Some Electronic Aspects of Biochemistry*

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IT is our deep belief that some of the most important developments in science in the next decade may come through the application of quantum theories to biology. The situation seems to be quite ripe for it. In particular, it appears to us that the most promising field for the extension and renewal of theoretical chemistry is biochemistry, and that results of incalculable value for the understanding of basic processes of life may be obtained by using the methods of molecular quantum mechanics in this field. For these reasons we started in our Laboratory, a few years ago, a detailed investigation of the electronic aspects of a number of fundamental biochemical problems. In this paper a fragmentary report of two of the problems that we are dealing with is presented.

I. ELECTRON TRANSFER IN BIOLOGICAL SYSTEMS AND MECHANISM OF ACTION OF OXIDO-REDUCTION ENZYMES

It appears more and more evident that a great number of biochemical processes occur through a mechanism involving an electron transfer from a donor molecule to an acceptor one. In this respect the recent discoveries by Isenberg and Szent-Gyorgyi[1] and by Harbury and Foley[2] of tryptophan-riboflavin or caffeine-riboflavin complexes, in which a riboflavin molecule has taken up an electron from tryptophan or caffeine, seem of particular significance. Thus the charge-transfer complexes as recently defined and extensively studied for many simple chemical systems by Mulliken[3] may be of fundamental biological importance. For this reason, it seemed useful to investigate the eventual electron-donor or acceptor properties of some principal types of biologically important groups of molecules, in particular, of systems containing conjugated electrons as these have the greatest chances of being involved in electron transfer phenomena. The usefulness of the quantum mechanical approach is, moreover, particularly evident in this field as no experimental data seem to be available about the molecular ionization potentials or electroaffinities of these large biochemical molecules, which could yield information about, respectively, their electron-donor or electron-acceptor abilities.

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[1] I. Isenberg and A. Szent-Gyorgyi, Proc. Natl. Acad. Sci. U. S., **44**, 857 (1958).

[2] H. A. Harbury and K. A. Foley, Proc. Natl. Acad. Sci. U. S. **44**, 663 (1958).

[3] R. S. Mulliken, J. Am. Chem. Soc. **72**, 600 (1950); 811 (1952); J. Phys. Chem. **56**, 801 (1952); Rec. trav. chim. **75**, 845 (1956).

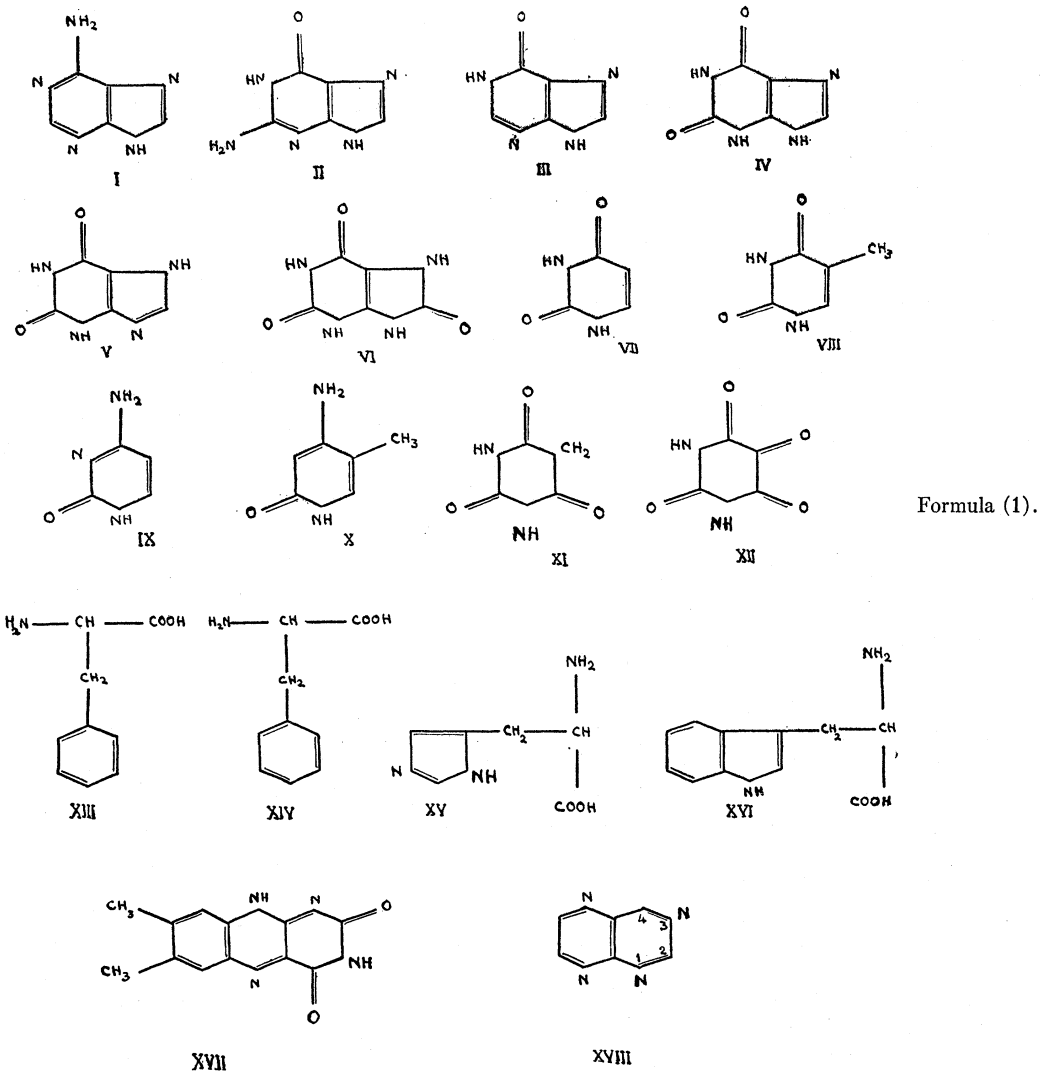
The quantum mechanical approach is based on the LCAO molecular orbital calculations of the distribution of electronic energy levels.[4] Such calculations yield the energies of the molecular orbitals of the mobile or π electrons of the system in the form $E_i=\alpha+K_i\beta$, where α is the Coulomb integral and β is the resonance integral of the method. Positive values of K_i correspond to occupied (bonding) orbitals, negative values of K_i to empty (antibonding) orbitals. The smallest positive value of K_i corresponds to the highest occupied molecular orbital (HOMO), and the comparison of the value of this parameter in a series of related compounds gives the relative value of their ionization potentials: the smaller the value, the lower the ionization potential and consequently, the greater the electron donor capacity of the molecule. The smallest negative value of K_i corresponds to the lowest empty molecular orbital (LEMO), and the comparison of the value of this parameter in a series of related compounds measures the relative value of their electroaffinity: the smaller the value, the greater the electroaffinity.

TABLE I. Energies of molecular orbitals.

Compound	Energy of HOMO	Energy of LEMO
Adenine (I)	0.486	−0.865
Guanine (II)	0.307	−1.050
Hypoxanthine (III)	0.402	−0.882
Xanthine (IV)	0.397	−1.197
Xanthine (V)	0.442	−1.005
Uric acid (VI)	0.172	−1.194
Uracil (VII)	0.597	−0.960
Thymine (VIII)	0.510	−0.958
Cytosine (IX)	0.595	−0.795
5-methylcytosine (X)	0.530	−0.796
Barbituric acid (XI)	1.033	−1.295
Alloxane (XII)	1.033	−0.757
Adenine-thymine pair	0.425	−0.874
Guanine-cytosine pair	0.308	−0.781
Phenylalanine (XIII)	0.908	−0.993
Tyrosine (XIV)	0.792	−1.000
Histidine (XV)	0.660	−1.160
Tryptophan (XVI)	0.534	−0.863
Riboflavin (XVII)	0.496	−0.343
Pteridine (XVIII)	0.864	−0.386
2-amino-4-hydroxypteridine	0.489	−0.650
2,4-diaminopteridine	0.544	−0.508
2,4-dihydroxypteridine	0.653	−0.663

Table I gives the results of the calculation of the energies of these two essential molecular orbitals in a

[4] See, e.g., B. Pullman and A. Pullman, *Les Theories Electroniques de la Chimie Organique* (Masson Ed., Paris, 1952).



Formula (1).

series of biologically fundamental molecules,[5] and the structural formulas are shown in Formula (1). These are:

(a) *Purines.* The compounds studied include adenine (I) and guanine (II), the fundamental bases which enter into the constitution of nucleic acids; hypoxanthine (III), which is a key compound in the *de novo* synthesis of these acids; and xanthine (IV, V) and uric acid (CI), which represent the essential products of metabolic degradation of these acids. Following physicochemical evidence discussed previously,[6] the oxygenated compounds were considered to exist essentially in the keto form. For xanthine, two tautomeric forms were considered, corresponding to the possibility of the hydrogen being attached to N_7 or N_9. Effectively in this molecule, N-methylation at the imidazole ring often results in an N_7-substituted compound (theophylline, caffeine, etc.).

The results show that *purines as a whole should be moderately good electron donors*, but that they should have only very restricted electron-acceptor properties. A particular case is that of uric acid which apparantly should be a much better electron donor than the remaining bases. In fact, the coefficient of the energy of its HOMO is sufficiently small to indicate that it should be a very good electron donor.

(b) *Pyrimidines.* The compounds studied include, in the first place, uracil (VII), thymine (VIII), cytosine (IX), and 5-methylcytosine (X), the four fundamental pyrimidines which enter into the constitution of nucleic acids. They obviously should be only moderate donors and moderate acceptors. *In particular, they should be poorer donors than the fundamental purines.* Moreover, it is interesting to observe that, in striking difference to the metabolic degradation products of purines, the similarly highly oxygenated metabolic degradation pro-

[5] B. Pullman and A. Pullman, Proc. Natl. Acad. Sci. U. S. **44**, 1197 (1958).

[6] A. Pullman and B. Pullman, Bull. soc. chim. France **1958**, 766.

ducts of pyrimidines, barbituric acid (XI), and alloxane (XII) should be very bad electron donors.

(c) *Purine-pyrimidine pairs of deoxyribonucleic acid* (DNA). It is particularly striking and may be particularly important to observe[7] that of the two possible purine-pyrimidine pairs, such as they occur in DNA, following the Watson and Crick model, it is the guanine-cytosine pair which should be at the same time a better electron donor and a better electron acceptor than the adenine-thymine pair.

(d) *Aromatic amino acids of proteins.* The four fundamental aromatic amino acids which enter into the constitution of proteins—phenylalanine (XIII), tyrosine (XIV), histidine (XV), and tryptophan (XVI)—should all be moderate π electron donors and rather poor electron acceptors. Among these molecules, tryptophan is the one which, by far, should be the best π electron donor.

(e) *Riboflavin and pteridines.* The striking fact about the isoalloxazine ring (XVII), which represents th π-electron system of the riboflavin molecule, is that while in the preceding compounds the energy coefficient of the HOMO was generally smaller in absolute value than the energy coefficient of the LEMO, the reverse is true of isoalloxazine. Moreover, for this molecule the coefficient of the LEMO is relatively very small. *Consequently, in contrast to all the preceding groups of compounds, riboflavin, while being a moderate electron donor, should be a good electron acceptor.*

Theoretical computations indicate that an analogous situation should exist to some extent in pteridines. Pteridine itself (XVIII) should, in fact, be nearly as good an electron acceptor as riboflavin. The same is not true about the 2-amino-4-hydroxypteridine, which represents the basic skeleton of natural pteridines, or about 2,4-diamino- or 2,4-dihydroxypteridines. Nevertheless, even in these molecules the coefficient of the LEMO is still small enough for these compounds to be relatively good electron acceptors. Similar properties may then be expected also for folic acid and for pteroic acid.

This assembly of theoretical data finds numerous applications in the study of different properties of the preceeding compounds. Thus, e.g., the electron-donor properties of purines seem to correlate extremely well with the solubility effect of these molecules towards aromatic hydrocarbons,[5] an effect which has been claimed to be of importance for the carcinogenic activity of these hydrocarbons.[8] It is also evident in the previously mentioned complex formation between purines and riboflavin, and in this connection studies on the quenching of fluorescence[9] indicate that purines (adenine, hypoxanthine, caffeine) form such complexes much more readily than pyrimidines (thymine, cytosine), a result in agreement with our calculations. Moreover, the predicted complete difference in electron-donor abilities between the highly oxygenated metabolic degradation products of purines and pyrimidines also appears to be confirmed by experiment: as judged from its solubilizing power toward aromatic hydrocarbons, amines, or benzacridines, uric acid seems to be an excellent electron donor; alloxane, on the contrary, may even rather act as an electron acceptor, e.g., when replacing DPN^+ in alcohol dehydrogenase.[10] The greater electron-donor and electron-acceptor power of the guanine-cytosine pair of DNA over that of the adenine-thymine pair may be of importance for the relative stability of nucleic acids rich in one of these pairs: the formation of a charge-transfer complex between the parallel-stacked pairs and the resultant stabilization of the macromolecule should be greater in nucleic acids rich in the guanine-cytosine pairs. Recent results by Marmur and Doty[11] effectively indicate that the denaturation temperature is appreciably higher for nucleic acids rich in the quanine-cytosine pair. Nevertheless there are also other probably more important reasons which contribute to the same practical situation. Thus, the calculations indicate that the resonance energy of the quanine-cytosine pair should be greater than that of the adenine-thymine pair by about 6–7 kcal/mole.[7]

The results concerning the moderate electron-donor properties of the aromatic amino acids which enter into the constitution of proteins are also verified by experiment. It is predicted that tryptophan should be the best donor by far among these molecules, and Isenberg and Szent-Gyorgyi[7] have effectively shown that this compound is, in fact, the only one of the four amino acids which forms an electron-transfer complex with riboflavin. The easy formation of such a complex with serotonin and different serotonin or tryptamine derivatives and with lysergic acid indicates that the transferred electron comes effectively from the π-electron pool of the indole system.

The electron-acceptor properties of riboflavin already have been mentioned on a number of occasions. The existence of similar but, in agreement with calculations, reduced properties in pteridines has been proved by Fuijmori.[12] These electron-acceptor properties of the pterdine ring seem to be related to the antitumour activity of the folic-acid antimetabolites.

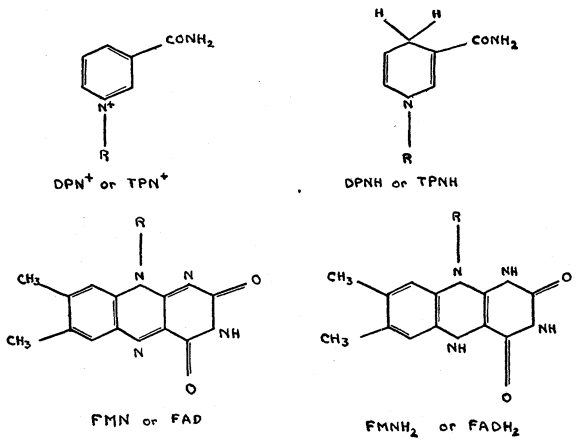
We now go over to a different although closely related subject of compounds whose biological role is related *both* to their electron-acceptor and electron-donor capacities. These are the respiratory enzymes

[7] B. Pullman and A. Pullman, Biochim. et Biophys. Acta **36,** 343 (1959).
[8] E. Boyland, in *Chemical Carcinogenesis,* A Ciba Foundation symposium (Churchill Ltd., London, 1959).
[9] G. Weber, Biochem. J. **7,** 114 (1950).
[10] See, e.g., K. J. Laidler, *Introduction to the Chemistry of Enzymes* (McGraw-Hill Book Company, Inc., New York, 1957) p. 85.
[11] J. Marmur and P. Doty, Nature **183,** 1427 (1959).
[12] E. Fujimori, Proc. Natl. Acad. Sci. U. S. **45,** 133 (1959).

which function as electron carriers in the oxido-reduction chain.

Among the most important of these oxidation-reduction enzymes are pyridine nucleotide enzymes and flavoproteins. In the first class of these compounds, the coenzyme is usually diphosphopyridine nucleotide (DPN) or triphosphopyridine nucleotide (TPN). In the second class, the coenzyme is usually a derivative of the vitamin riboflavin, either flavin mononucleotide (FMN) or flavin-adenine dinucleotide (FAD). In the pyridine nucleotides the reversible reduction occurs at the nicotinamide residue; in the riboflavin derivatives it occurs at the isoalloxazine ring.

The notation given in Formula (2) are used for the oxidized and reduced forms of these electron carriers:



Formula (2).

It may now be shown that the *oxidation-reduction mechanism of the respiratory coenzymes also may be related to the energies of the highest occupied and the lowest empty molecular orbitals of the π electrons of these compounds.*[13]

These energies are reproduced in Table II for the couples DPN^+-DPNH and FMN–$FMNH_2$ (the same results holding for the couples TPN^+–TPNH and FAD–$FADH_2$). Both in DPN^+ and in FMN the lowest empty orbital is a relatively low-lying one (energy coefficient $|K|<0.4$), a result which signifies that these molecules must possess a relatively strong electroaffinity. Consequently, these oxidized forms of the coenzymes are excellent electron acceptors. Their highest filled molecular orbital is also a rather low-lying one (especially that of DPN^+), which means that these molecules are rather poor electron donors.

A complete reversal of this distribution of the energies of the preceeding two orbitals is observed in the reduced forms of the two coenzymes. Their lowest empty orbitals are raised appreciably ($|K|>0.7$), which signifies the disappearance of their electron-acceptor properties. On the other hand, the reduction

TABLE II. Energies of molecular orbitals.

Compound	Energy of HOMO	Energy of LEMO
DPN^+	1.032	−0.356
DPNH	0.298	−0.915
FMN	0.496	−0.343
$FMNH_2$	−0.105	−0.949

is accompanied by a strong elevation of the highest filled molecular orbital ($K<0.3$), a phenomenon which confers on the molecules a great electron-donor power.

Thus, in both couples, FMN-$FMNH_2$ and DPN^+-DPNH, the oxido-reduction is accompanied by an instantaneous redistribution of the energies of the molecular orbitals and, in particular, of those of the lowest empty and the highest filled orbitals in such a way that, in each case, a particularly low-lying empty orbital is associated with the oxidized form and a particularly high-lying filled orbital is associated with the reduced form. The oxidized form thus has a natural tendency to accept electrons and the reduced form to give them up.

Moreover, this natural oscillation process finds a complementary driving force in a very particular property which is associated with $FMNH_2$. This molecule seems to possess a very unusual characteristic which has not yet been observed in another compound, namely, that its highest filled molecular orbital is an antibonding one ($K=-0.105$), the sign of its energy coefficient being that generally associated with orbitals which may be occupied only in the excited state of molecules. This signifies that the occupation of this orbital in the ground state of the molecule represents a fundamentally unstable arrangement and that $FMNH_2$ thus has a particularly strong natural tendency to expel the electrons located at that orbital. This particular result thus accounts not only for the outstanding electron-donor properties of this substance but also for its autoxidability. (In the nonautoxidable DPNH, the highest filled orbital is still a bonding one and its reoxidation necessitates a system with a higher potential, in fact, a flavoprotein.)

This study is being presently extended in our Laboratory to the next group of electron carriers, the cytochromes.

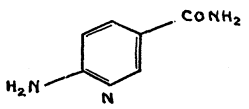
In connection with the curious observation that the highest filled molecular orbital of $FMNH_2$ or $FADH_2$ is an antibonding one, the problem of the possible existence of other compounds possessing the same unusual property has been investigated. It was shown that leucomethylene blue is such a compound, the antibonding character of its highest filled orbital being even more pronounced than that of $FMNH_2$.[14] As a matter of fact, the property should manifest itself in a number of dyes of the phenothiazine series. It occurs in chloropromazine, a powerful drug acting on the nervous system,

[13] B. Pullman and A. Pullman, Proc. Natl. Acad. Sci. U. S. **45**, 136 (1959).

[14] B. Pullman and A. Pullman, Biochim. et Biophys. Acta **35**, 535 (1959).

and it has been suggested that the mechanism of action of such drugs is related to their electron-donor capacities.[15]

Before leaving this field of the respiratory coenzymes it may be interesting to quote an example of the application of the ideas developed here to the problem of cancer chemotherapy. The example is concerned with the 6-aminonicotinamide antagonism of DPN-dependent enzymatic systems.[16] Thus, it has been shown recently[17] that 6-aminonicotinamide (6-AN) [Formula (3)], which is a powerful niacin antagonist, is capable



6-AN

Formula (3).

of causing a marked regression of the 755 tumor in mice. The same authors have also shown that this 6-AN is converted *in vivo* to a 6-AN analog of DPN^+ or TPN^+, which is then postulated to become bound to available apo-dehydrogenases, producing, thus, unusual and ineffective holo-enzymes. It is supposed that in this antagonism, inhibition probably occurs at the initial step of electron transport in the mitochondria, the unusual enzymes being incapable of functioning in the normal electron- and hydrogen-transfer reactions essential for the normal growth of the cell. This would result in the blocking of the oxidative phosphorylation with, as a consequence, an impaired synthesis of ATP, the selective toxicity to tumors being due to quantitative differences in the DPN content of the normal and malignant tissues, the faulty and normal pyridine nucleotides competing for the apo-dehydrogenases. This hypothesis concerning the inability of the 6-AN analog of DPN^+ or TPN^+ to function as normal electron carriers receives confirmation and explanation from the study of the distribution of the energies of the highest filled and the lowest empty molecular orbitals in this compound. These energies are shown in the last column of Table III, from which it can be seen that the substitution of an amino group at the 6-carbon atom of DPN^+ involves an elevation of the energies of both the highest occupied and the lowest empty molecular orbitals. The essential result is the elevation (increase in the absolute value of K_i) of the lowest empty molecular orbital, since it is the energy of this orbital which determines the biological functioning of DPN^+ as an electron acceptor. If the relatively important increase in the absolute value of K_i for this orbital is taken into account, the amino substitution signifies an important diminution of the electron-acceptor properties of the substance, if not their entire disappearance.

TABLE III. Energies of molecular orbitals

Compound	Energy of the highest filled orbital	Energy of the lowest empty orbital
DPN^+	1.032	−0.356
6-AN-DPN^+	0.735	−0.471

The antitumor activity of the antagonist may be considered as resulting effectively from this loss of the electron-accepting capacity.

II. ELECTRONIC STRUCTURE AND CANCER CHEMOTHERAPY

We have been investigating for many years the mechanism of the chemical induction of cancer and have established a relation between the electronic structure of molecules and their ability to induce tumors.[18] In the past few years this research has been extended to the problem of establishing a correlation between the electronic constitution and the antitumor activity of certain molecules. We summarize here very briefly data which have been the subject of a series of papers from our Laboratory.[19–21]

The most significant results have been obtained in the field of the *antimetabolites*. This type of chemotherapeutic agents includes, in the field of cancer, antagonists of purines, pyrimidines, folic acid, glutamine, etc. We illustrate the mode of approach to the problem and the nature of the results that may be obtained on the example of *the purine antimetabolites*.

The LCAO approximation of the molecular-orbital method has been used for the calculation of the different types of possible electronic characteristics of these molecules: energies of the molecular orbitals, charge distribution, bond orders, free valences, etc. The compounds studied included essentially:

(1) *Reference compounds*. These are adenine (XIX) and guanine (XX) [see Formula (4)], the two fundamental purine bases which enter into the constitution of nucleic acids, and hypoxanthine (XXI), a key compound in the *de novo* synthesis of these acids [the antitumor activity of purine antimetabolites being most probably related to the inhibition of the conversion of inosinic acid into adenylic or guanylic acids in this *de novo* synthesis (Fig. 1)].

(2) *Compounds showing antitumor activity*. These are 6-mercaptopurine (XXII), thioguanine (XXIII), 2,6-diaminopurine (XXIV), 8-azaguanine (XXV),

[15] G. Karreman, I. Isenberg, and A. Szent-Gyorgyi, Science (in press).

[16] B. Pullman and A. Pullman, Cancer Research **19**, 337 (1959).

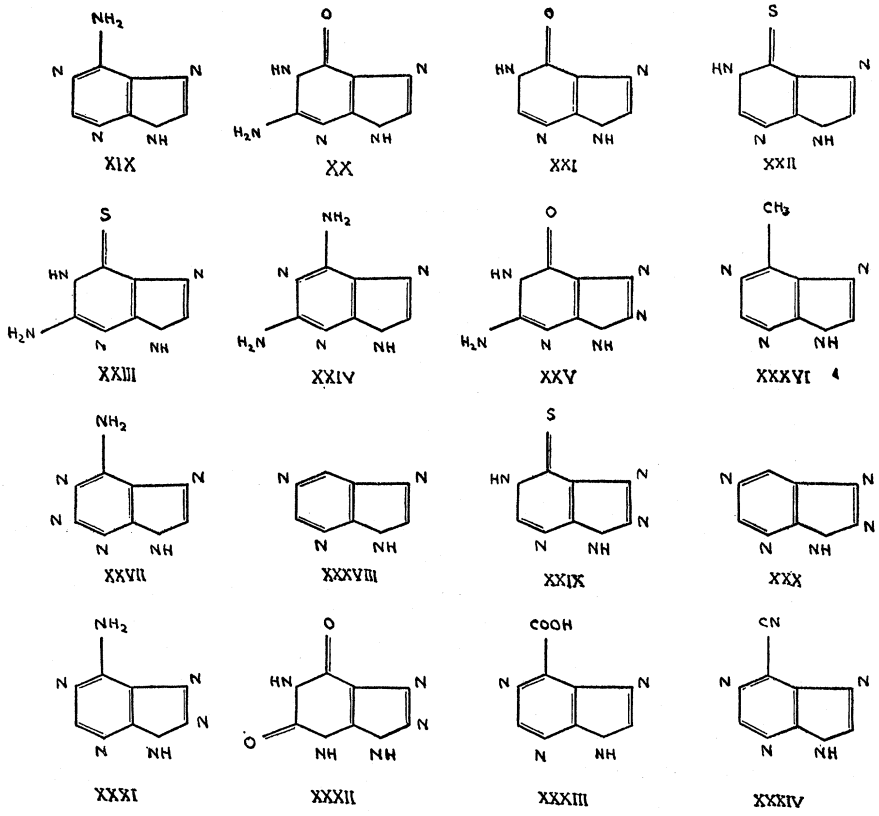
[17] L. S. Dietrich, L. A. Kaplan, I. M. Friedland, and D. S. Martin, Cancer Research **18**, 1272 (1958).

[18] A. Pullman and B. Pullman, *Cancérisation par les substances chimiques et structure moléculaire* (Masson Ed., Paris, 1955).

[19] A. Pullman and B. Pullman, Bull. soc. chim. France **1958**, 766, 973; **1959**, 594.

[20] T. Nakajima and B. Pullman, Bull. soc. chim. France **1959**, 1502.

[21] A. Pullman, B. Pullman, and T. Nakajima, Bull. soc. chim. France **1959**, 590.



Formula (4).

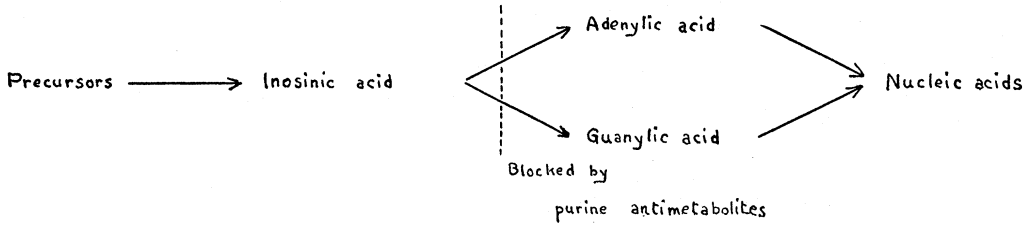


FIG. 1. *De novo* synthesis of nucleic acids.

6-methylpurine (XXVI), 2-azaadenine (XXVII), and purine (XXVIII). These are, in fact, all the essential compounds in this group.

(3) *Compounds closely related to the preceeding ones but devoid of antitumor activity.* These are 8-aza-6-mercaptopurine (XXIX), 8-aza-purine (XXX), 8-aza-adenine (XXXI), 8-azaxanthine (XXXII), 6-carboxy purine (XXXIII), and 6-cyanopurine (XXXIV). Many other potential purine antimetabolites did not show any antitumor activity, but their structure is generally more different from that of the active antimetabolites than that of the compounds which we have studied. It was tentatively assumed that the most important information about the mechanism of action may be obtained if the inactive compounds differ as little as possible in their apparent molecular structure from the active ones, many "trivial" reasons being possibly responsible for the absence of antitumor activity in compounds whose molecular configuration differs to a large extent from that of the natural bases.

A detailed examination of the results obtained led to the conclusion that a definite correlation seems to exist between the structure of the antimetabolites at the electronic level, and the existence of an antitumor activity. *The correlation seems to involve the ring nitrogens of the purine skeleton and* is of a twofold nature (Fig. 2).

(1) *Relation between the electrical charge of the hydrogen-bearing N_9 and the antitumor activity.* Because of the participation of its lone pair in the conjugation of the π-electron pool of the purine ring, this nitrogen bears, in all the compounds studied, a formal positive charge.

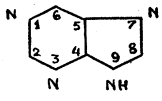


FIG. 2. Numbering of atoms in the purine skeleton.

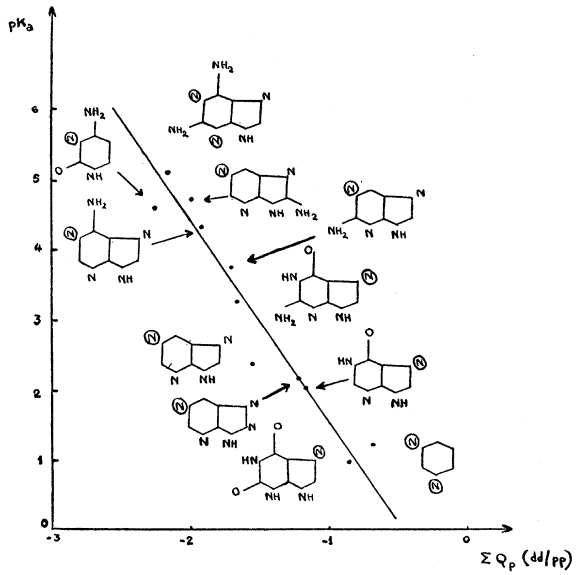


FIG. 3. Calculated versus experimental basicities in purines and pyrimidines.

This charge lies in the limits of $+0.400e$ to $+0.419e$ in the reference compounds, in the limits of $+0.399e$ to $+0.431e$ in the active antimetabolites and in the limits of $+0.437e$ to $+0.441e$ in the inactive ones. Thus, as a general rule, *the positive formal charge of N_9 is greater in the inactive antimetabolites than in the active ones, and in those latter compounds it is of the same order of magnitude as in the natural bases.* This correlation receives a simple biochemical interpretation: N_9 is the place of the metabolic ribosidation (and ribotidation) of the bases, and it may be shown[22] that the stability of the ribosides should be the greater the smaller the positive charge on this nitrogen. The ribosides of the active antimetabolites should thus be more stable (and of stability comparable to that of the ribosides of the natural bases) than those of the inactive ones. The experimental evidence seems effectively to indicate[23] that the potential purine antagonists can exhibit antitumor activity only after their transformation into the corresponding ribosides (or ribotides), and it appears even that the development of resistance on behalf of the organism to the action of the drugs is related to the loss of its ability to riboside them.[24]

(2) *Relation between the basicity of the remaining ring nitrogens and the antitumor activity.* The remaining hydrogen-free ring nitrogens bear a formal negative charge. It is frequently supposed that their relative basicities should depend directly on this charge, which is supposed to give a measure of their attraction toward a proton. A more refined recent study of the problem has shown, however,[25] that in such polyaza-compounds the basicity is, in fact, determined by a more complicated expression of the type:

$$B = C^{2+} + \sum_{p \neq d} Q_p (dd \mid pp),$$

where Q_p is the formal charge of the nitrogen atom and (dd/pp) the Coulomb integral between an electron of the lone pair of the N atom and the π electron of atom p, the summation being carried out on all the atoms p of the cycle. This formula seems to give excellent results in predicting the relative basicities in related compounds as may be seen from Fig. 3, in which the quantity $\sum_{p \neq d} Q_N (dd/pp)$ is plotted against the experimental pK_a's of a series of biologically important purines and pyrimidines. (The circles on the formulas indicate the most basic nitrogen in each compound.)

TABLE IV. Basicity and antitumor activity.

Compound	Position of the most basic nitrogen	$\Sigma_{p \neq d} Q_p$ $(dd \mid pp)$	Exptl. pK_a	Anti-tumor activity
2,6-diaminopurine	N_1	−2.21	5.09	+
2-azaadenine	N_1	−1.96		+
Adenine	N_1	−1.91	4.22	Natural
Guanine	N_7	−1.67	3.3	bases
8-azaadenine	N_1	−1.65		−
Purine	N_1	−1.52	2.39	+
8-azaguanine	N_7	−1.46		+
6-methylpurine	N_1	−1.4	2.6	+
6-mercaptopurine	N_1	>-1.35	<2.5	+
8-azapurine	N_1	−1.22	2.12	−
6-cyanopurine	N_1		0.7	−
8-azaxanthine	N_7	−0.55		−

When considered in relation to the antitumor activity, these results lead, as can be inferred from Table IV, to the conclusion that there seems to exist a rather satisfactory correlation between this activity and the basicity. Thus, *all the compounds which manifest antitumor activity are more basic than those which are devoid of such an activity.* Perhaps a more significant way of presenting the correlation may be to say that the basicity *of the active antimetabolites seems to be closer around that of the natural bases, adenine and guanine, than that of the inactive compounds.* Moreover, it should be observed *that the most basic ring nitrogen of the active antimetabolities is predicted to be either N_1 or N_7, which are also the most basic nitrogens of, respectively, adenine and guanine.*

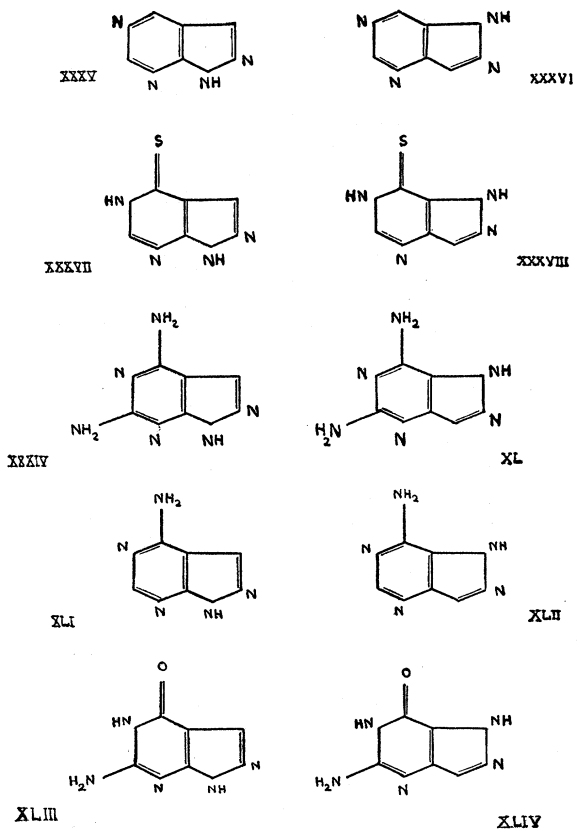
The importance of this correlation between antitumor activity and basicity (basic *strength* and *position* of the most basic ring nitrogen) received strong support from a recent investigation on the antitumor activity of

[22] A. Pullman and B. Pullman, Proc. Natl. Acad. Sci. U. S. **45**, 1572 (1959).

[23] G. B. Brown and M. Balis, *The Leukemias*, Henry Ford Hospital International Symposium (Academic Press, Inc., New York, 1957), p. 541; J. M. Buchanan, *ibid.* p. 552; B. R. Baker in *Chemistry and Biology of Purines*, A Ciba Foundation Symposium (Churchill Ltd., London, 1957), p. 270; J. J. Biesele, *Mitotic Poisons and the Cancer Problem* (Elsevier Publishing Company, Inc., Amsterdam, 1958).

[24] See, e.g., R. W. Brockman, H. E. Skipper, and J. R. Thomson, Proc. Am. Assoc. Cancer Research **2**, 284 (1958).

[25] T. Nakajima and A. Pullman, J. chim. phys. **56**, 493 (1958).



Formula (5).

pyrazolopyrimidines. Pyrazolopyrimidines are isomers of purine differing from this last compound only in the position of the nitrogen atoms in the imidazole ring. Two fundamental pyrazolopyrimidine rings are known, XXXV and XXXVI [see Formula (5)]. A great number of their derivatives have been investigated experimentally[26] in view of an antitumor activity, the compounds studied including the analogs of the active purines, e.g., the analogs XXXVII and XXXVIII of 6-mercaptopurine or the analogs XXXIX and XL of 2,6-diaminopurine. The analogs XLI and XLII of adenine and XLIII and XLIV of guanine also have been investigated. The important result was obtained that out of about a hundred molecules studied, only the adenine analog (XLIII) (and some of its close derivatives) have exhibited antitumor activity.

These results become immediately understandable if examined in the light of our present findings on the relation between antitumor activity and basicity.[21] It is then found theoretically that in all the pyrazolopyrimides studied, with the exception of the pyrazolopyrimidine XXXV and the adenine analog XLIII (and some of its close derivatives), the most basic ring nitrogen is at position 3 and no more at positions 1 or 7 as it is in the natural bases, adenine and guanine, or in the active "classical" purine antimetabolites. It is only in XXV and XLIII that the most basic nitrogen is predicted to be at N_1. Moreover, it is predicted that while the basicity of the active adenine analog XLIII should be close to that of adenine and thus lie in the interval of basicities of the active "classical" antimetabolites, that of the inactive XXXV should be much smaller and lie outside this interval (the values of the $\sum Q_p(dd/pp)$ for these two compounds are computed to be, respectively, -1.67 and -1.23). Thus, the preceeding correlation between basicity and antitumor activity appears to be an excellent selection rule permitting us to pick out the active compound from a large group of inactive ones. Very recently the basicities of the pyrazolopyrimidines have been determined experimentally[27] and the authors of this determination have also arrived at the conclusion that there is a relation between the basicity of the compounds and their antitumor activity. Nevertheless, these authors had quite a number of exceptions in their correlation which are not exceptions in our proposition. This is due to the fact that they have considered only the correlation between the basic strength and antitumor activity, while we have also taken into consideration in this respect the *position* of the most basic nitrogen on the molecular periphery. As we have seen, the most basic nitrogen of the pyrazolopyrimidines should most commonly be N_8, in which case the compounds should, following our proposition, be devoid of antitumor activity. In connection with this result the question may be raised about the reason for which the most basic ring nitrogen should be localized at N_1 (or eventually at N_7) in the active antimetabolites. Although no definite answer can be given as yet to that question, it may nevertheless be remarked that N_1 of the purine skeleton is the nitrogen which participates, through a hydrogen bond, in the formation of the purine-pyrimidine pairs of DNA. It may thus tentatively be supposed that the ability of the potential analogs to enter into the nucleic acids in place of the natural bases (a procedure may be related to their antitumor activity) depends both on the absolute and the relative value of the basicity of that nitrogen. As to the importance of N_7, this may perhaps be related to the participation of this position in an eventual complex formation with the active centers of enzymes or with metal ions.[19,20]

Finally, it may be added that no correlation exists in the series of the pyrazolopyrimidines between their antitumor activity and the charge of their hydrogen bearing N_9. Correlatively, experimental data indicate that in these molecules, ribosidation is not a necessary condition for the existence of antitumor activity.

These researches on the relation between submolecular structure and antitumor activity are prac-

[26] H. E. Skipper, R. R. Robins, J. R. Thomson, C. C. Cheng, R. W. Brockman, and M. Schabel, Cancer Research **17**, 579 (1957).

[27] B. M. Lynch, R. K. Robins, and C. C. Cheng, J. Chem. Soc. (**1958**), 2973.

TABLE V.

Atom	δ	Bond	η
$=\ddot{N}-$	0, 4	$C=\ddot{N}-$	1
$-\ddot{N}-$ |	1	$C-\ddot{N}-$ |	0, 9
$=N-$ |$^+$	2[a]	$C=N-$ |$^+$	1
$=O$	1, 2	$C=O$	2
$-\ddot{O}-$	2	$C-\ddot{O}-$	0, 9
$=S$	0	$C=S$	1, 2
$-\ddot{S}-$	0	$C-\ddot{S}-$	0, 6
$C_{arom}-C_{aliph}\equiv H_3$	$C_{arom}=-0.1$ $C_{aliph}=-0.2$	$C-C$ $C\equiv H_3$	0, 1 2

[a] In this case there are also δ's=0.3 on the C atoms adjacent to $=N-$ \|$^+$.

tically in their very beginnings. The results obtained so far seem greatly encouraging. It is hoped that a better understanding of this relation may lead to a rational approach to chemotherapy in place of the purely empirical present one. In the field of chemical carcinogens, the success of the theory was such that it led to the creation of more powerful carcinogens than the previously known ones. It is hoped that one day the same might be done in the field of cancer chemotherapy.

CONCLUSIONS

We have chosen two subjects among those which are presently being studied in our Laboratory in the field of electronic biochemistry. Among other important problems on which work is far advanced but which, cannot be described here are, e.g., studies on the electronic structure of the energy-rich phosphates, on the mechanism of action of hydrolytic enzymes, on the mechanism of action of decarboxylating enzymes, on the electronic structure of the bile pigments, on the electronic structure and biochemical role of porphyrins, etc. We are also continuing our work on the relation between electronic structure and carcinogenic activity, especially in the field of the aromatic amines and the aza-compounds.

Electronic biochemistry appears as a most thrilling development of theoretical chemistry and also is a wide-open gateway to submolecular biology through which a fundamentally better understanding of the secrets of life should be reached.

APPENDIX

The energies of the Coulomb and the exchange integrals involving heteratoms being of the type

$$\alpha_r=\alpha_c+\delta\beta_{c=c}$$

and

$$\beta_{r-c}=\eta\beta_{c=c},$$

the set of δ's and η's in Table V has been used in the calculations referred to in this paper.

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Semiempirical Theory of Vibronic Interactions in Some Simple Conjugated Hydrocarbons*

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FOR most problems in the theory of molecular structure, the electronic and nuclear motions may be viewed separately. However, for the comprehension of the appearance of certain weak electronic absorption bands and of the geometrical instability of particular molecular conformations of degenerate electronic systems, this is no longer possible. To see this let us first consider the variation of the electronic energy of $C_6H_6^+$ with nuclear displacements. The charge density functions are as pictured in Fig. 1 and a few selected permissible nuclear displacements are shown in Fig. 2.

If the nuclei composing the $C_6H_6^+$ molecule are displaced, the Coulombic potential energy V changes; and the magnitude of this change, for small displacements, is given by the first few terms in the Taylor series expansion

$$V_{\text{displaced}}=V^0+\sum_j S_jV_j'+\tfrac{1}{2}\sum_{j>k} S_jS_kV_{jk}''+\cdots, \quad (1)$$

or

$$V_{\text{displaced}}-V^0=\Delta V=\sum_j S_jV_j'+\tfrac{1}{2}\sum_{j>k} S_jS_kV_{jk}''+\cdots. \quad (2)$$

As the change in the electronic energy engendered by this change in the Coulombic potential energy ΔV is given by the summation of the charge density times the

* Presented at the William E. Moffitt Memorial Session.