QUANTUM THEORY PROJECT
FOR RESEARCH IN ATOMIC, MOLECULAR AND SOLID STATE
CHEMISTRY AND PHYSICS
UNIVERSITY OF FLORIDA, GAINESVILLE, FLORIDA

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Per-Olov Löwdin

PREPRINT NO. 98
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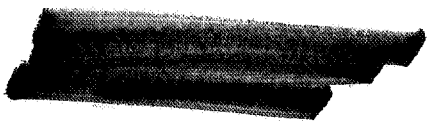
*This paper will be published in the Proceedings from the Study Week on Intermolecular Forces at the Pontifical Academy at the Vatican, Rome, April 18-24, 1966.



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1. INTRODUCTION

During the last three decades, there has been a very strong tendency towards the unification of all the natural sciences: physics, chemistry, and biology. After the discovery of modern quantum theory in 1925, physics and chemistry were unified in 1928 through the quantum-mechanical explanation of the nature of the covalent chemical bond by Heitler and London[1] leading to the development of quantum chemistry. In cellular biology, most of the important achievements have been obtained by considering the cells as huge chemical and physical systems subjects to the ordinary laws of chemistry and physics, which has lead to the development of biochemistry and biophysics. In the recent development, more and more emphasis has been put on the fundamental molecular structure of the living systems and on the detailed electronic and protonic structure of the molecules involved leading to the development of molecular and submolecular biology[2]. The electrons and protons are fundamental particl which do not obey the laws of classical physics but the laws of modern quantum mechanics, and the detailed structure of the biologically interesting molecules has hence to be treated by quantum chemistry which has lead to the opening of a new field which should perhaps be called "quantum biology". In describing nature in terms of a few elementary particles, physics, chemistry, and biology have hence established a common basis, and we note that this unification has become conceivable only through the development of modern quantum theory in addition to and as a consequence of the fundamental experimental discoveries.

It is clear that, also in the biological systems, the question of intermolecular forces must be of essential importance and, in this paper, we will concentrate our interest on a particular link between molecules which is known as the "hydrogen bond". The fundamental importance of this bond in biology seems first have been clearly pointed out by Pauling[3] and his associates, who used it to explain the stability of the secondary structure of the proteins in the helical polypeptides. Today it is believed that the hydrogen bond plays a fundamental role in many of the most important aspects of biochemistry, and in this paper we will particularly discuss the properties of the hydrogen bond in connection with questions concerning the structure and stability of the genetic code.

2. STRUCTURE AND STABILITY OF DNA; INTRODUCTION

The development in biology during the last three decades has shown that the genetic message in the chromosomes is contained in a giant molecule called deoxyribonucleic acid (DNA). Of the landmarks of the history of this fascinating discovery we will here mention only Griffith and his genetic transformation principle in 1928, Avery, Mac Leod, and McCarty and their identification of the compound carrying the transformation and the genetic message with the well-known deoxyribonucleic acid in 1944, Chargaff and his pioneering studies of the base contents of DNA in 1950, Wilkins and his X-ray investigations of the stereo structure of DNA in 1953, and finally the Watson-Crick stereo model of DNA in the same year. For a more complete account, we would like to refer the reader to another paper[4]. According to the Watson-Crick[5] model, the DNA molecule is a double helix consisting of two intertwined helical sugar-phosphate chains held together by a sequence of pairs of nitrogen bases joined by hydrogen bonds. There are only four such nitrogen bases involved, namely adenine A, guanine G, thymine T, and cytosine C. Depending on the special structure of these hydrogen bonds, the pairing of the nitrogen bases in DNA is specific according to the scheme A-T, G-C, and each base pair consists of two "complementary" bases. There is strong evidence for believing that the genetic information is contained in the sequence of base pairs along the DNA-molecule or, which is apparently the same, in the sequence of bases along a single strand, i.e. in the form of a "chemical script" of four symbols, e.g. ATTAGCATG ... . Even for such a simple organism as E. Coli, the script contains about two million "signs".

Before the cell duplication, the DNA-molecule has to replicate itself and, in this process, the hydrogen bonds are released, the two DNA strands become at least partly free, and each one serves as a template for the formation of a new complementary strand formed from mutation material in the environment, so that two complete DNA molecules with the same base-pair sequence as the original one are produced. The schematic structure of DNA, the rough template character of the base pairs, and the selection of complementary bases at DNA-replication are illustrated in Figs. 1-3, respectively.

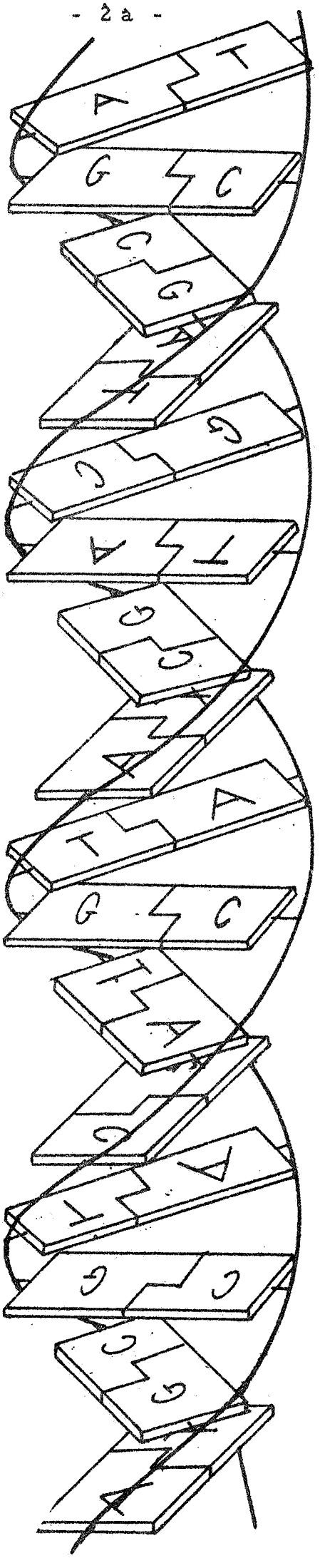


Fig. 1 a. Schematic structure of the DNA molecule carrying the genetic code.

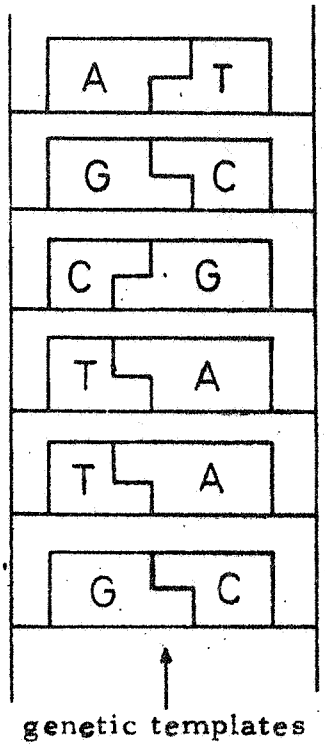


Fig. 1 b. The structure of the genetic code unwound. The genetic templates are the surfaces in the middle indicated by the broken lines; they are held together by hydrogen bonds.

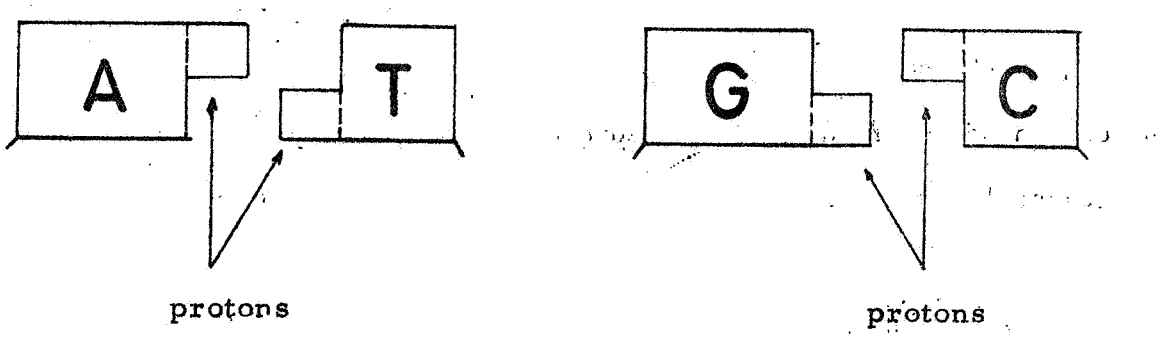


Fig. 2. Schematic form of the templates formed by the normal bases occurring in DNA.

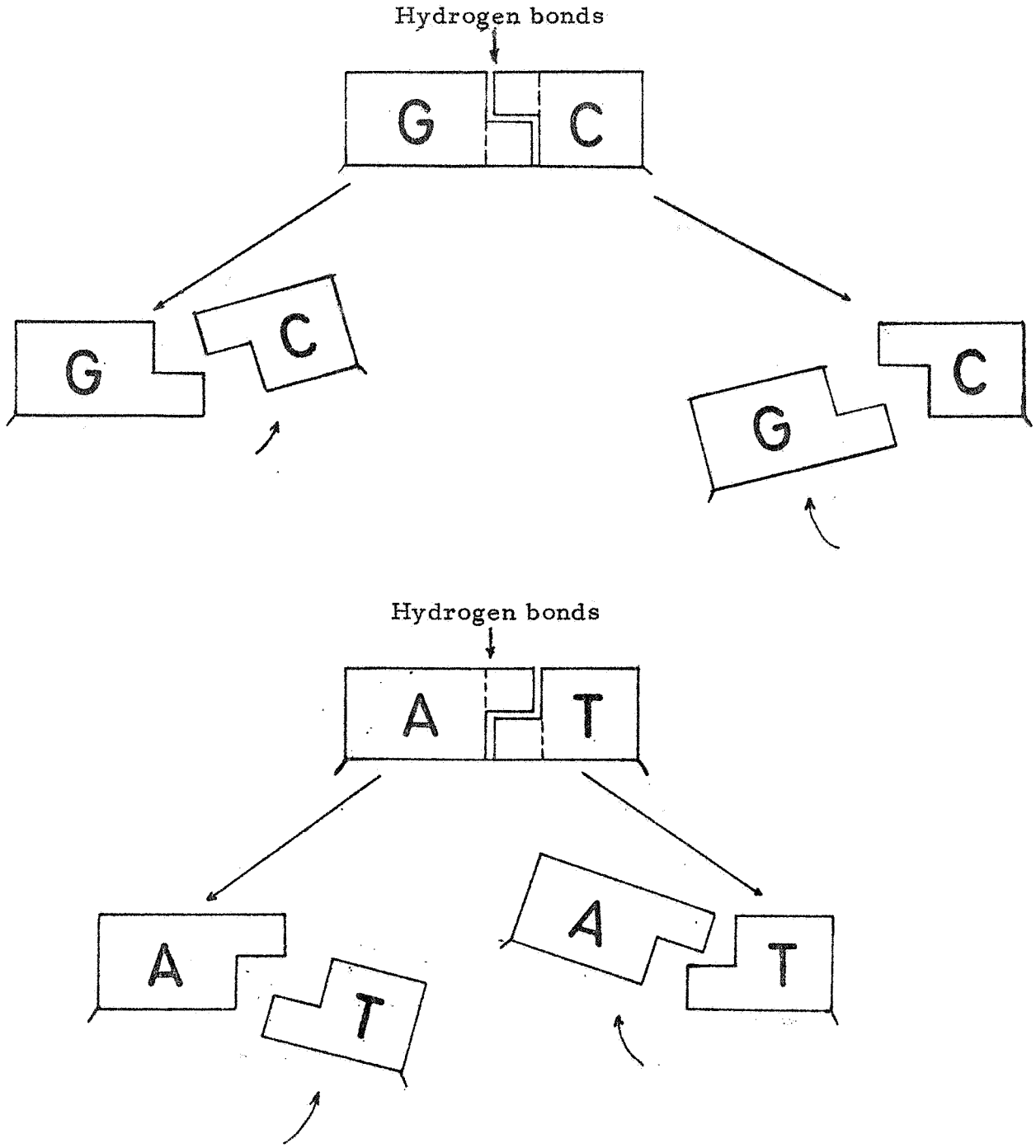


Fig. 3. Selection of complementary bases at the replication of DNA; curved arrows indicate that the bases come from the environment.

Watson and Crick also pointed out that the nitrogen bases may occur in certain rare tautomeric forms A^*, T^*, G^*, and C^*, which have another pairing scheme A^*-C, G^*-T, etc. depending on the change of the template; see Fig. 4. Incorporation of these tautomeric forms may then cause errors in the genetic code leading to mutations; see Fig. 5.

The template idea of the genetic code may to a certain extent be tested by treating DNA with certain chemicals which change the template in a specific way, as e.g. nitrous acid. This compound causes an "oxidative deamination" of the bases which influences the template, so that, for instance, adenine goes over into hypoxanthine with a guanine-like template. It turns out that nitrous acid, as well as other compounds with a similar template effect, are strong mutagens[6].

The Watson-Crick model of DNA, the complementary concept, and the template idea have been of fundamental importance in the recent development of biochemistry and medicine. Even if these ideas have been almost universally accepted, they have also been criticized by some prominent scientists who have questioned whether the template really has such physical and chemical properties that it explains the enormous stability of the genetic code over thousands and thousands of years. Commoner[7] has expressed the opinion that, at the normal temperature of the human body ($T = 310^{\circ}$ K), the thermal disturbances of the order $kT = 0,03$ eV are enough to destroy in the long run any perfect order of electrical charges in the template, and that any theory of the stability of the genetic code has to take this fact into account. Heitler[8] - one of the fathers of quantum chemistry - chose in 1964 the DNA-replication as an example of a biological process which has such an enormous accuracy that it can hardly be understood on the basis of the present laws of physics and chemistry.

The problem of the occurrence of incorporation errors in the DNA replication(see Fig. 5) was further emphasized by some recent measurements of the tautomeric equilibrium constants of some of the nitrogen basis by Katrizky and Waring[9] which indicated that somewhat less than 1 o/oo of the "short" bases may occur in the rare tautomeric forms.

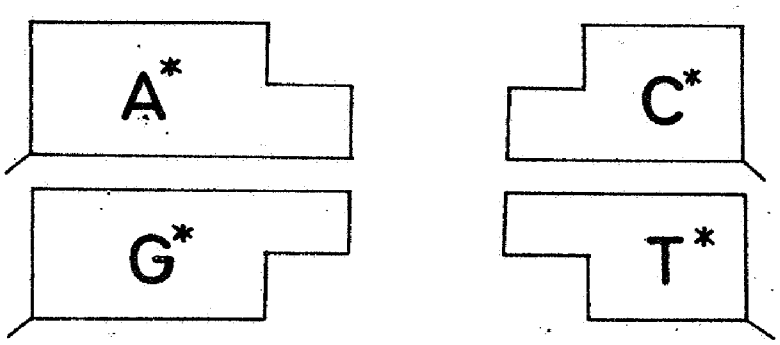


Fig. 4. Schematic form of the templates of the rare tautomeric forms of the bases occurring in DNA.

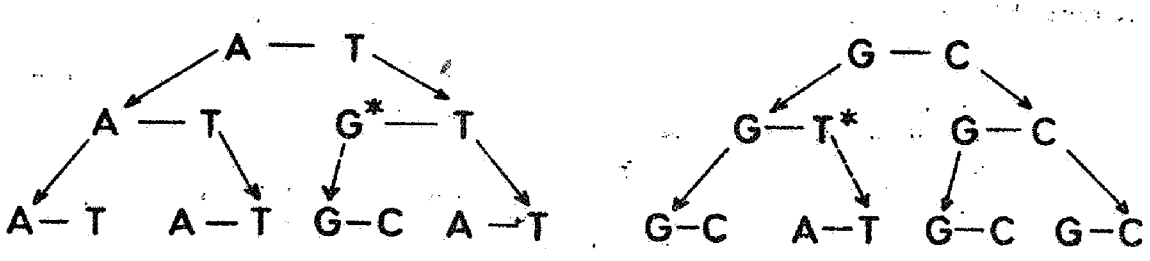


Fig. 5. Effect of incorporation errors; note that diagrams are only very schematic, and that the processes in reality are considerably more complicated (see also Fig. 30).

This implies that, since a DNA molecule usually consists of more than a million base pairs, there may be several hundred tautomeric errors per replication. Experience tells us, however, that the replication mechanism is remarkably exact, and that such a large number of incorporation errors in some way is avoided.

It is hard to give reliable figures for the internal stability of DNA or for the over-all accuracy at a DNA-replication. After careful studies of experimental mutation rates, Freese[10] has found that the probability for a spontaneous error per base pair and generation should be in the range 10^{-8} to 10^{-11}, which means that spontaneous mutations are exceedingly rare events and that the genetic code has a most unusual internal and replication stability.

Since the Watson-Crick model is of such a fundamental importance, the author felt that it may be worthwhile to investigate the problem of the stability of the genetic code as expressed in the template idea in greater theoretical detail. A preliminary study[11] of the problem showed that there is an additional element of instability in the hydrogen bonds themselves. In classical chemistry, a hydrogen bond is a hydrogen atom simultaneously attracted to two electronegative atoms, but, in modern terminology, a hydrogen bond is a proton shared between two electron lone pairs. Each electron pair gives rise to a potential well for the proton, and the proton is hence usually situated in a "double-well" potential with two classical equilibrium points. The proton may jump from one of these equilibrium positions to the other by going over the potential barrier between them[12], or it may move through the barrier by means of the quantum-mechanical tunnel effect as emphasized by the author[11]. If a proton changes place, it is often causing another proton to move to keep the gross electric neutrality, and the total effect will be a change of the genetic templates which is equivalent to an internal tautomeric shift; see Figs. 6 and 7. This effect is temperature dependent and, at $T = 310^{\circ}K$, this proton transfer above or through the barrier may be a very important source of error which may greatly disturb the stability of the genetic code. In our study, it seemed hence necessary to take the possibility of such proton jumps into full account.

Fig. 6. Internal tautomeric shift in a base pair in DNA through proton exchange by means of the quantum-mechanical tunnel effect or above the barrier.

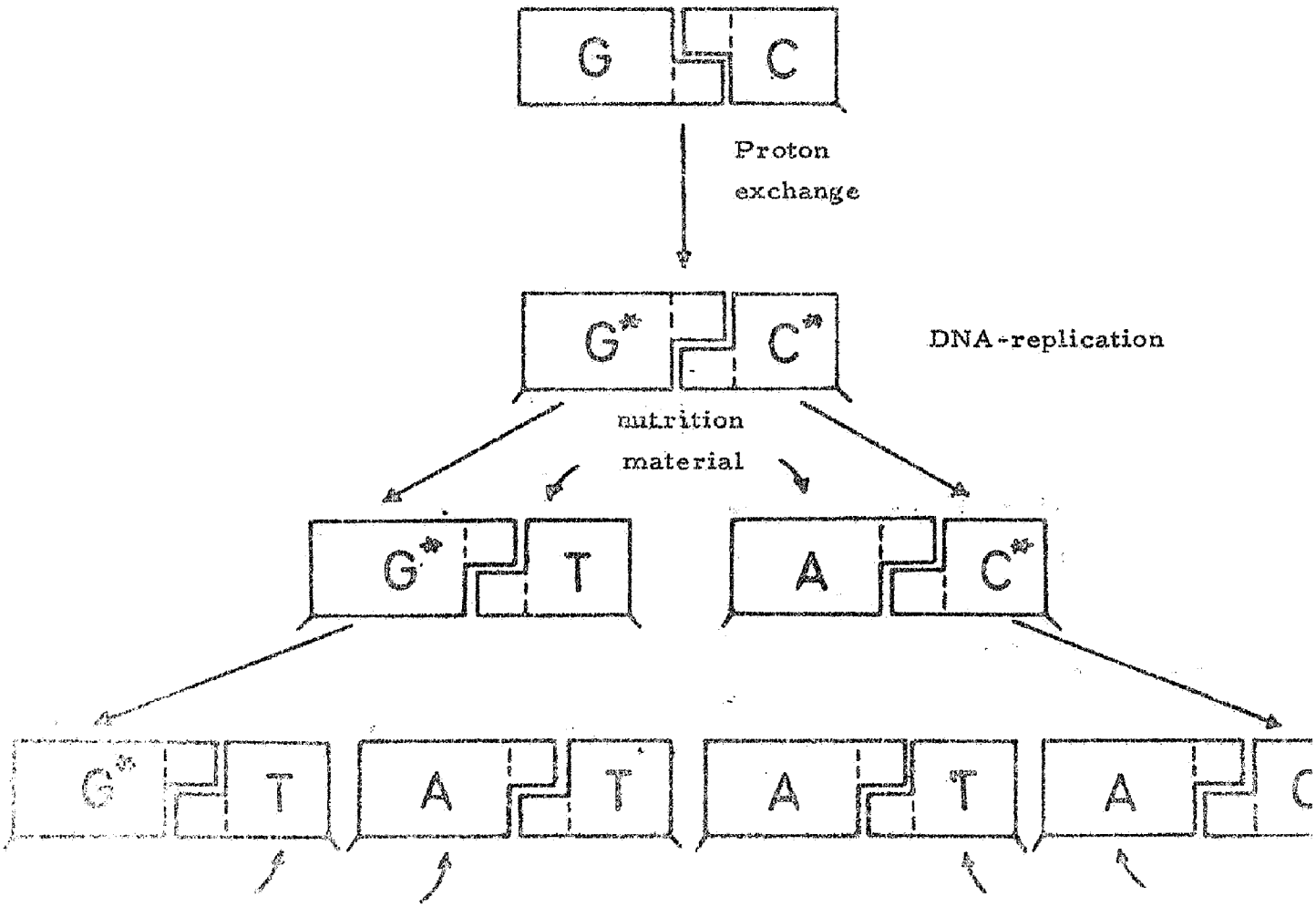


Fig. 7. Formation of rare base pairs through proton exchange and the associated replication scheme.

Theoretical investigations of the stability of the genetic code have been carried out at the Quantum Chemistry Group of Uppsala University during the last three years. Even if the results I can report here are only of a preliminary nature, they are still highly favourable in the sense that they indicate that the genetic code in the Watson-Crick model seems to have enough long-time stability to correspond to the experimental experience.

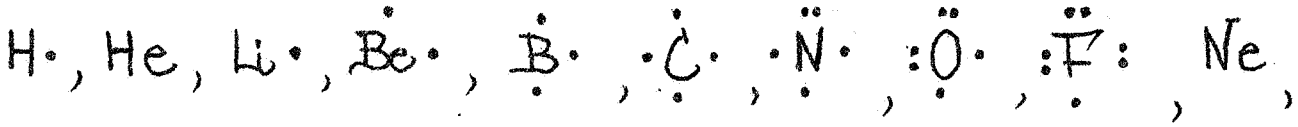
It should perhaps be pointed out that our main problem has not been to find new mutation mechanisms but to explain why spontaneous mutations as a whole are as rare as they are, in spite of all possible disturbances of the genetic code. In this connection, we believe that we have clarified at least some of the possible mechanisms for spontaneous mutations as well as some specific models for induced mutations caused by radiation or the influence of specific chemicals. All theoretical results obtained so far confirm the template idea and the importance of the hydrogen bonds in storing and transferring the genetic information.

3. THEORETICAL ANALYSIS OF THE CHARACTER OF THE GENETIC CODE

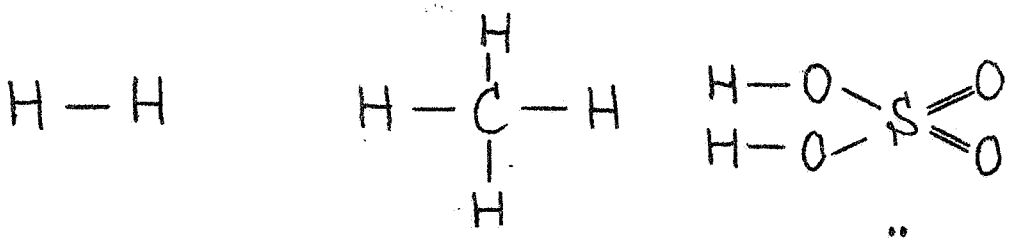
In the previous section, we have only indicated the genetic template in a very crude way, and it is now desirable to analyze it in greater detail. The nucleic acids were discovered by Miescher in 1868, and their chemical structure had been revealed by skilful chemical methods by Hammarsten, Todd, Caspersson, and many others[13] long before one had realized their fundamental importance in genetics. It had been found that they consisted of sugar-phosphate chains to which their were four nitrogen bases (A, G, T, C) attached. In the "tetranucleotide hypothesis", it was assumed that these bases occurred repeatedly in a regular order, but Chargaff could show that this is not the case and that, for DNA, one has only the rule $[A] = [T]$, $[G] = [C]$ for the molar contents. In the Watson-Crick model of DNA, the nitrogen bases may occur in an arbitrary order corresponding to the "genetic script", and the Chargaff rule forms the essential experimental basis for the specific base pairing A-T, G-C in linking two chains together in the double helix.

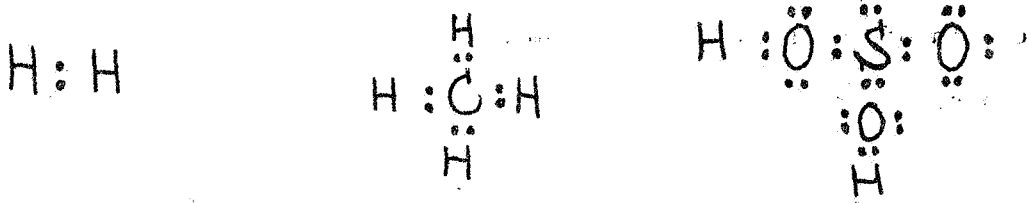
The stereostructure of DNA is illustrated in Figs. 8 and 9. We will here particularly emphasize the nature of the hydrogen bonds, and we note that the A-T pair has two and the G-C pair three such bonds consisting of hydrogen atoms shared between the electronegative atoms nitrogen and oxygen in the combinations NH --- N or NH--- O.

In order to proceed, it is necessary to study these hydrogen bonds in greater detail, and we will then also consider their electronic structure. According to Lewis, it is convenient to let a chemical symbol denote the atomic nucleus plus the electronic rare-gas shell and indicate the valence electrons with additional dots. In this notation, the symbol H means hence a proton, whereas the hydrogen atom is denoted by H•. For the lightest elements, one obtaines the notations:

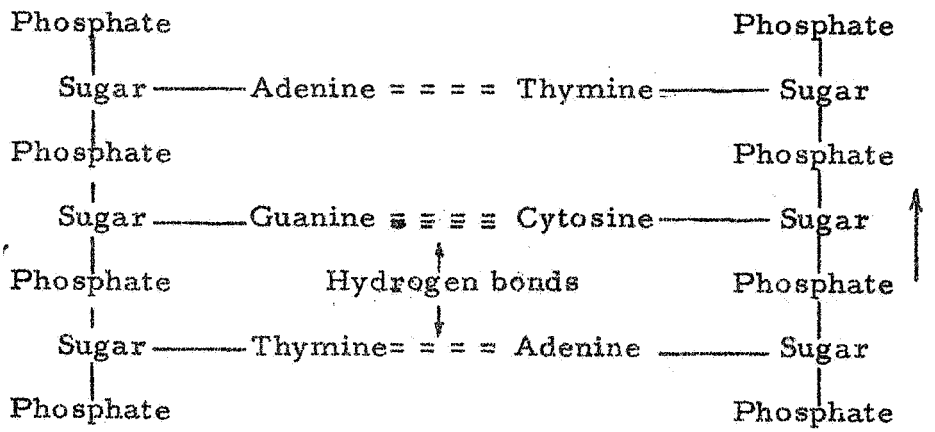


Lewis also observed in 1916 that, in a chemical compound, each covalent bond was in some way associated with an electron pair: shared between the two atoms linked by the bond, for instance:





The deeper explanation of this bond was later given by quantum-mechanical methods by Heitler and London[1]. Each bond has further a particular direction, and this directional property was treated quantum-mechanically by the idea of orbital hybridization introduced independently by Slater[14] and Pauling[15] in 1932. An atomic s-orbital is spherically symmetric around the nucleus, whereas a p-orbital has an angular distribution in the form of dumble consisting of two spheres with different signs; see Fig. 10. By a superposition of an s-orbital and a p_k-orbital,



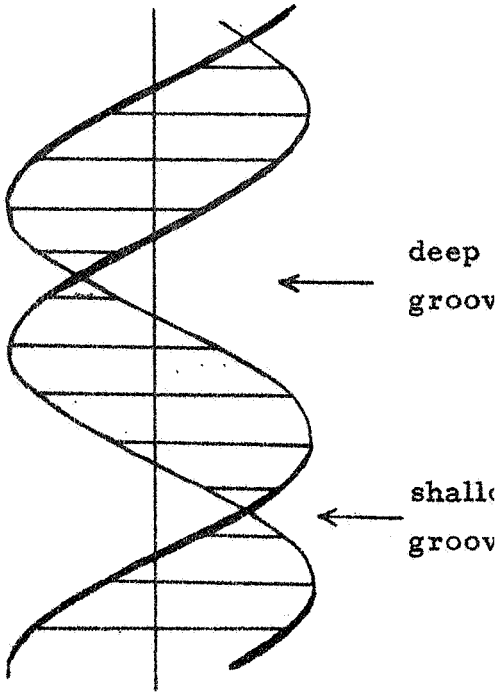
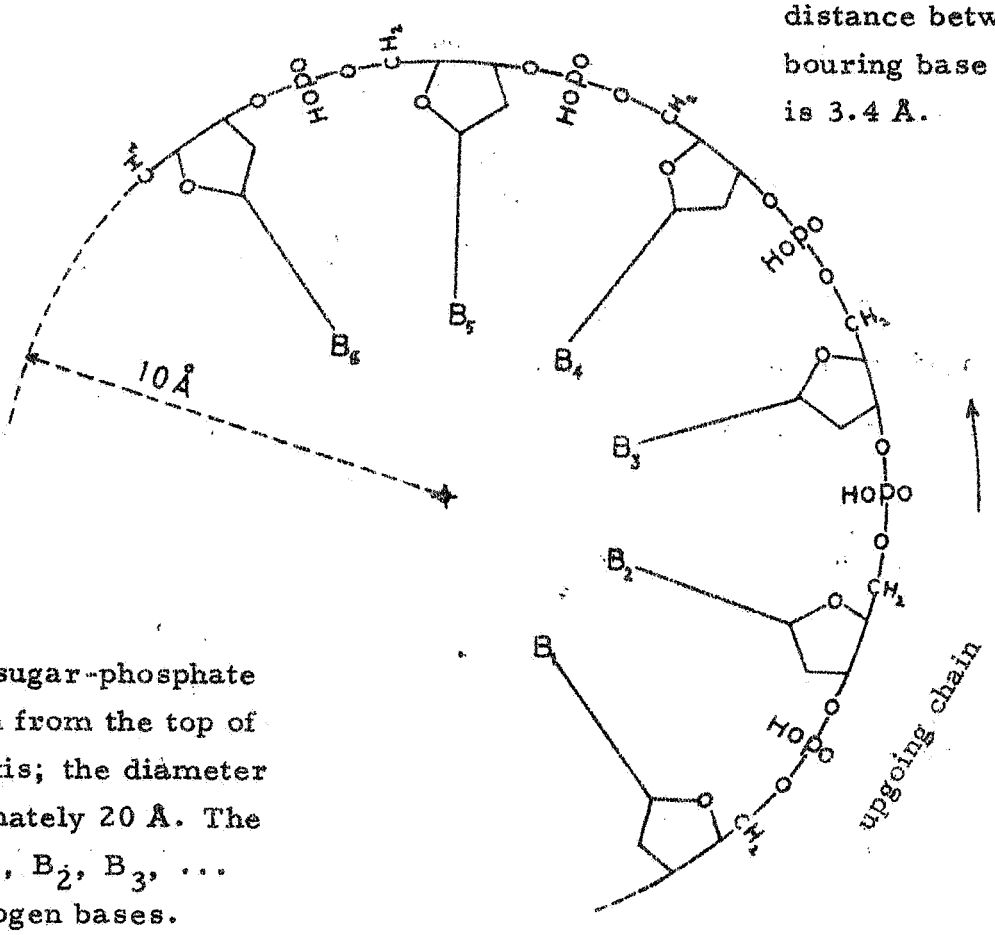


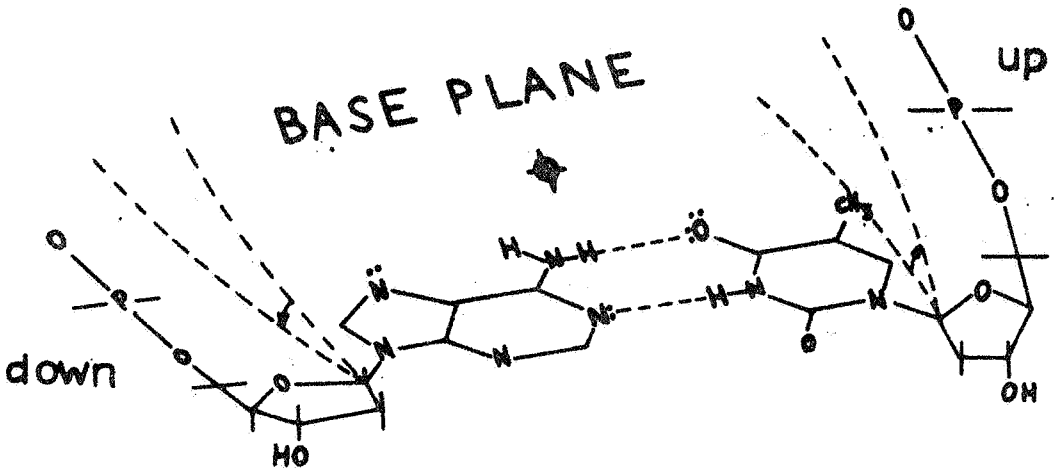
Fig. 8. Schematic structure of DNA:

a) Linear representation of the DNA molecule.

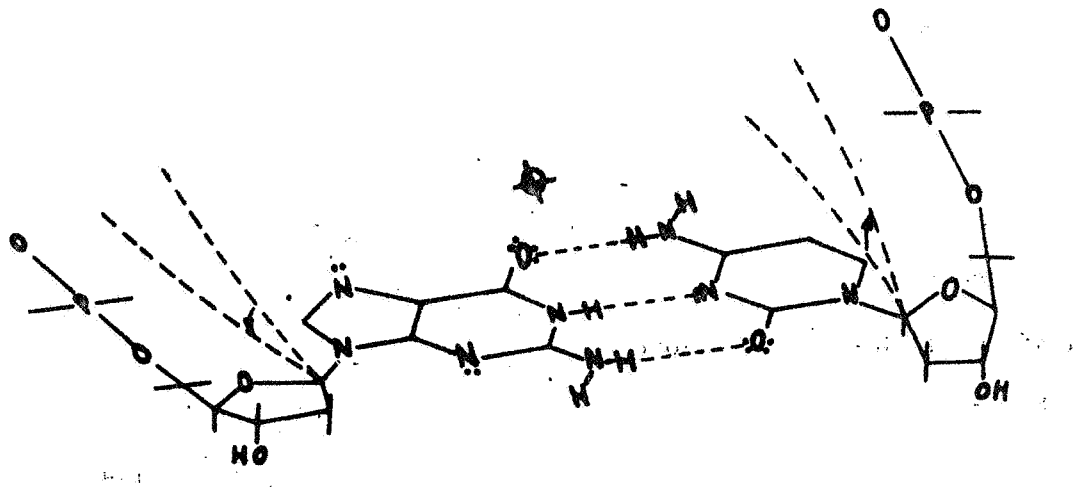
b) Double helix of DNA. Note that there are ten base pairs per full revolution, and that the distance between neighbouring base pair planes is 3.4 Å.



c) One of the sugar-phosphate chains seen from the top of the helix axis; the diameter is approximately 20 Å. The symbols B_1, B_2, B_3, ... denote nitrogen bases.



(a)



(b)

Fig. 9. The normal base pairs in DNA:

a) A-T base pair with two hydrogen bonds.

b) G-C base pair with three hydrogen bonds.

one obtains a hybrid

$$h = \alpha s + \beta p_k , \qquad (\alpha > 0, \beta > 0) \qquad (1)$$

which has its maximum electron density in the k direction. The normalization gives $\alpha^2 + \beta^2 = 1$, and the ration $n = \beta^2/\alpha^2$ is often used to characterize the hybrid which is given the symbol sp^n. We note further that the geometrical angle θ_{12} between two hybrides sp^{n_1} and sp^{n_2} is given by the formula [16]

$$\cos\theta_{12} = -(n_1 n_2)^{-1/2} , \qquad (2)$$

giving the connection between the geometrical structure of the molecule and the amount of hybridization of various bonds. We note finally that, if one has four hybrids characterized by the numbers n_1, n_2, n_3, and n_4, one has the additional relation $\alpha_1^2 + \alpha_2^2 + \alpha_3^2 + \alpha_4^2 = 1$, since the total amount of s character has to be used up by the hybrids. This gives the auxiliary condition:

$$(n_1+1)^{-1} + (n_2+1)^{-1} + (n_3+1)^{-1} + (n_4+1)^{-1} = 1 , \qquad (3)$$

The carbon atom in the methane molecule CH_4 is characterized by four equivalent valence arms having $n = 3$ and the hybridization sp^3. On the other hand, the carbon atom in the benzene molecule C_6H_6 has three equivalent valence arms in a plane forming an angle of 120° with each other, and application of (2) gives then $n = 2$ and the hybridization sp^2. For the fourth hybrid, one obtains according to (3) that $n_4 = \infty$, which means that this hybrid is pure p-orbital perpendicular to the molecular plane; see Fig. 11. The benzene molecule contains a total of $6 \times 4 + 6 = 30$ valence electrons, of which 24 are contained in the 12 single bonds in the σ-skeleton formed by the sp^2 hybrides, whereas 6 are assigned to the so-called π-electron cloud associated with the six pure $2p_z$-orbitals. According to the new ideas developed by Hückel [17] in 1931-32, one may construct six molecular orbitals associated with the benzene ring by linear combination of the

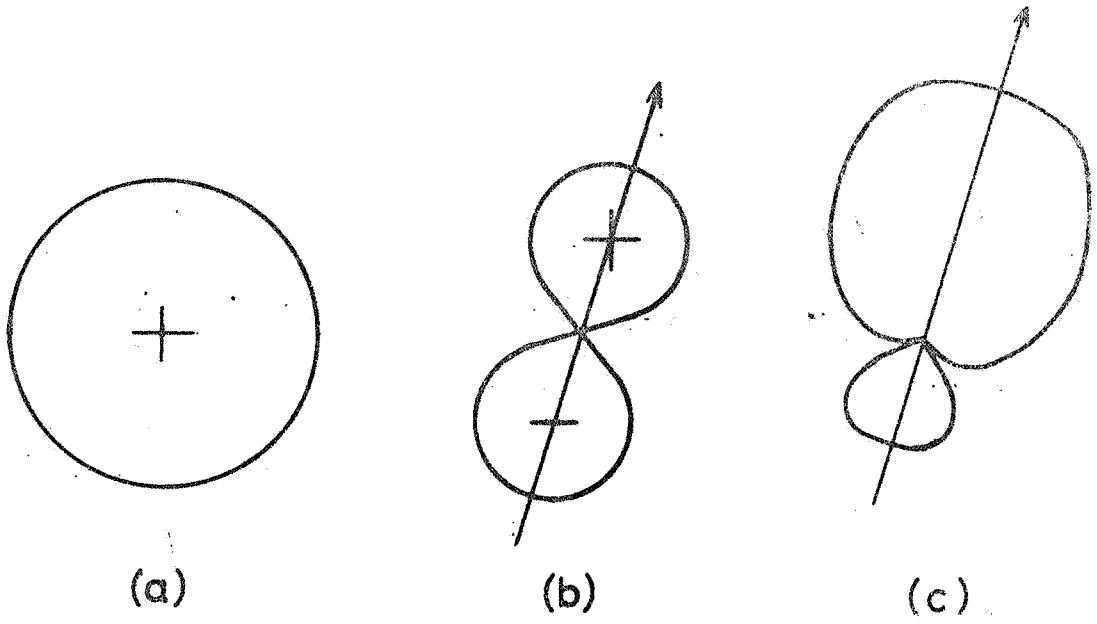


Fig. 10. Schematic diagrams of angular distributions of various orbitals: (a) s-orbital, (b) p-orbital and (c) hybrid.

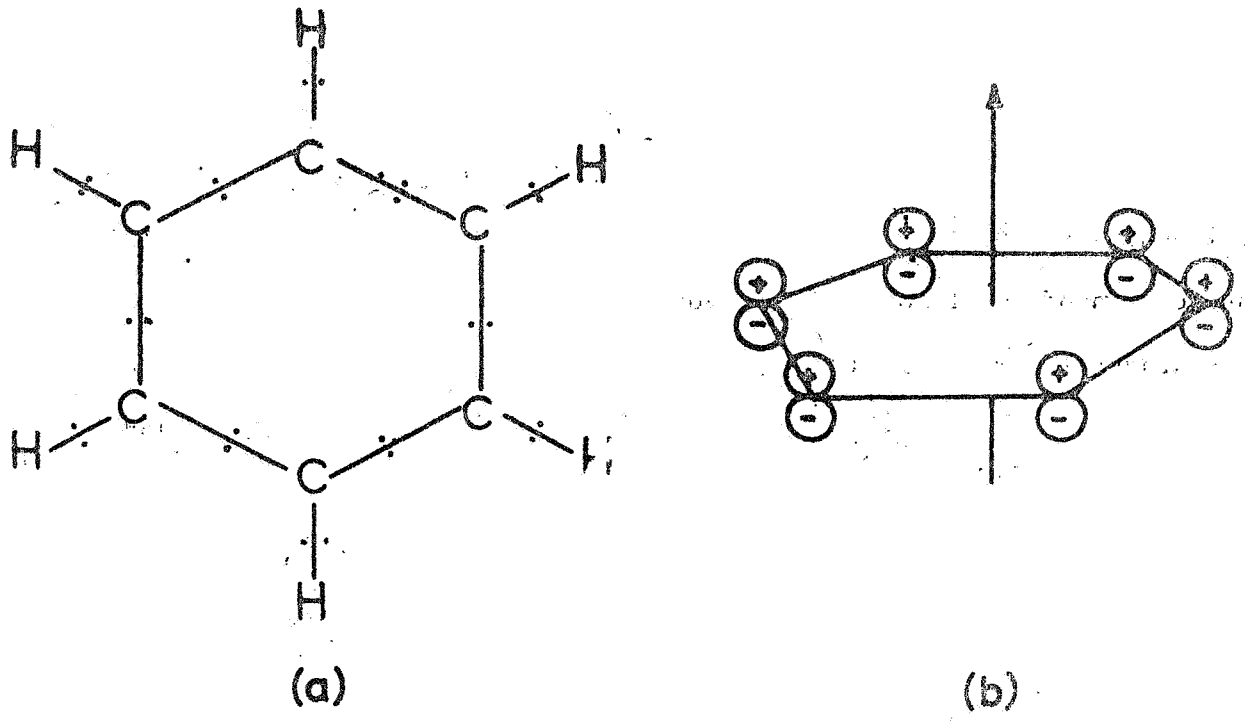


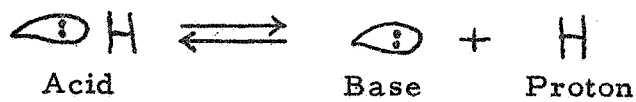
Fig. 11. Electronic distribution in the benzene molecule C_6H_6: (a) σ-skeleton, (b) π-electron cloud.

six atomic $2p_z$-orbitals, perpendicular to the ring , and the six π-electrons are then assigned to the three molecular orbitals having the lowest energy with two electrons in each. There are hence three empty molecular π-orbitals, and there is a considerable energy gap between the highest occupied orbital and the lowest empty orbital.

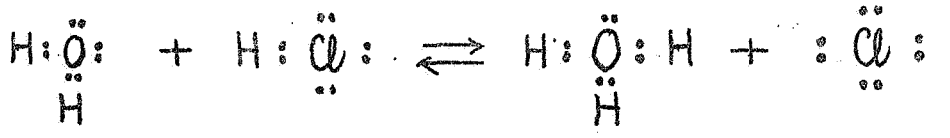
This quantum-mechanical picture gives an excellent explanation of the saturated character of the benzene molecule and its aromatic properties. The Hückel scheme with mobile π-electrons around the ring replaces thus the classical chemical picture with alternating single and double bonds. This model holds not only for the benzene molecule but for the conjugated systems in general, and we will in the following deal with such a system by considering the σ-skeleton formed by the single bonds in the plane of the molecule and with the π-electron cloud replacing the classical double bonds.

This separation of the electrons into two groups is very important also for the analysis of the character of the genetic code. In order to approach this problem, we will now carry out a thought experiment consisting of dropping one of the protons in the benzene ring in Fig. 11 into the neighbouring carbon nucleus giving rise to a nitrogen nucleus; see Fig. 12. This leads to the formation of a pyridine molecule characterized by having an electron lone pair sitting in a sp^2 hybrid on the nitrogen atom, and this lone pair will attract every proton or positive group in the neighbourhood. This tendency of pyridine to try to catch a proton and become a pyridinium ion $C_5NH_6^+$ gives it a characteristic "base" character.

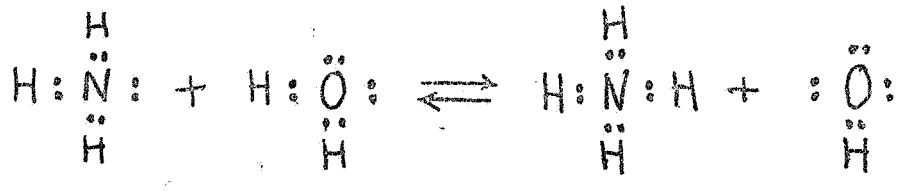
In general, protons are usually attached to molecules by means of electron pairs and, in many cases, the bonding is so strong that it is very hard to remove the protons. According to Lewis, however, there is a very important class of chemical compounds in which the easy removal or attachement of protons is one of the fundamental chemical properties, namely the acids and the bases. They are characterized by the reaction:



The main feature of the base is hence that it has at least one electron lone pair available, and the hybridization theory gives a much better understanding of the properties of localized electron pairs than was possible before modern quantum theory. An acid is characterized by having "loose" protons which are more or less easily removed. There are also molecules which like water are both proton donors and acceptors, and one and the same molecule may hence have both properties . An electrolytic dissociation of the type $HCl \rightleftarrows H^+ + Cl^-$ is much better understood if one considers the fact that it occurs when hydrogen chloride is solved in water, and that a molecule of the latter steals a proton from a molecule of the former, so that $H_2O + HCl \rightleftarrows H_3O^+ + Cl^-$, or



The hydrogen chloride HCl is hence a proton donor or a Lewis acid, and we note that the hydroniumion H_3O^+ is here a very natural consequence of the hybridization theory. For the dissociation associated with the solution of ammonia H_3N in water, one has similarly
$H_3N + H_2O \rightleftarrows H_4N^+ + OH^-$, or



which means that H_3N is a proton acceptor or a Lewis base.

A very interesting phenomenon occurs in a liquid or solid system of molecules having free electron lone pairs, since there will then be a competition between the molecules to catch any available free or loose protons in the environment. If two molecules through their electron lone pairs are attracted to one and the same proton, the molecules are said to be joined by a hydrogen bond. In modern terminology, a hydrogen bond is hence a link between two molecules which consists of a proton shared between two electron lone pairs. A hydrogen bond between two pyridine molecules is illustrated in Fig. 13.

A hydrogen bond is a comparatively weak chemical bond having a binding energy of the order 6-12 kcal/mole. It is of fundamental importance

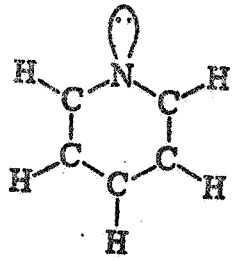


Fig. 12. Pyridine molecule with an electron lone pair on the nitrogen atom pointing out from the ring.

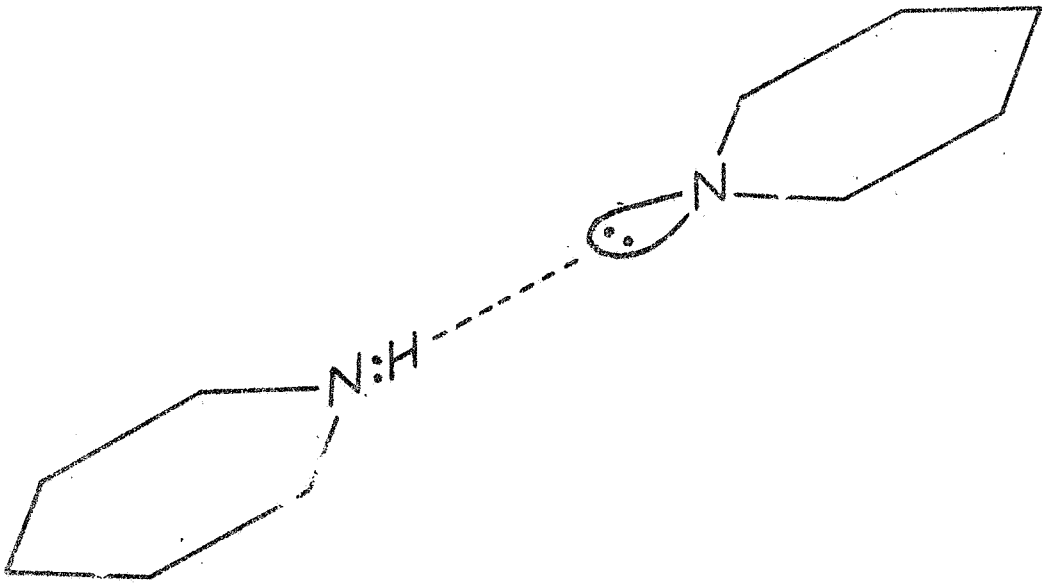


Fig. 13. Hydrogen bond between two pyridine molecules consisting of a proton H shared between two electron pairs.

in ordinary chemistry as well as in biochemistry, and here we will study its properties in connection with DNA and the genetic code.

The genetic code as a proton-electronpair pattern. - Let us now consider the hydrogen bonds between the base pairs in DNA and resolve the two hydrogen bonds in the adenine-thymine pair and the three hydrogen bonds in the guanine-cytosine pair into their proton and electronpair components. From the chemical structures in Fig. 9, we obtain the results indicated in Fig. 14, which imply that the genetic code consists of a pattern of elementary particles, and that each base has a characteristic template consisting of protons and electronpairs as outlined in Fig. 15. These detailed patterns may now be compared with the schematic templates used in the introductory part in Fig. 2.

If a proton is moved from its normal electronpair to a free electronpair within the same template, one obtains a tautomeric form which may be important as a possible cause of errors in the DNA replication mechanism. The proton-electronpair codes of these tautomeric forms are outlined in Fig. 16, and they should be compared with the schematic templates illustrated in Fig. 4. There are, of course, many other tautomers which may be of importance in other connections, but we will here limit our discussion to the main forms listed in Fig. 16.

It is perhaps somewhat surprising that the genetic code ultimately consists of a rather simple combination of elementary particles, and we will now show that this may have important consequences.

Hydrogen bond as a double-well potential; tunnel effect

Each electron pair in a hydrogen bond attracts the proton, and this attraction may physically be described as a potential well with a specific minimum representing the classical equilibrium position of the proton. Since there are two electron pairs involved, the proton in a hydrogen bond feels a potential which is the superposition of two single-well potentials with different minima and which is hence usually a double-well potential; see Fig. 17. The two minima in these potential are

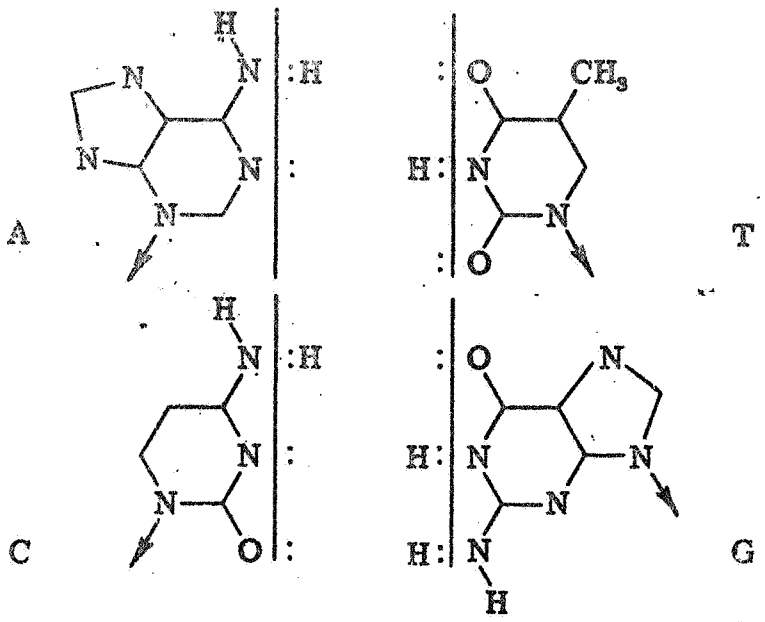


Fig. 14. The hydrogen bonds between the normal base pairs in DNA resolved into their proton-electronpair components; note that here H denotes a proton and the symbol : an electron pair according to Lewis.

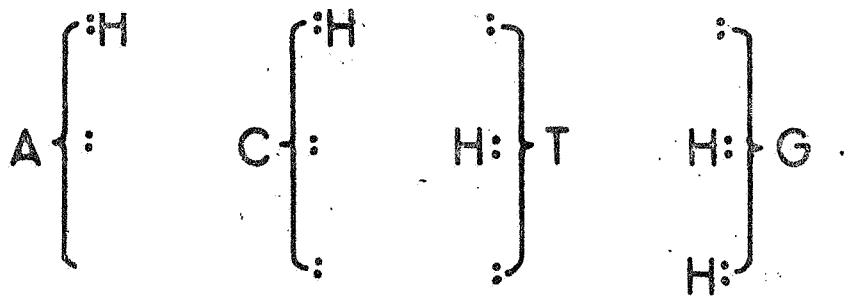


Fig. 15. The proton-electronpair code of the normal nitrogen bases; compare the schematic templates in Fig. 2.

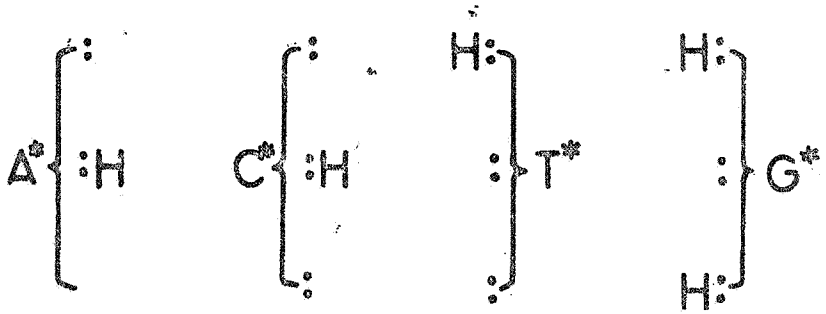


Fig. 16. The proton-electronpair code of some rare tautomeric forms of the nitrogen bases; compare the schematic templates in Fig. 4.

usually not of the same height, but we will discuss both the symmetric and asymmetric case. In classical physics, a particle would stay in one of the wells, unless it has sufficient energy to pass the "potential barrier" in the form of the maximum separating the two minima.

In modern physics, the circumstances are rather different since the proton is an elementary particle which obeys the laws of quantum theory and may be described as a "wave packet" having the ability to penetrate also into classically forbidden regions. This phenomenon is an immediate consequence of Schrödinger's wave equation, and it implies that, if two classically permitted regions are separated by a potential barrier leading to a classically forbidden region, an elementary particle may penetrate the classically forbidden region from one permitted region to another; see Fig. 18. This so-called tunnel effect was discovered independently by Gamow[18] and by Gurney and Condon[19] in 1928-29 in explaining the general phenomenon of radioactivity which involves the escape of certain particles through nuclear potential barriers. The tunnel effect is now a well-known phenomenon in physics, as e.g. in the predissociation of molecules in molecular spectroscopy, in the ammonia clock, in the tunneling diodes, in corrosion, etc.

The tunnel effect is treated in most textbooks in quantum theory. The probability that the wave packet will pass the potential barrier upon hitting it is called the transmission coefficient g, and this quantity is given approximately by the formula:

$$g \approx e^{-2K}, \qquad K = \frac{2\pi}{h} \int_{x_1}^{x_3} \left\{ 2m \left[E - V(x) \right] \right\}^{1/2} dx , \tag{4}$$

with the notations used in Fig. 18; m is the mass of the particle, and h is Planck's constant.

It is instructive to start with the problem of tunneling out of a single well; see Fig. 19. The particle is from the very beginning in the left-hand well, where it oscillates with the frequency ν_1 and hits the barrier ν_1 times per second. It is convenient to introduce the notations

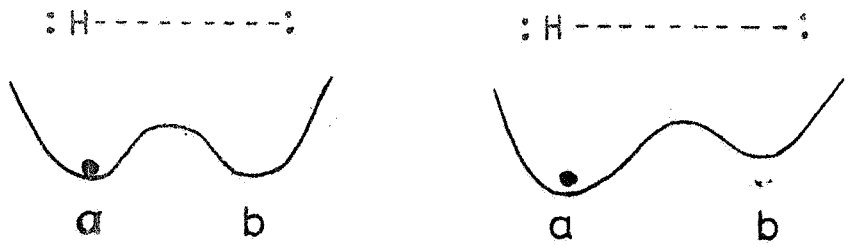


Fig. 17. Typical double-well potentials felt by a proton in a hydrogen bond; the left-hand potential is symmetric, and the right-hand potential is asymmetric.

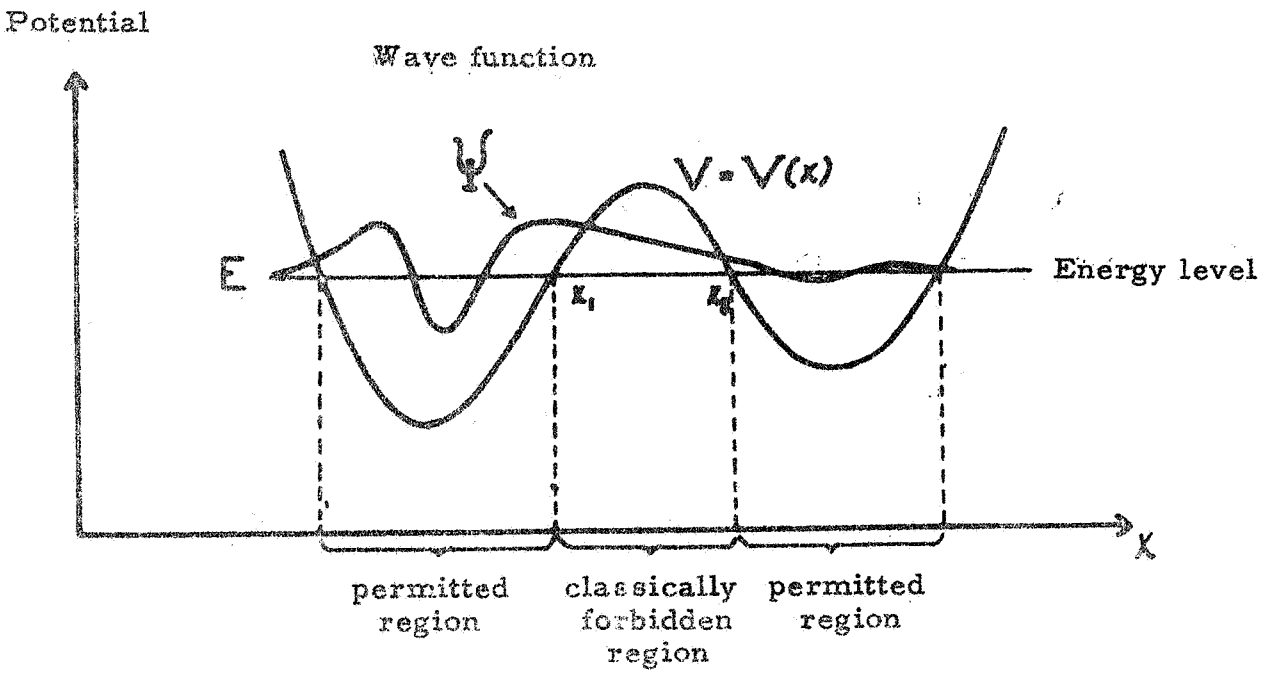


Fig. 18. Tunneling of a wave packet through a classically forbidden region; transmission coefficient $g \approx \exp(-2K)$ according to formula (4).

$$c_1 = \nu_1 g \,, \qquad \tau_1 = 1/c_1 = 1/\nu_1 g \,, \tag{5}$$

and c_1 will be called the "tunneling rate" and τ_1 the "tunneling time" with a physical meaning which will be described below. In our applications, the transmission coefficient g will be very small, so that $g \ll 1$.

At every hit of the wave packet, the population in a particular energy level of the left-hand well will be decreased by a factor $(1-g)$, and, after p hits, the decrease will hence be described by the factor $(1-g)^p$. If the energy level population is originally a_1, the population after t secs will thus be

$$n_1(t) = a_1 (1-g)^{[\nu_1 t]} \,, \tag{6}$$

which is a step function. However, if the transmission coefficient g is sufficiently small, the quantity $(1-1/g^{-1})^{g^{-1}}$ may be approximated by the number e^{-1}, which leads to the "smeared out" formula:

$$n_1(t) = a_1 \left\{ (1-1/g^{-1})^{g^{-1}} \right\}^{g[\nu_1 t]} \approx \tag{7}$$
$$\approx a_1 e^{-c_1 t} \,.$$

This means that the step function may be replaced by a function with a continuous derivative satisfying the simple differential equation:

$$\frac{dn_1}{dt} = -c_1 n_1 \,. \tag{8}$$

The part of the population which has passed the barrier during the time t is hence:

$$n_3(t) = a_1 \left(1 - e^{-t/\tau_1}\right) \,. \tag{9}$$

The "tunneling time" τ_1 is the time requested for the original population to decrease by a factor e^{-1} and, for very small times $t \ll \tau_1$, one may use the linear relation $n_3(t) \approx a_1 t/\tau_1$.

In order to get an idea of the tunneling times for various potential barriers, we have in Table I tabulated τ_1 as a function of the barrier width a_o (in Å) and barrier height V_o (in eV) for a parabolic barrier for a proton and a deuteron under the assumption that the proton frequency in the well is approximately $\nu_1 = 10^{13}$ sec^{-1}. The quantity τ_1 turns out to be a function of the parameter $\chi_o = a_o \sqrt{V_o}$, which varies from $\tau_1 = 10^{-13}$ sec for $\chi_o = 0$ to $\tau_1 = 3 \times 10^{9}$ years for $\chi_o = 2$; the latter time corresponds to the age of the universe according to the modern estimates just presented here in Dr. Sandage's lecture.

If the thermal equilibrium is quickly reached in the left-hand well, the population will assume a Boltzmann distribution over the various energy levels proportional to the Boltzmann factor $B = \exp\{ - \Delta V/k\,T \}$. The Boltzmann factor is tabulated in Table II for three typical temperatures: T = 273°K, 310°K, and 373°K.

After these preparations, we will now study the tunneling in a double-well potential, in which case particular attention has to be devoted to the question of "back-tunneling" from the more shallow to the deeper well. Let us assume that initially at the time $t = 0$, the particle is entirely in the left-hand well having the part $a_1(E)$ of its population in a specific energy level E with the transmission coefficient $g = g(E)$. If $n_1 = n_1(t)$ and $n_3 = n_3(t)$ denote the parts of the population in this level at time t in the two wells, respectively, one obtains by generalizing (8) a system of differential equations:

$$\begin{cases} \dfrac{dn_1}{dt} = - c_1 n_1 + c_3 n_3 , \\ \dfrac{dn_3}{dt} = c_1 n_1 - c_3 n_3 , \end{cases} \tag{10}$$

where $c_1 = \nu_1 g$ and $c_3 = \nu_3 g$ are the rates for tunneling in the two opposite directions. The initial condition $n_1(0) = a_1$, $n_3(0) = 0$ corresponds to the solution

$$n_1(t) = \frac{c_3 a_1}{c_1+c_3} + \frac{c_1 a_1}{c_1+c_3} e^{-(c_1+c_3)t},$$

$$n_3(t) = \frac{c_1 a_1}{c_1+c_3} - \frac{c_1 a_1}{c_1+c_3} e^{-(c_1+c_3)t}. \qquad (11)$$

The time $\tau_{1,3} = (c_1 + c_3)^{-1}$ may be denoted as the relaxation time characteristic for the double-well potential. For small times, $t \ll \tau_{1,3}$, the tunneling part of the population is given by the formula:

$$n_3 \approx a_1 c_1 t - a_1 c_1 (c_1+c_3) \frac{t^2}{2!} + \cdots, \qquad (12)$$

where the linear term is associated with only left-right tunneling, and the quadratic term shows also the first effect of the "back-tunneling".

For $t \gg \tau_{1,3}$, the solutions have an entirely different character, since $n_1(t)$ and $n_3(t)$ quickly approach the constant values:

$$n_1 = \frac{\nu_3 a_1}{\nu_1+\nu_3}, \quad n_3 = \frac{\nu_1 a_1}{\nu_1+\nu_3}, \qquad (13)$$

which are essentially independent of the value of the transmission coefficient g. The quantities (13) correspond to an equilibrium situation, in which one has $\nu_1 n_1 = \nu_3 n_3$. In this case, the system is approaching a thermal equilibrium over the energy levels of the double well with the population of each level divided into the fractions $\nu_3 : \nu_1$ over the two separate wells. This results imply that, in a first approximation, the final distribution of the particle population is independent of the particle mass m. This similarity between the proton and the deuteron for $t \gg \tau_{1,3}$ has to be remembered in interpreting such mutation experiments as are aimed at studying the tunneling phenomena in general by using deuterated organisms[20].

Table I. Tunneling times τ_1 for the proton and the deuteron as functions of the barrier width a_o(in Å) and barrier height V_o (in eV).

$a_o\sqrt{V_o}$	Proton τ_1	Deuteron τ_1
0	10^{-13} sec	10^{-13} sec
0.1	$10^{-11.5}$ sec	10^{-11} sec
0.2	10^{-10} sec	10^{-9} sec
0.3	$10^{-8.5}$ sec	10^{-6} sec
0.4	10^{-7} sec	10^{-4} sec
0.5	$10^{-5.5}$ sec	10^{-2} sec
0.6	10^{-4} sec	1.3 sec
0.7	10^{-2}	2 min
0.8	10^{-1}	3.7 hrs
0.9	3 sec	20 days
1.0	2 min	8 years
1.1	53 min	10^3 years
1.2	1.16 days	1.3×10^5 years
1.3	1.22 months	1.7×10^7 years
1.4	3 years	2.2×10^9 years
1.5	100 years	
1.6	3000 years	
1.7	10^5 years	
1.8	3×10^6 years	
1.9	10^8 years	
2.0	3×10^9 years	

Table II. Boltzmann factors $B = \exp\{-\Delta V/kT\}$ for three temperatures T as functions of ΔV(in eV).

V	T=273°K $^{10}\log B$	T=310°K $^{10}\log B$	T=373°K $^{10}\log B$
0	0	0	0
0.1	-1.846	-1.626	-1.351
0.2	-3.692	-3.252	-2.702
0.3	-5.538	-4.878	-4.053
0.4	-7.384	-6.504	-5.404
0.5	-9.230	-8.130	-6.755
0.6	-11.076	-9.756	-8.106
0.7	-12.922	-11.382	-9.457
0.8	-14.768	-13.008	-10.808
0.9	-16.614	-14.634	-12.159
1.0	-18.460	-16.260	-13.510
1.5	-27.690	-24.390	-20.265
2.0	-36.920	-32.520	-27.020
2.5	-46.150	-40.650	-33.775
3.0	-55.380	-48.780	-40.530
3.5	-64.610	-56.910	-47.285
4.0	-73.840	-65.040	-54.040
4.5	-83.070	-73.170	-60.795
5.0	-92.300	-81.300	-67.550

Spontaneous proton tunneling in DNA. - Let us now consider a DNA molecule in its stable form during a period when it is biologically inactive so that the genetic message is not undergoing replication or transcription. According to Fig. 14, one can express the essential properties of the base pairing through hydrogen bonds as shown in Fig. 19.

Even if the double-well potentials in each hydrogen bonds are rather asymmetric, one can expect that there will always be a small probability for proton transfer in each bond through (and above) the potential barrier in accordance with the results discussed previously. If the two bases are originally equally charged, the tunneling of one proton in one direction will very likely induce a tunneling of the other proton in the reverse direction to keep balance between the gross electric charges. This "simultaneous" proton exchange implies hence the base transitions:

$$A-T \longrightarrow A^*-T^*, \quad G-C \longrightarrow G^*-C^*$$

which lead to the production of pairs of tautomeric bases. If the hydrogen bonds are released in this position, the tautomeric forms will lead to errors in the next replication, i.e. to mutations, as indicated in Fig. 6. This means that it is necessary to consider also the possibility of an internal proton exchange in studying the stability of the genetic code in DNA.

The problem is complicated by the fact that, in each base pair, there are at least two hydrogen bonds involved, which means that one has to study the motion and stability of two protons in a four-well potential. Since the movements of the protons will further influence and polarize both the σ and π electron clouds, a complete solution involves a solution of a quantum-mechanical many-body problem. Before treating this problem, we will now first discuss the influence of the incorporation errors on the stability of DNA.

4. STABILITY OF GENETIC CODE AT REPLICATION

In connection with the DNA replication process, Watson and Crick[5] suggested that the hydrogen bonds between the base pairs in some way were released, that the two DNA strands become at least partly free, and that each one of them serves as a template for the formation of a new complementary strand formed from nutrition material in the environment, so that two complete DNA molecules with the same base-pair sequence as the original one are produced. However, Watson and Crick did not discuss the obviously rather complicated unwinding - winding mechanism involved in this replication, and this problem has later been discussed by many authors[21].

Through the experiments by Meselson and Stahl[22], Taylor[23], Yoshikawa and Sueoka[24], Nagata[25], and Cairns[26], it has been shown rather conclusively that each one of the daughter molecules actually contains one strand from the parents molecule of DNA. As to the unwinding-winding mechanism, one has now essentially adopted the Y-model or "speedometer-cable" model first suggested by Delbrück and Stent[21], and experimental support for this model has also been obtained through Cairn's pictures of the replication process produced by autoradiography[26]. It should be observed, however, that there are still some difficulties in this model associated with the enzymes necessary to build the two new sugar-phosphate chains of different polarity simultaneously, and particularly the work by Kornberg[27] is of importance in this connection.

Here we will adopt the Y-model as the starting point for our study, and we will particularly focus the interest on the replication zone itself. The results have previously been reported by the author[28] at the Ravello Conference in 1963. Following a suggestion by Crick[29], we will assume that the genetic code is opened up to the environment by rotating one of the strands 180° with respect to the axis of the helix; see Figs. 20 and 21. Two new nucleotides may now be added from nutrition material in the environment, so that there are a total of four nucleotide bases in the replication region which may be characterized as a "replication plane", even if the exact steric conditions may be rather varying. Depending on the attachments to the sugar-phosphate chains, the four bases will in the next stage of the process be separated into two base pairs in such a way that each daughter molecule will get one old base and one new base in

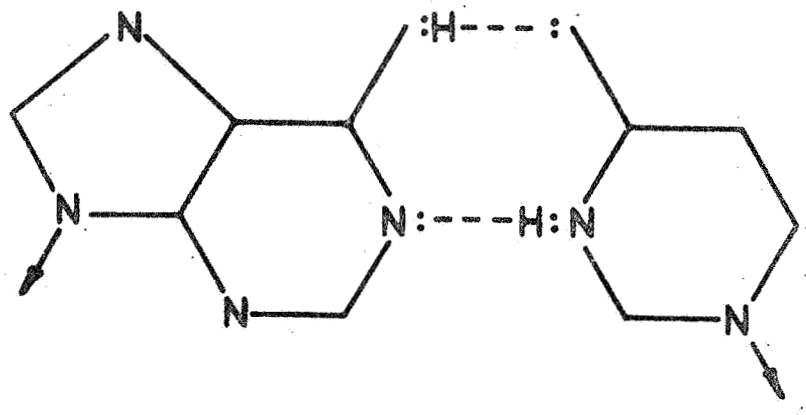


Fig. 19. Typical base pairing through hydrogen bonds in DNA.

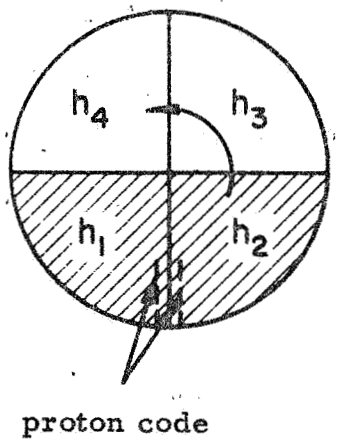


Fig. 20. Geometry of the base plane in DNA and opening of the genetic code by rotating one strand 180° around the helical axis.

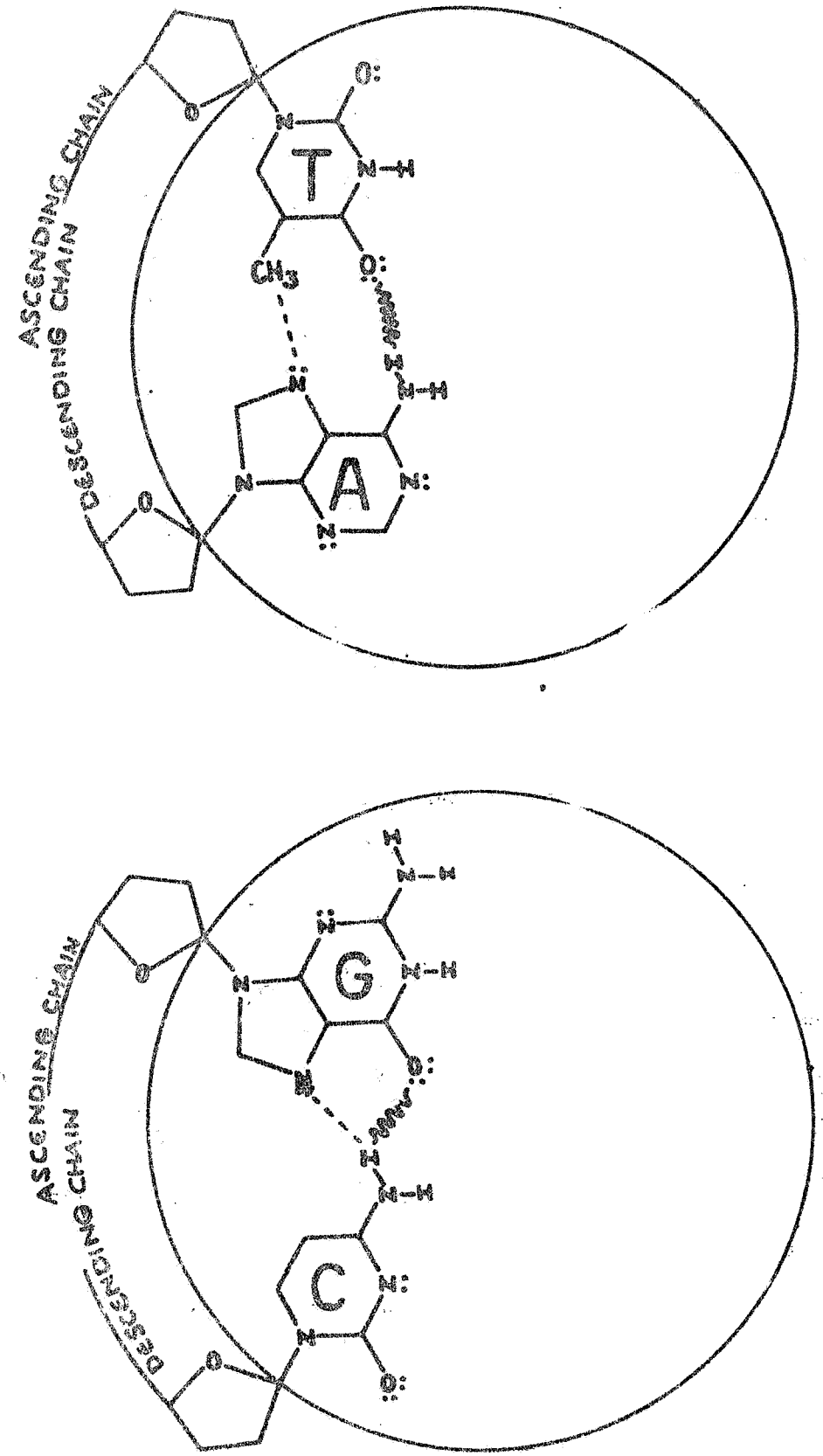


Fig. 21. Following a suggestion by Crick, the genetic code may be opened to the environment by rotating one strand 180° around the helical axis; the figure illustrates the two nucleotide base pairs in the "open" position ready for the addition of a pair of complementary bases. The symbol ~~~ indicates a temporary hydrogen bond.

complete agreement with the original Watson-Crick model; see Fig. 22.

It is now instructive to study the detailed structure of the "replication plane" consisting of four nucleotide bases, and the result is illustrated in Fig. 23. The figures indicate that, in normal replication, the replication plane may be stabilized by at least two temporary hydrogen bonds and that, around the helical axis, one has a parallelogram consisting of two amino groups and two keto groups situated in opposite corners.

It is interesting to analyze also the "replication plane" which occurs if one tries to incorporate a rare tautomeric base from the environment; see Fig. 24. The normal hydrogen bonds will fit as before, but we note that, instead of the two temporary hydrogen bonds, one will obtain only one such bond and an antibonding combination in which either two protons or two electron lone pairs will meet. The appearance of the various parallelograms around the helical axis is indicated in Fig. 25. Since a replication plane with a single tautomeric error has an energy which is several electron volts higher than the normal replication plane, the probability for the incorporation of a single rare tautomer is correspondingly small, as may be seen from the Boltzmann factors in Table II.

Actually such a "purification" procedure in the DNA synthesis seems to be strongly needed in order to prevent an excess of incorporation errors, since the tautomeric forms of the nucleotide bases - as we have pointed out before - do not seem to be as "rare" as was originally anticipated. Particularly for some of the pyrimidine bases, the measured equilibrium constants K are comparatively high. For methylcytosine, Kenner[30] et al. has found the value $K = 10^{-4.7}$, and, for uracil and bromuracil, Katrizky and Waring[30] have found the values $K = 10^{-3.3}$ and $K = 10^{-1.7}$, respectively. One cannot expect that the nutrition material should necessarily be in tautomeric equilibrium, but the figures give some indications of the orders of magnitude. The occurrence of a "replication plane" in the Y-model of the DNA synthesis may hence be of essential importance for the accuracy of the DNA replication.

In concluding this section, it should be observed that the mechanism of the replication plane does not prevent the incorporation of "tautomeric pairs of complementary bases". The probability of such an event is to

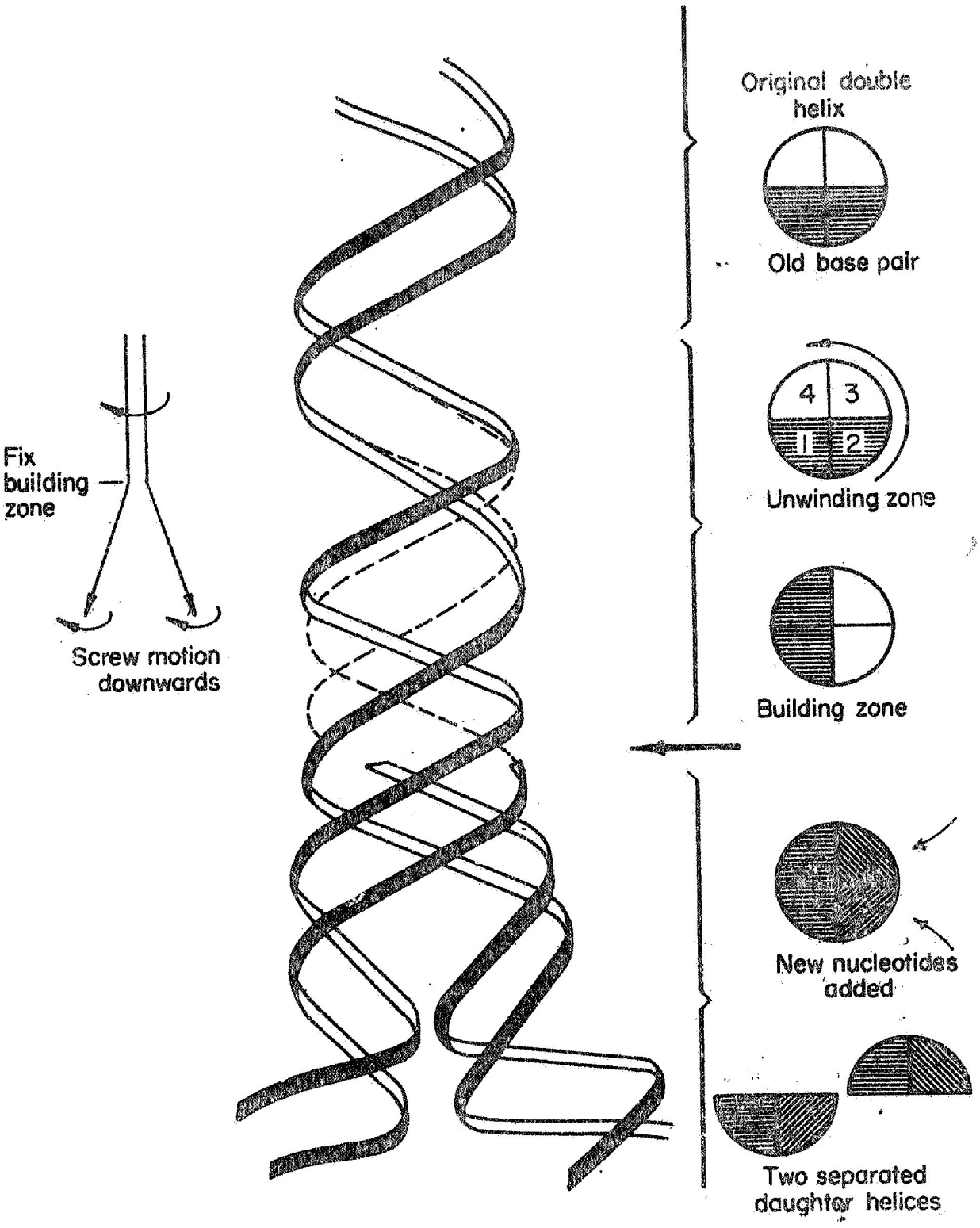


Fig. 22. Study of the Y-model combined with the idea that the genetic code is opened by rotating one of the strands 180° around the helical axis in the replication zone. Note particularly the occurrence of four nucleotides together in the "replication plane".

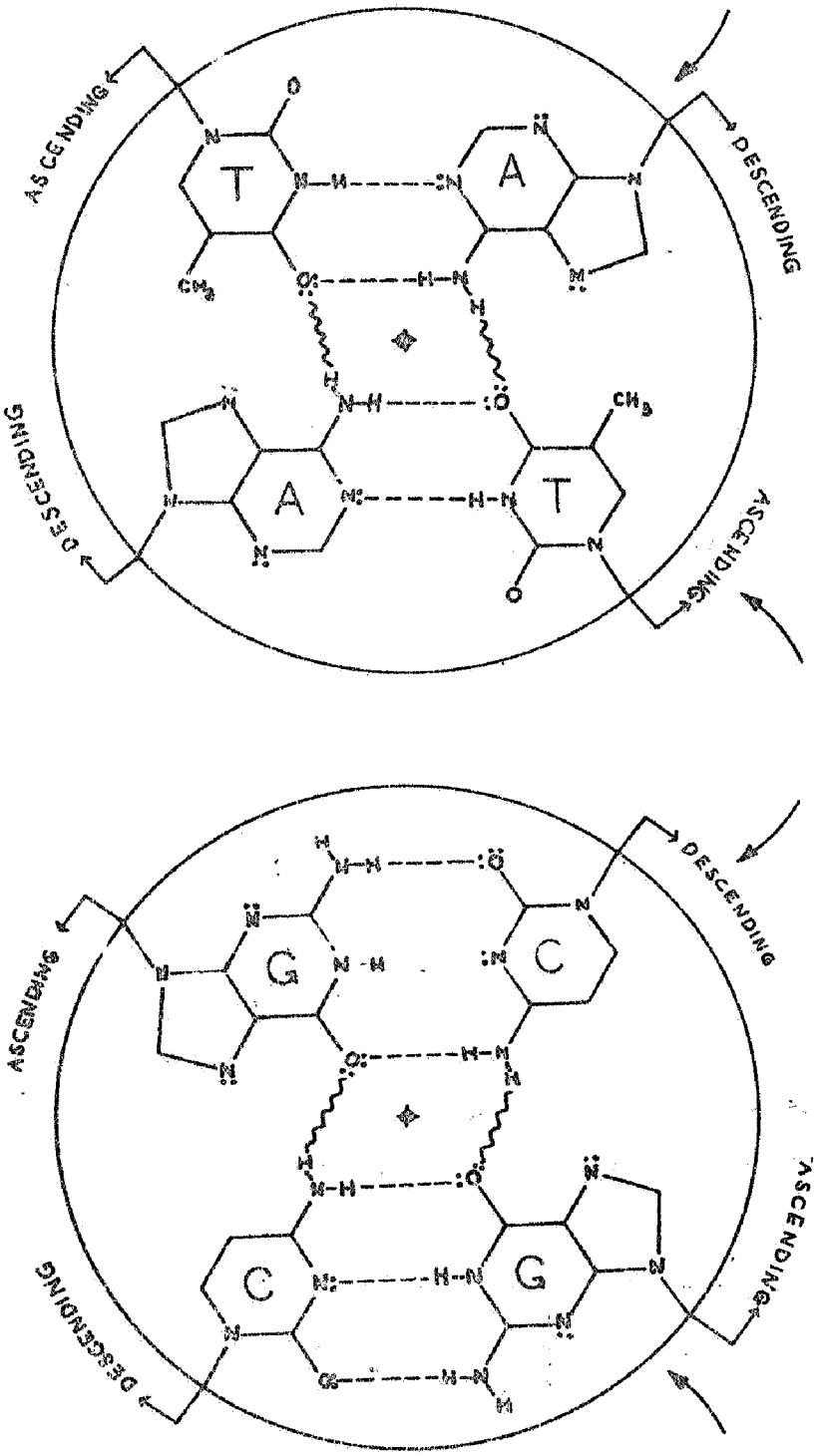


Fig. 23. The four bases in the "replication plane" after the addition of two new nucleotides from the environment. Note the occurrence of a parallelogram consisting of two aminogroups and two keto-groups around the helical axis and the temporary hydrogen bonds denoted by ∿∿∿ .

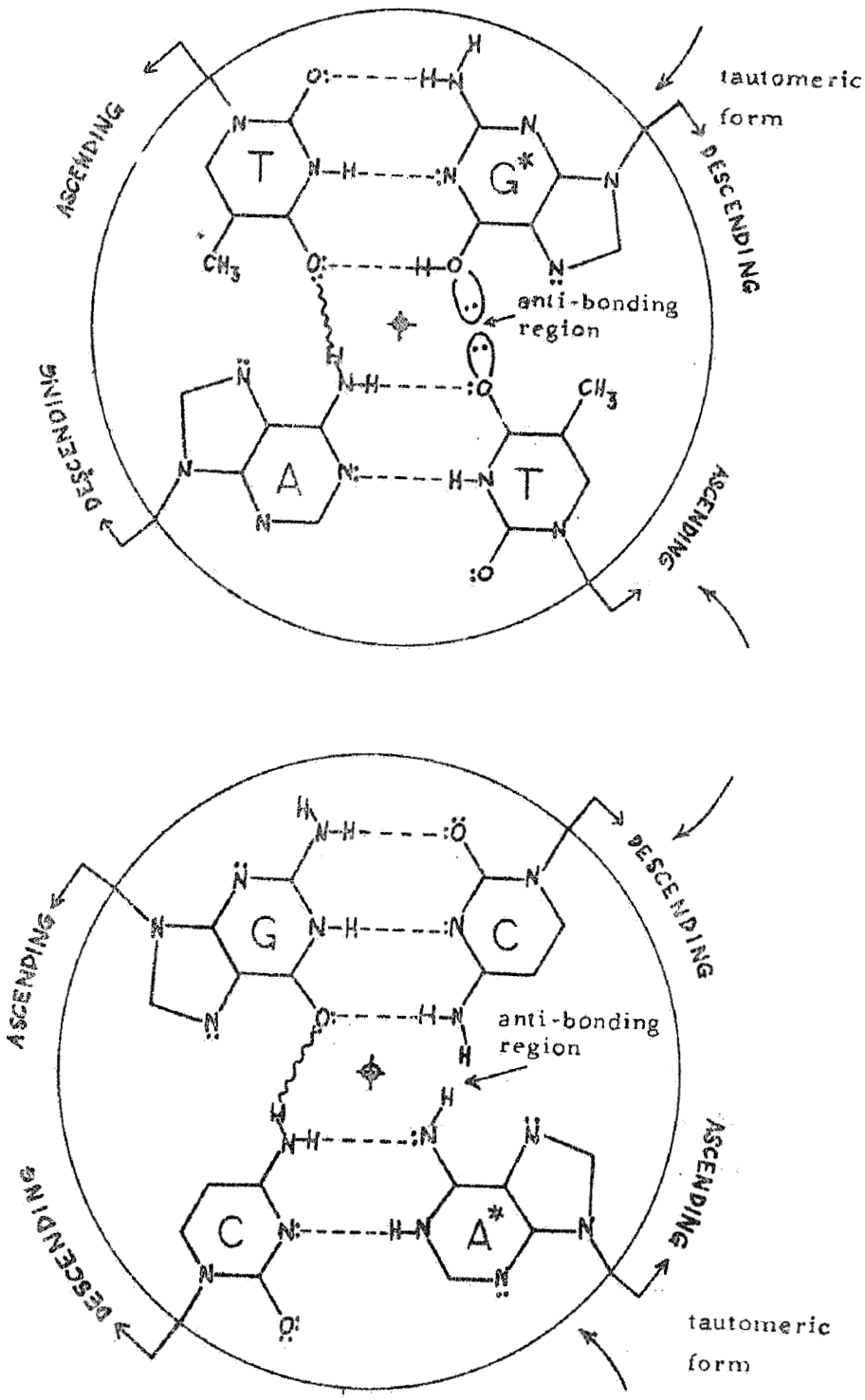


Fig. 24. The four nitrogen bases in the replication plane in the case of the incorporation of a single tautomeric base from the environm

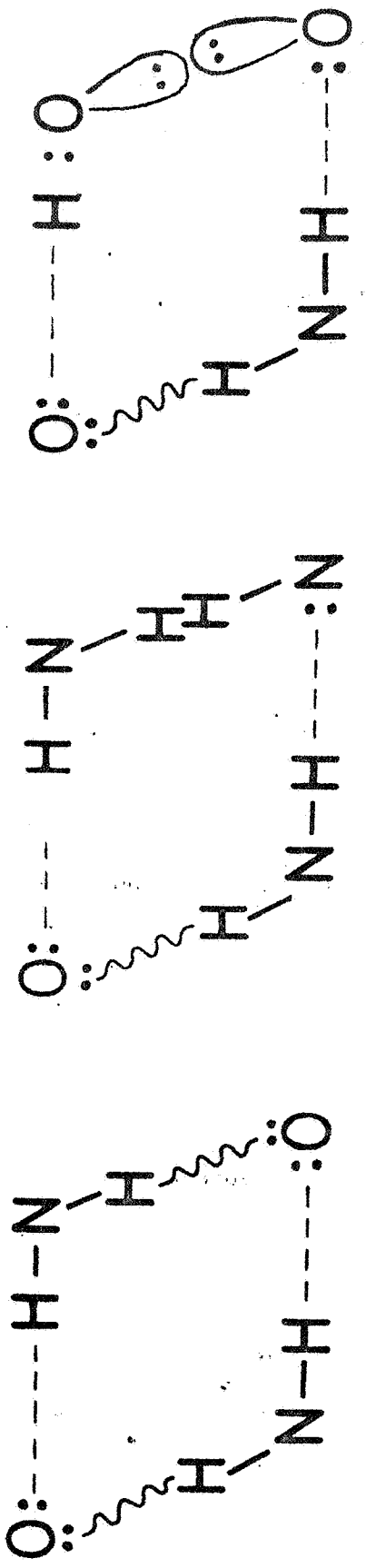


Fig. 25. The four key groups around the center of the replication plane in the normal case and in the case of the incorporation of a single tautomeric error: (a) TA-AT plane, (b) TA-AC* plane, (c) TG* -AT plane.

some extent regulated by the product $K \times K'$, where K and K' are the tautomeric equilibrium constants for a complementary pair of nitrogen bases. The purine bases have many tautomeric forms, and the equilibrium constants for the forms characterized by the codes outlined in Fig. 16 have not yet been accurately measured. It does not seem unreasonable, however, to expect that the value of the product $K \times K'$ will be of the same order of magnitude as the spontaneous mutation probabilities per base pair and generation, i.e. in the range 10^{-8} to 10^{-11} as previously mentioned. The replication scheme associated with the incorporation of a tautomeric pair of complementary bases is not as straightforward as one may first expect, however, and this problem will be further discussed at the end of next section; see particularly Fig. 30.

5. INTERNAL STABILITY OF THE GENETIC CODE IN DNA

In order to investigate the internal stability of the genetic code in the base pairs in DNA, it is necessary to study the problem of the proton exchange as outlined in Sec. 3. This problem can only be solved by evaluating the potential energy surfaces for the protons by calculating the complete charge distributions in the G-C and A-T base pairs for various positions of the protons in the hydrogen bonds. Since the protons in their motion are going to strongly polarize the clouds of the mobile π-electrons as well as the σ-electrons in the lone pairs, this is a rather formidable problem even with the help of modern electronic computers.

One may get a first rough idea of the shape and depth of the potential wells by considering the binding of the proton in some simple inorganic compounds, as water and ammonia. According to Pauling, for example, the average energy of the OH bond in water is 110.6 kcal/mole or 4.794 eV, the bond distance is 0.96 Å, and the stretching frequency is 3657.05 cm^{-1} or $\nu = 1.0963 \times 10^{14}$ sec^{-1}. The average energy of the NH bond in ammonia is 93.4 kcal/mole or 4.049 eV, the bond distance is 1.01 Å, and the stretching frequency is 3336.7 cm^{-1} or $\nu = 1.0094 \times 10^{14}$ sec^{-1}. However, it should be observed that, in the DNA base pairs, these bonds are essentially modified by the fact that the oxygen and nitrogen atoms belong

to conjugated systems with mobile π -electrons, which are easily polarized by the protons. The figures mentioned above give anyway an idea of the orders of magnitude one can expect to meet for the fundamental quantities involved.

The charge and bond orders of the G-C and A-T base pairs in the Hückel scheme were first evaluated by the Pullmans[31]. Using these data, the author[28] made a first rough estimate which indicated that it may be of interest to start the entire investigation by evaluating the double-well potential for the proton in the middle bond in the G-C base pair.

At this time, it was also felt that, in order to get reliable results. it would be necessary to refine the π-electron calculations beyond the Hückel scheme, and a semiempirical MO-LCAO-SCF method for studying the base pairs was developed by Rein and Ladik[32]. Using the charge orders for the G-C pair obtained in this way, Ladik[33] calculated the potential felt by the proton in the middle hydrogen bond under the assumption that both the π - and the σ -electrons stayed unpolarized, and he found a double-well potential with an energy difference of 2.72 eV between the two minima and a potential barrier of 3.40 eV with respect to the lowest minimum. It was clear that, even if this result was qualitatively correct, the figures were greatly exaggerated depending on the neglect of polarization.

In order to take the polarizing effect of the proton into full account, Rein and Harris[34] developed a refined MO-LCAO-SCF method in which one considered explicitly the twentyfour π-electrons of the G-C base pair as well as the four σ -electrons of the hydrogen bond under consideration; the calculations contain still one adjustable parameter K of the type introduced by Wolfsberg and Helmholtz[35]. The polarizing effect of the proton was studied by solving the SCF equations repeatedly for twelve subsequent protonic positions along the hydrogen bond. The resulting potential was again a double-well potential with the energy difference 1.4 eV between the two minima and the barrier 1.8 eV with respect to the deepest minimum. Even the excited and ionic states of the G-C pair have been treated in a similar way[36], and the results indicate that proton

transfer in the hydrogen bonds may be an important factor in the theory of induced mutations caused by radiation or by charge-transfer compounds.

It has been pointed out in Sec. 3. that, if a proton moves from one equilibrium position to the other in a hydrogen bond, it is likely to induce the reverse motion of a proton in another hydrogen bond in order to maintain the gross electric neutrality of the system. The first investigation of the potential energy surface associated with the motion of two protons was carried out by Rein and Harris[37] in a study of the two "upper" hydrogen bonds in the G-C base pair. The MO-LCAO-SCF equations for this system of twentyfour π-electrons and twelve σ-electrons were solved for 74 configurations of the two protons in the N:H ... : N bond and the upper O: ... H:N bond. The calculations were carried out for several values of the Wolfsberg-Helmholtz parameter K, and finally comparatively high values ($K \approx 1.5$) were chosen. The potential energy surface has four minima corresponding to different metastable tautomeric forms, and the equilibration among the tautomers through proton tunneling is estimated to be comparatively fast with a time constant of 3.8×10^{-5} sec; see Fig. 26. The energy difference between the normal form G-C and the tautomer form $G^* - C^*$ obtained by double proton exchange along the two hydrogen bonds is 0.65 eV, and the characteristic potential for simultaneous proton exchange is illustrated in Fig. 27. We note that the equilibrium is quickly achieved, and that the energy difference 0.65 eV at $310^{\circ}K$ according to Table II corresponds to a Boltzmann factor of $B = 10^{-10.5}$. For a more detailed discussion, the reader is referred to reference 27.

Using essentially the technique and computational programs developed in this way, Lunell and Sperber[38] have evaluated the potential energy surface for the motion of the two protons in the hydrogen bonds of the A-T base pair, and the results are illustrated in Fig. 28. There are apparently three equilibrium positions, and the curve along the diagonal is given in more detail in Fig. 29. Since the potential barriers are comparatively high (≈ 2.7 eV), it is easily shown that only a negligible amount of the proton population may pass over the barrier, whereas the tautomeric equilibrium is instead quickly achieved through the tunnel effect. The energy difference between the normal form A-T and the main tautomeric form $A^* - T^*$ obtained through double proton exchange

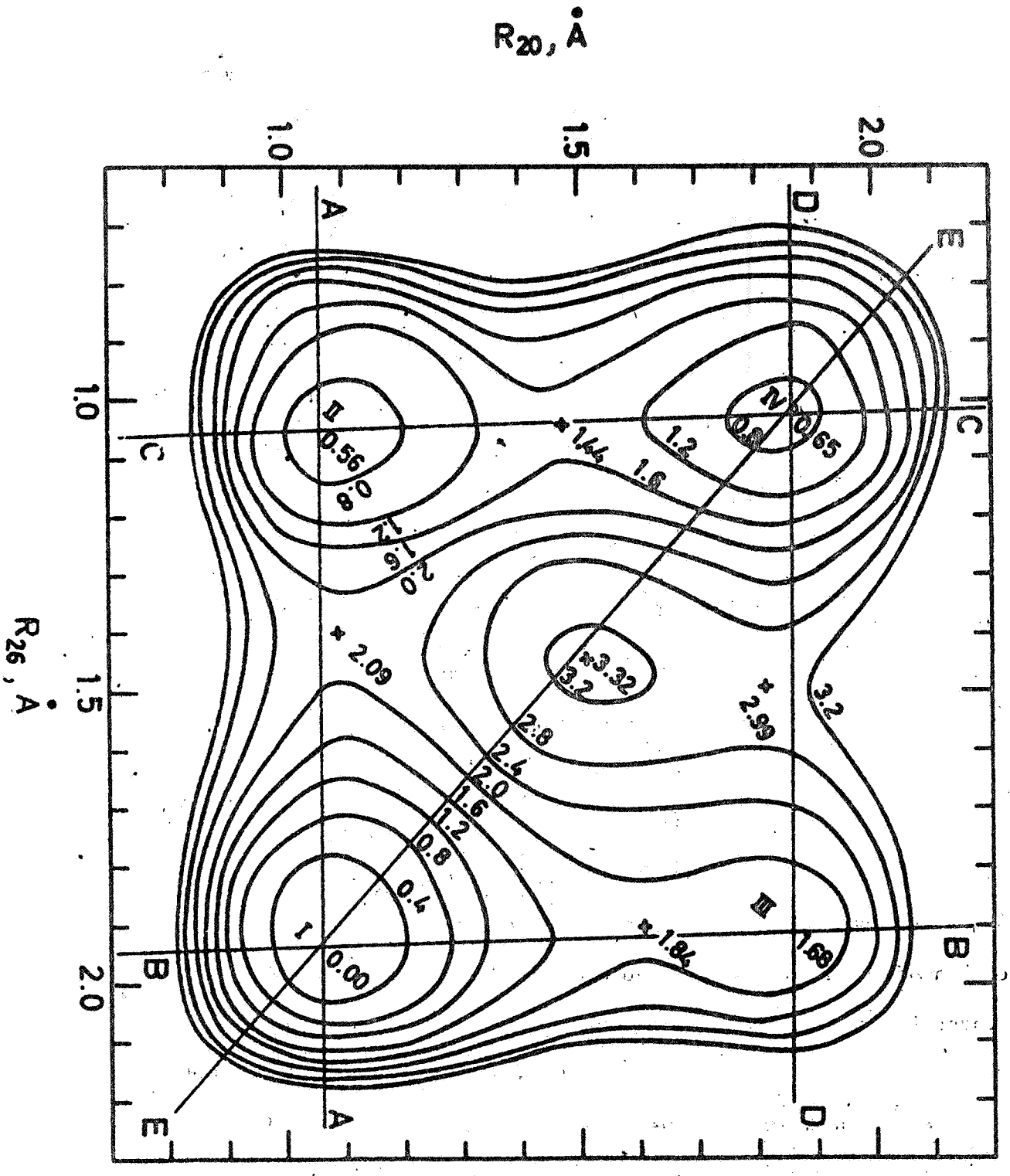


Fig. 26. Potential energy surface for the motion of the two protons in the two upper hydrogen bonds of the quanine-cytosine base pair according to Rein and Harris (reference 37). All energies are expressed in electron volts with respect to the deepest minimum. The coordinates R_{26} and R_{20} indicates the distances from the guanine molecule for the upper proton and the middle proton, respectively.

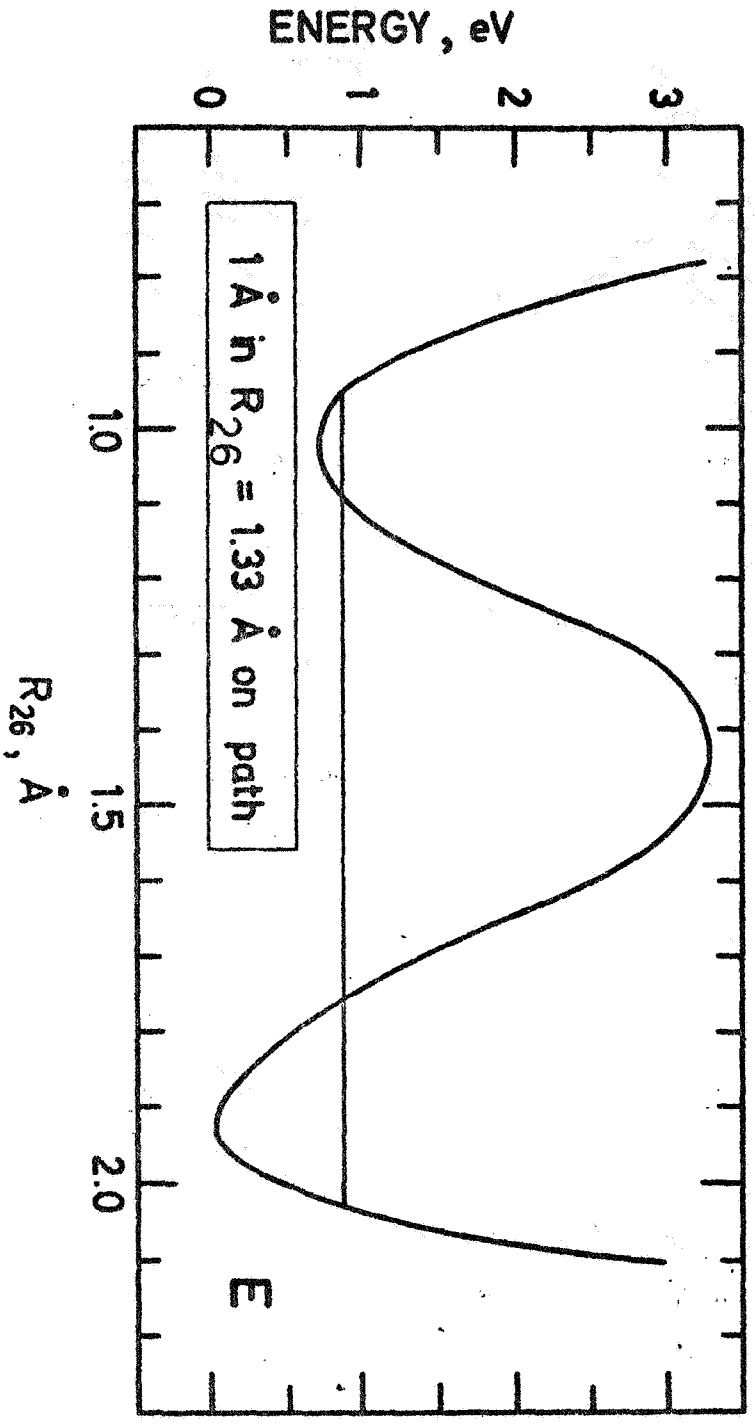


Fig. 27. Potential energy curve for the simultaneous motion of two protons in the upper hydrogen bonds of the guanine-cytosine base pair according to Rein and Harris (reference 37). Note that an energy difference of 0.65 eV at 310°K corresponds to a Boltzmann factor of $B = 10^{-10.5}$.

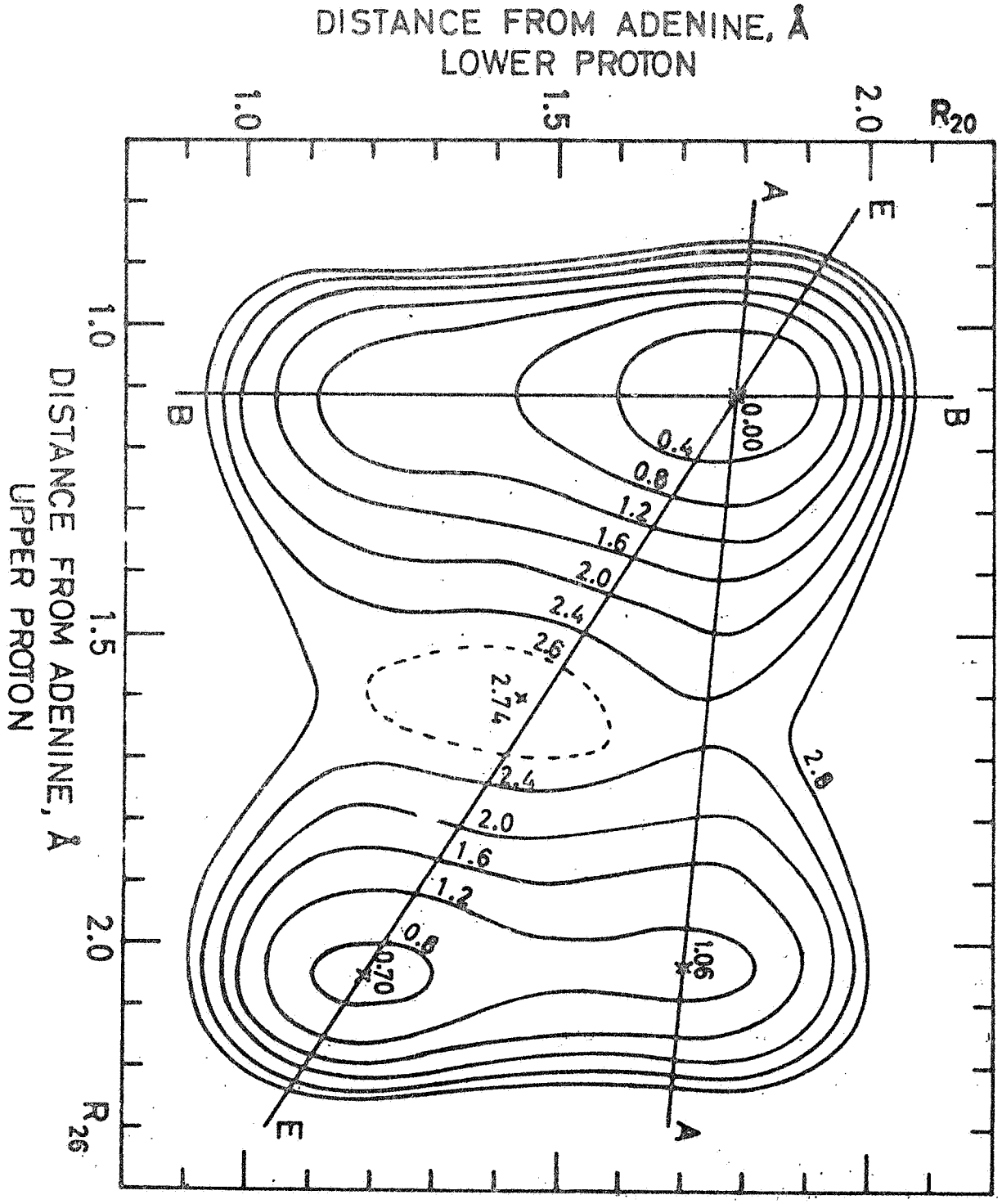


Fig. 28. Potential energy surface for the motion of the two protons in the hydrogen bonds of the adenine-thymine base pair according to Lunell and Sperber (reference 38). All energies are expressed in electron volts with respect to the deepest minimum.

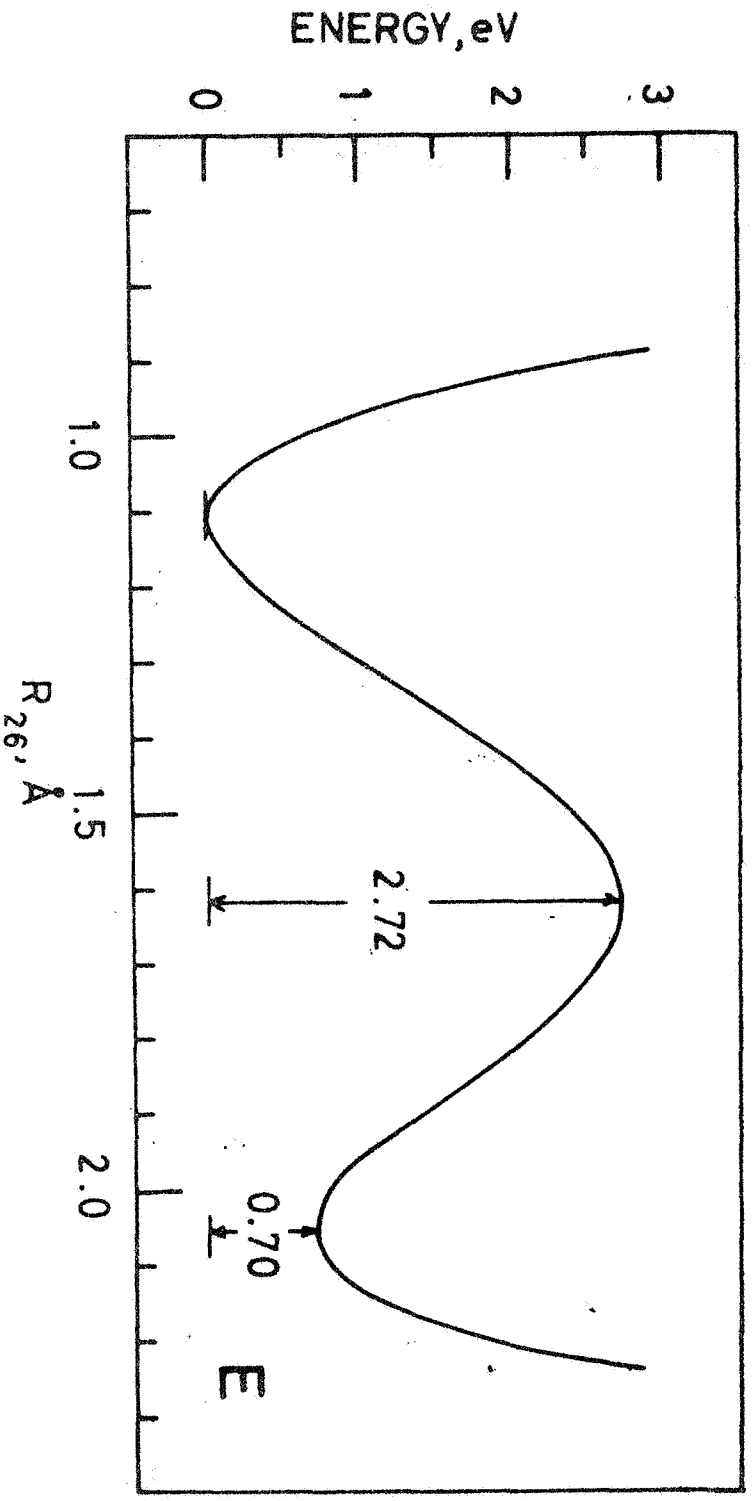


Fig. 29. Potential energy curve for the simultaneous motion of two protons in the hydrogen bonds of the adenine-thymine base pair according to Lunell and Sperber (reference 38). Note that an energy difference of 0.7 eV at 310°K corresponds to a Boltzmann factor of $B = 10^{-11.4}$.

is 0.7 eV, which at $T = 310^{\circ}$ K corresponds to a Boltzmann factor of $B = 10^{-11.4}$. This result implies that the A-T base pair has a somewhat higher internal stability against mutations than the G-C base pair, in full agreement with data obtained by the Pullmans[39] in an entirely different way.

These calculations have been carried out by means of the IBM 7090 computer at FOA in Stockholm and the CD 3600 computer at the University of Uppsala. Even if these calculations are some of the most elaborate studies of simple conjugated systems performed so far, they are far from being sufficiently accurate for our purpose, and it is particularly desirable to refine the treatment of the lone pairs and the σ-skeleton in general and eliminate the use of the Wolfsberg-Helmholtz parameter K. Work along these lines is in progress.

Even in their present form, the figures obtained are probably accurate enough to permit at least a qualitative discussion of the problem of the internal stability of the genetic code in DNA. First of all, we observe that the potential barriers seem to be high enough to prevent any significant part of the proton population to pass above the barriers, and that essentially all proton transfer occurs through the tunnel effect with comparatively short tunneling times ($\approx 10^{-5}$ sec), so that the tautomeric equilibria are quickly achieved. The energy difference between the normal base pairs and the tautomeric base pairs obtained through proton exchange is 0.65 eV and 0.7 eV for the G-C and A-T pairs, respectively, and these figures correspond to the Boltzmann factors $10^{-10.5}$ and $10^{-11.4}$. These factors should be compared with the spontaneous mutation probabilities per base pair and generation, which experimentally lie in the range between 10^{-8} and 10^{-11} according to Freese[10]. Such an agreement is certainly "too good to be true", and we have all reasons to be critical against so good results. However, it seems anyway rather satisfying that the genetic code in DNA even within rather wide margins seems to be sufficiently stable even with respect to proton tunneling. So far, all data obtained confirm the Watson-Crick model.

Proton-tunneling in the "rare" base pairs. - At normal temperature, say $T = 310^{\circ}K$, proton exchange in the normal base pairs G-C and A-T is a quick but exceedingly rare event leading to the formation of the tautomeric pairs G^*-C^* and A^*-T^*. It is now of interest to study the phenomenon of proton tunneling in the four anomalous base pairs:

$$A^*-C,\ A-C^*,\ G^*-T,\ G-T^*,$$

which are related two and two by double proton exchange. A study[40] of the π-electron energies favors the forms A-C^* and G^*-T, in which the bases A and T are normal. In time for the Study Week, Lunell and Sperber[38] have concluded a calculation of the double-well potential for the A^*-C base pair and found that the form A-C^* has a total energy which is 0.5 eV lower than the form A^*-C and that the potential barrier in the middle is 1.05 eV with respect to the higher minimum. This result implies that the form A^*-C is unstable with respect to double proton exchange and quickly goes over practically completely to the form A-C^*. Preliminary calculations indicate that a similar result holds also for the G-T^* pair which quickly through double proton exchange goes over into the form G^*-T.

This double proton exchange in the "rare" base pairs seems to be of essential importance in discussing the effect of incorporation errors at DNA replication. One can expect that the nutrition material at the replication contains all the nucleosidetriphosphates of the bases A, T, G, C and their "rare" tautomeric forms A^*, T^*, G^*, C^* of the type described in Fig. 16, as well as other tautomeric forms. One may get an idea of the probability distribution over the various forms by calculating their total energies. However, since only the difference in energy between the normal and the tautomeric form is of importance, one may, in a first approximation, calculate the difference between the energies of the mobile electrons, since the change in polarization of these electrons is one of the main effects of the replacement of the proton in the tautomeric shift.

The π-electron energies of the nitrogen bases and their tautomeric forms were first evaluated in the Hückel scheme by the Pullmans[31].

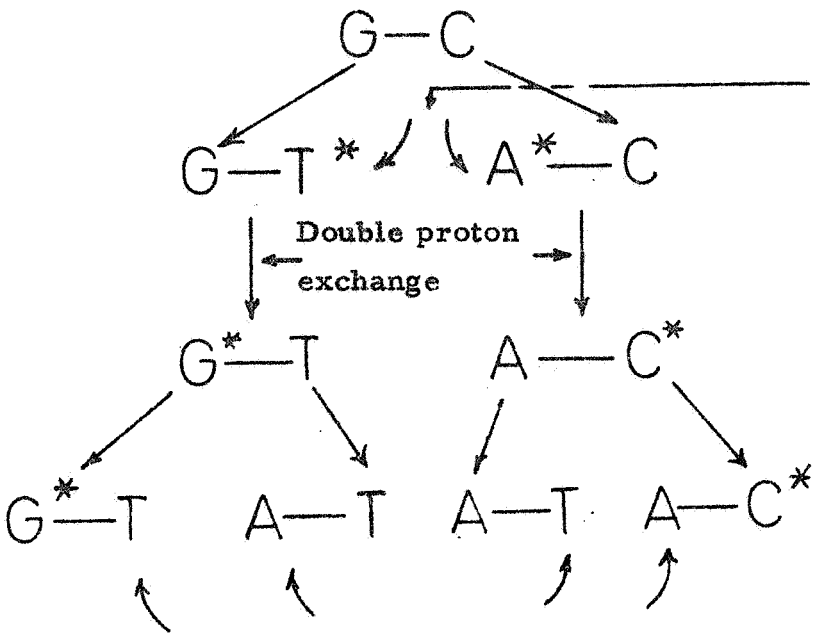
Using slightly modified data from reference 4, p. 296, one gets the following result:

	A	T	G	C
Normal form:	$12\alpha + 17.214\beta$	$12\alpha + 22.512\beta$	$14\alpha + 22.094\beta$	$10\alpha + 16.096\beta$
Tautomeric form:	$12\alpha + 16.940\beta$	$12\alpha + 21.798\beta$	$14\alpha + 21.482\beta$	$10\alpha + 15.962\beta$
Difference:	0.274β	0.714β	0.612β	0.032β

where β is the Hückel bond integral with the approximate value $\beta \approx -18$ kcal/mole $= -0.78$ eV. The results imply that, of all four nitrogen bases, cytosine C is most likely to occur in its tautomeric form C^* - a result already obtained by the Pullmans[39] - and the other bases come then in the order adenine A, guanine G, and thymine T.

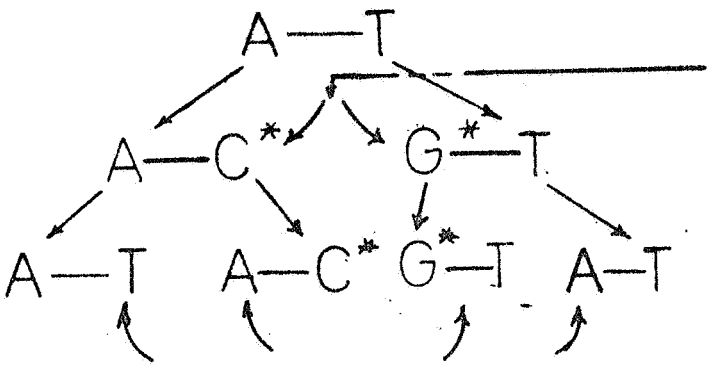
In sec. 4, it was shown that the incorporation of a single tautomeric base in the "replication plane" was highly improbable, but that one could not exclude the incorporation of a tautomeric pair of complementary bases (A^*, T^*) or (G^*, C^*). The π-electron data given above indicate that the probability for the occurrence of a complementary tautomeric pair is much smaller for (A^*, T^*) than for (G^*, C^*), and the essential incorporation error involves the latter pair.

It should be observed, however, that the replication schemes will be modified by the previously mentioned unstability of A^*-C and G-T^* with respect to double proton exchange. In Fig. 30 a, we have outlined the replication scheme of a G-C base pair after the incorporation of the tautomeric pair (T^*, A^*) in the replication plane, and we note that the process leads to a mutation G-C → A-T which undergoes "biological amplification" by the factors 2, 4, 8, 16, 32, ... etc. In Fig. 30 b, we have sketched the replication scheme of an A-T base pair after incorporation of the tautomeric pair (C^*, G^*), and we note that the process does not lead to a mutation until the forms C^* and G^* in some way have undergone a tautomeric shift and gone over into the normal forms. Available data indicate that, if the pair (C^*, G^*) is very rare at tautomeric equilibrium, the pair (T^*, A^*) should be exceedingly rare, and the processes discussed above may then also be of importance in the



Incorporation of a tautomeric pair (T^*, A^*) from the environment in the replication plane

(a)



Incorporation of a tautomeric pair (C^*, G^*) from the environment in the replication plane

(b)

Fig. 30. Replication scheme for the normal base pairs under incorporation of tautomeric pairs of complementary bases; note that the pair (T^*, A^*) occurring in (a) is apparently rare in comparison to the pair (C^*, G^*) occurring in (b).

purification of DNA replication with respect to errors leading to mutations and in explaining "why spontaneous mutations are so rare". The figures in this section are, of course, highly preliminary, and it seems essential to study these problems with considerably more accuracy in greater detail.

6. BIOLOGICAL SIGNIFICANCE OF THE STABILITY OF DNA

The DNA molecule carrying the genetic message is situated in the chromosomes in the cell nucleus, and from this position it regulates the entire metabolism of the cell, particularly the protein synthesis occurring in the ribosomes in the cytoplasm. The genetic instruction is carried from the DNA molecule to the various parts of the cell by a certain compound called messenger-RNA. This is also a ribonucleic acid which seems to be essentially one-stranded and contains the ordinary four nitrogen bases but with thymine T replaced by uracil U, which is analogous to thymine having the methyl group replaced by hydrogen. It seems clear that, in the transcription of the genetic message, messenger-RNA is synthesized in the deep groove of DNA by means of the template principle, but it is not yet experimentally known whether this involves a temporary opening of the genetic code by releasing the hydrogen bonds or whether the genetic message is "read" in a proton-electronpair "copy" associated with the 6-amino and 6-keto groups in the deep groove of DNA; see Fig. 14. For a survey of the literature in this field, we would like to refer to reference 4.

The messenger-RNA regulates again the protein synthesis in the ribosomes by the template principle. Each one of the twenty amino acids is attached to an "adapter" in the form of a comparatively small molecule of soluble RNA (s-RNA) which carries the amino acid to its correct place on the messenger RNA by means of complementarity between a small nucleotide part of the adapter and the template. There are further good reasons for believing that a triplet of consecutive bases in the genetic message of DNA corresponds to one amino acid, and a great deal of research is currently devoted to this so-called coding problem. The metabolism

of the cell seems hence to be essentially regulated by the simple rule:

$$\text{DNA} \longrightarrow \text{RNA} \longrightarrow \text{Proteins},$$

which is now almost universally accepted as the basis for the entire molecular biology.

Mutations and Evolution of Life. - As mentioned in the introduction, one has strong experimental evidence that the change of the genetic template of a single base pair may lead to a change of one amino acid in a specific protein, which means that the change of a single sign in the genetic code may lead to a mutation[6]. Since the introduction of the Watson-Crick model of DNA, it has seemed clear that the occurrence of spontaneous mutations in some way should be associated with the natural occurrence of tautomeric forms of the base pair, and the main problem has not been to find possible mutation mechanisms but instead to explain "why spontaneous mutations are as rare events as they are". The purpose of the study reported here has also been to show that the genetic code has sufficient stability against incorporation errors as well as against proton exchange through tunnel effects.

At the same time, it is rather fascinating to realize that the tautomeric forms differ from the normal forms only by having a single proton replaced a distance which is approximately 2, 3 Å, or that tautomeric forms may be produced by having a proton changing its place by jumping about 1 Å. One of the strongest driving forces in the evolution of living organisms on the earth through mutations may hence be proton jumps over a distance of 1 Å = 10^{-8} cm facilitated by the quantum-mechanical tunnel effect.

The spontaneous mutations involve changes of base pairs of the type A-T $\rightleftarrows$ G-C which are reversible. Our study indicates that the A-T pair should have higher stability than the G-C pair, which implies that the spontaneous mutations should in the long run favor the direction G-C $\longrightarrow$ A-T. It is perhaps not a mere coincidence that Chargaff's investigations of the base contents of DNA show that the higher organism have a considerably larger content of A-T pairs than G-C pairs in

their DNA-molecules.

Our result that the genetic template in DNA consists essentially of a pattern of protons and electron pairs is of importance also in discussing mechanisms for induced mutations. If one of the strands of DNA becomes electrically charged - through weak UV-radiation, through ionizing radiation, or through charge-transfer reactions - it seems very likely that all protons of the hydrogen bonds in the base pairs may end up on one and the same side leading to ionic tautomeric forms which will then cause deletions at the DNA-replication. For a more detailed account, we will refer to references 4, 33, 36, and 37.

In connection with mutagens having a certain steric structure, as e.g. acridine, it is interesting to study to what an extent such compounds may be incorporated in the replication planes instead of new nucleotides (see Figs. 21 and 23), since this would lead to a simple interchelation mechanism. Errors of this type lead to mutations which are irreversible, since they depend on deletions in the genetic message.

Aging. - Even the aging of biological organisms is today a phenomenon which is often considered on the basis of molecular and submolecular biology. Several authors have emphasized the importance of possible damage to the genetic code and the occurrence of somatic mutations as causes of aging[41].

In this connection, there is a distinct difference between unicellular and multicellular organisms. A unicellular organism propagates by cell division and, since one cell suddenly becomes two, two become four, etc., a cell lacks individual identity and cannot age in the ordinary sense, particularly if the time span between two cell divisions is comparatively short. If such a cell becomes damaged, it will either die directly or lose out in the struggle for existence in competition with normal cells. This implies that a system of such cells will usually not show any loss of vigor with time.

In multicellular organisms, the circumstances are rather different. The metabolism of each cell is regulated by its genetic code which is read over and over again in the formation of messenger-RNA necessary for the protein synthesis. The transcription is carried out by means of such templates that hydrogen bonds are formed and broken again. Our study in

Sec. 5 as to the double-well potentials indicates that each hydrogen bond between complementary base pairs contains inevitably a "proton error" of the order of magnitude 10^{-10} to 10^{-11}, which becomes manifested when the bond is broken, since the protons are then forced to "choose side". This means that every transcription introduces necessarily a small error in the proton-electronpair code of DNA which has been utilized for this purpose, i.e. there is a specific loss of genetic information of the cell concerned which is essentially proportional to the number of transcriptions, i.e. to the rate of metabolism of the cell. Since the proton error mentioned above is certainly only a lower bound, it seems worthwhile to investigate whether this loss of genetic information through repeated transcriptions in any way may be related to the primary cause of the phenomenon of aging.

It is rather remarkable that, in multicellular organisms, the gametes are set aside in an early stage of life and are not brought to full metabolism and maturity until the time they are really needed.

In this connection, one should observe that the multicellular organisms have two types of tissues, which will here be denoted by I and II, and which differ by their modes of replication. Tissues of type I renew themselves periodically by cell replication, whereas, in tissues of type II, the cells have stopped their replication at a certain stage of life, so that such a tissue belongs to the mature organism once and for all. Nerve cells and brain cells are typical examples of the second type.

In tissues of type I, where the cells divide frequently, a large damage to the genetic code will usually cause only little harm; since the corresponding cell will immediately lose out in competition with the normal cells. On the other hand, there is a certain "average" loss of the genetic code in all the cells through the metabolism which may cause tissues of this type to age, even if half of the DNA-molecule are sorted out at every replication.

In tissues of type II, where the cells belong to the mature organism once and for all, the loss of the genetic information through the repeated transcriptions necessary for the protein synthesis and the general metabolism is a still more serious phenomenon, since the cell may finally lose the information necessary to produce vital structural proteins or enzymes

and will finally die. It does not seem unreasonable to think that the primary cause of aging of multicellular organisms would be concentrated to such cells, and it seems at least worthwhile to study these problems in greater detail, and particularly a closer investigation of the transcription process would be of essential importance.

Occurrence of Tumors and Cancer. - A tumor may be described as an "individual within the individual", and it seems rather natural that many authors have discussed the possibility that tumors and cancer may be caused by somatic mutations, i.e. changes in the genetic code in the cells of the body. Cancer may be described as the growth of abnormal cells in the organism which have a higher rate of metabolism and replication power than the normal cells and which hence take over the organic material in large areas and form malignant tumors. It is characteristic for the cancer cells that they often seem to lose the specificity of the tissue from which they originate, that they easily form daughter tumors (metastasis), and that they do not seem to activate the ordinary immunological reactions of the organism.

The theory of the cause of cancer is complicated by the fact that many chemical compounds which are strong mutagens are not carcinogenic, and vice versa, and today it seems hardly justified to assume that somatic mutations associated with certain changes in the genetic code would be the only cause of tumors. In the deletion hypothesis, cancer is assumed to be essentially caused by the fact that some growth - controlling factors in the cell or in the organism - enzymes, hormons, etc. - for some reasons have been deleted. This may depend on an error in the DNA molecule itself, e.g. through a somatic mutation, or it may depend on other errors along the basic assembly line DNA $\rightarrow$ RNA $\rightarrow$ proteins in the hormon producing cells or in the cells of the tissue directly concerned.

In discussing these problems, it is important to understand how the cell differentiation in a multicellular organism is achieved. One knows from experience that each cell of the organism has the same complete genetic message contained in a system of DNA molecules but also that, in the differentiated cells, only a very small part of this information is utilized for the synthesis of the structural proteins and enzymes which are needed.

Platt[42] has compared the system of DNA molecules with a book with many chapters, in which only a specific chapter is "read" and utilized for controlling the metabolism in a special type of cell, whereas all the other chapters are "closed". The reading of the open chapter of the book is regulated by processes of the type successfully investigated by Jacob and Monod[43], but one still does not know whether similar processes regulate also the closed chapters, or whether they are simply "closed" by certain molecules blocking the reading of the deep groove in DNA.

One may wonder how a cell obtains such a specific message as "read chapter one", "read chapter two", etc. The simplest mechanism is based on the idea that a certain information I, which originally may have been obtained e.g. by reading a single strand of DNA, is not duplicated before a cell division but is instead divided into two parts, $I = I_1 + I_2$, going into different daughter cells. This leads to a replication scheme which is schematically illustrated in Fig. 31.

When the material in a tissue is renewed, it is essential that both the DNA molecule as well as the molecules regulating the reading of DNA are properly duplicated before cell division. The DNA molecule has a stabilizing protein (histone) in its shallow groove, and it seems very likely that it has a similar protein cover also in the deep groove, which may be removed for replication and transcription of the genetic message. If, at a cell replication, some of the molecules which eventually block certain parts of the deep groove of DNA would be deleted, the cell would lose part of its differentiation and probably get a foreign appearence in the tissue. However, since the cell does not contain any molecules which are foreign to the organism, the usual immunological reactions are not immediately activated. For the moment, one does not know what happens when a skin cell starts reading the chapter about brain cells, but it seems certainly worthwhile to investigate the existence and properties of partly "dedifferentiated" cells in connection with the problem of tumors and cancer. It is also important to consider the DNA molecule with its protein cover, i.e. as a nucleoprotein, and we note that a DNA molecule without its proper framework may be very dangerous to the organism - not because of lack of genetic information, but because of too much information simultaneously.

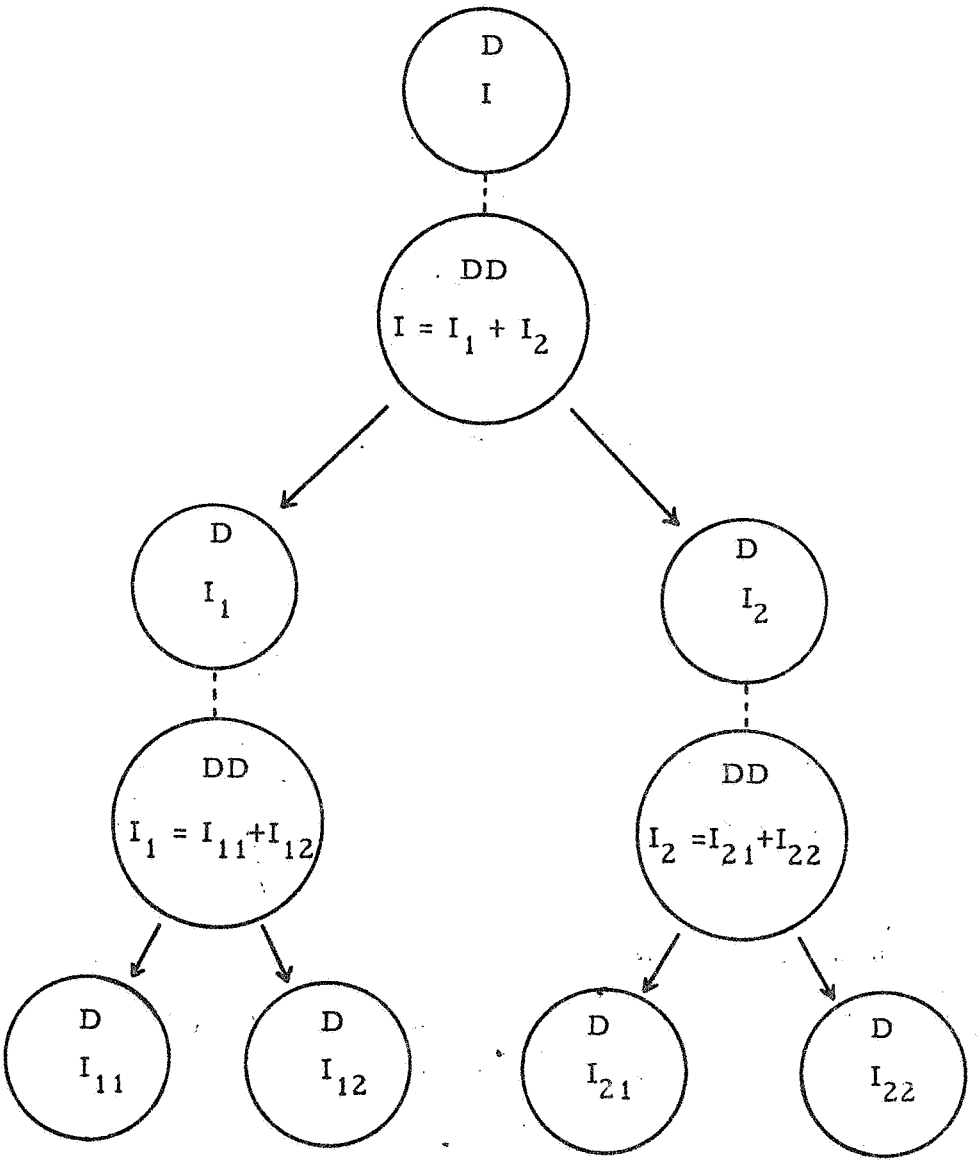


Fig. 31. Cell replication leading to a differentation of the cells. D = DNA content, I = Information for reading DNA.

This variant of the deletion hypothesis is appealing from the point of view that it explains the tendency of cancer to form daughter tumors through defect nucleoproteins as well as the lack of strong immunological reactions. There may be many reasons for the deletion of the transcription-controlling molecules: there may be a basic enzyme missing through aging or through damage to the genetic code caused by radiation or chemicals, or the controlling molecules themselves may have been damaged by radiation or by chemicals (including external viruses). This is certainly an immense area of research, and we have here only tried to emphasize the fact that, in connection with tumors and cancer, one should not only investigate the genetic message but also the instructions for reading this message, including the "blocking" of a large part of the genetic code in any differentiated cell.

The interest is again focused on the genetic code and the reading and blocking of the message in the deep groove of DNA, and it seems rather obvious that the templates formed by proton-electronpair patterns and the molding of new templates through hydrogen bonds are going to play a fundamental role also in this connection.

Memory. - Our analysis of the genetic code in Sec. 3 shows that, in the DNA and RNA molecules, nature has found an excellent mechanism for the long-time storage of information in the form of a proton-electronpair code of the type

Counting the proton, one gets the symbols O or 1, and such a storage is hence completely analogous to the storage mechanism in a modern electronic computer, with the difference that the "microcomponents" used by nature can hardly be beaten by any technical development. The question is now whether nature is using a similar type of device also for storing short-time information of the type usually referred to as "memory" - the ordinary conscious memory, the reflex memory, the "chemical memory" rendering immunity against diseases, etc. There are strong indications that nature is utilizing DNA and RNA molecules also for this purpose, but very little is known about the detailed mechanism. It seems very likely,

however, that the hydrogen bonds are of basic importance also in this important field of biology.

Conclusions. - In this section, we have discussed certain fundamental biological problems from the point of view of the Watson-Crick model and the study of the stability of the genetic code carried out in Secs. 3-5. There is hardly any question that all these problems have been greatly oversimplified, and that a complete discussion has to include not only the properties of the DNA molecule and the metabolic assembly line DNA → RNA → proteins, but the cell and the organism as a whole. It is clear that a great deal of additional experimental information is needed to give the theory a firmer ground to work on. It is evident, however, that hydrogen bonds are going to play a fundamental role even in the refined theories to come.

7. ACKNOWLEDGEMENTS

The program for the research as to the nature of the genetic code in DNA and its stability was outlined by the author in the Technical Note No. 85 which was dedicated to His Majesty, the Swedish King Gustaf VI Adolf, on his 80-year birthday on November 11, 1962. The generous support from King Gustaf VI Adolf's 70-Years Fund for Swedish Culture and Knut and Alice Wallenberg's Foundation for this research is gratefully acknowledged. The research program was later modified, and the modernized version has appeared as Technical Note QB 7 (1963); a revised version was finally published in 1965 in reference 4.

The study of the proton exchange in the base pairs of DNA involves the evolution of rather complicated potential energy surfaces. Even with the help of modern electronic computers, this is a rather formidable problem which was undertaken as a group effort by the Uppsala Quantum Chemistry Group. The author would like to express his sincere gratitude to the U.S. Cancer Institute for a research grant which made this investigation possible. Computer time was generously granted by FOA, Stockholm,

and by Uppsala University, which is also gratefully acknowledged.

The author is greatly indebted to Drs. Janos Ladik, Robert Rein, Frank Harris, Klaus Appel, Sten Lunell, Gunnar Sperber, and others for carrying out various parts of the numerical calculations and the necessary improvements of the basic theory of conjugated systems. He would also like to thank Dr. Olle Mårtensson for his most valuable assistance with the literature in the field.

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