 14) Orita, Y., Urakabe, S., Shirai, D., Furukawa, T., and Abe, H., Studies on the movement of urea related compounds in renal medulla of rabbits. *Jap. J. Nephrology*. **7**: 111-112, 1965 (in Japanese)

- 15) Rabinowitz, L., Mechanism of renal excretion of methylurea and acetamide in the dog. *Am. J. Physiol.* **209**: 188-194, 1965.
- 16) Bray, G. A., Distribution of urea, thiourea-C¹⁴ and sucrose-C¹⁴ in dog kidney during antidiuresis. *Am. J. Physiol.* **199**: 1211-1214, 1960.
- 17) Schmidt-Nielsen, B., and Shrauger, C. R., Handling of urea and related compound by the renal tubules of the frog. *Am. J. Physiol.* **295**: 483-488, 1963.
- 18) Yonezawa, T., Nagata, C., Kato, H., Imamura, A., and Morokuma, K., Introduction to quantum-chemistry, Part-1, 1955, 5th Ed. Kagakudozin, Kyoto. (in Japanese) p. 39-p. 65
- 19) Yomosa, H., Electronic structure of the peptide group and hydrogenbonded polypeptides. *Biopolymers*, **1**: 1-33, 1964.
- 20) Streitwieser, A. Jr., Molecular orbital theory for organic chemists, Wiley, 1961. New York. p. 135
- 21) Pauling, L., The nature of chemical bond and the structure of molecules and crystals; an introduction to modern structural chemistry, 1960, 3rd. Ed. Cornell Univ. Press. (Jap. Ed) p.235, p.204~p. 427,
- 22) Brodsky, W. W., Pehm, W. S., Dennis, W. H., and Miller, D. C., Thermodynamic analysis of the intracellular osmotic gradient hypothesis of active water transport. *Science* **12**: 302-306, 1955.
- 23) Davies, R. E., The mechanism of hydrochloric acid production by the stomach. *Biol. Rev.* **26**: 87-120, 1951.
- 24) Robinson, R. R., and Schmidt-Nielsen, B., Distribution of arginase within the kidneys of several vertebrates species. *J. Cell. Comparat. Physiol.* **62**: 147-157, 1963.
- 25) Bernal, J. D., and Fowler, R. H., *J. Chem. Physics.* **1**: 515, 1933. (cited from reference No. 21)
- 26) Pogosova, A. V., Kritzman, M. G., and Konikova, A. S., The effect of urea and temperature on amino acid incorporation into isolated proteins. *Biochemistry* **28**: 13-17, 1962.
- 27) Kleinzeller, S. A., and Kotyk, A., Symposium of Membrane Transport and Metabolism. (Prague, 1960) Acad. Press. 1961, New York.