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HYSTERESIS AND MOLECULAR MEMORY RECORD†

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The problem of memory and learning is discussed on the basis of a concise review of our present knowledge in this intriguing field of behavioral research. The results of a great variety of biochemical and biophysical studies support the current comprehension that biological information storage has a chemical molecular basis. Before a biosynthetic consolidation of phenotypic memory takes, however, place, there must be a physical interaction between the electric impulses of the neuronal network with an already existing molecular matrix. A mechanism proposed for fast imprint with low energy expenditure comprises conformational metastability (in certain cases leading to hysteretic phenomena) and conformational changes in macromolecules and macromolecular organizations such as membranes. Some physico-chemical aspects of a molecular memory system are discussed and a biopolyelectrolytic model system is described.

1. INTRODUCTION

Since the earliest times man has been trying to understand the processes of memory and learning. Studies in this fascinating field suffer, however, from the prime difficulty that there is no direct way to observe memory. We derive the existence of memory from observations of the changes in certain behavioral patterns which occur under specific conditions during a training period. However, behavioral results are difficult to analyze since the performance of living beings is affected by such numerous factors as previous experience, motivation, or boredom, stimulus or fear. Although memory is determined by genetic as well as environmental factors, we shall consider here only some phenotypic aspects of memorizing individual experience.

According to von Foerster (1968), learning, in an advanced sense, is not only the capacity to record experience in a memory store, but the ability to manipulate symbols, first inductively by computing generalities from particulars, and then deductively by reconstructing particulars from generalities. These faculties are developed during the learning process, thus indicating that memory is a complex structure which may be related to a change in the pattern of a distributed computer—a change which facilitates inductive operation.

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Indeed, the term 'memory' comprises multifunctionality. In analogy to a computer system, in memory we can logically distinguish recording, storage, read out and recognition of information stored in previous records. In contradistinction to computer technology, however, the memory components of living organisms are rather complex and clear cut isolation of the elements is difficult. Moreover, it is claimed that memory is based on a process of selection from an enormous number of bits of information perceived by the sensory organs. This selection may be of primary importance for individual memorizing. Obviously in the screening process a weight function is attributed to different elements of perception, which determines the imprint and the decay rate of memory traces.

Since the genetic record is molecular and based on changes in the covalent chemical structure of DNA, it seems natural to follow Semon's hypothesis that there exists a material 'engramme' also for the phenotypic experience (Lashley, 1950). Semon (1904) placed the engrammes in the Nissl bodies of the nerve cells; his idea was, however, challenged by Lashley, who tried in vain by ablation experiments to find the specific site of memory in the brain. Lashley was able to show that memory is proportional only to the total brain material. Milner and Penfield (1955) found that the hippocampal region is important for the facilitation of the memory imprint, but the hippocampus too does not appear to be the locus of memory storage.

Thus biological memory does not seem to be localized in particular parts of the brain but rather is distributed over the brain as a whole, resembling a network of the type recently discussed by Uttley (1970) in his paper on the Informon. This network is dynamic and statistical, having elements and connectivities which change with time. But, dependent on experience, it can extract pertinent information from the noisy background.

In this context, it is of value to cite a statement of Uttley: 'If a pattern has to occur for a very long time, the pathways have to be given an additional property *akin to magnetic hysteresis*—and then the informon becomes a permanent discriminator of the pattern to which it is adapted'.

The present-day view is therefore that the basic process of memorizing is a change in the statistical activity of population of neurons, due to previous experience. There is no doubt that these changes are based on a microscopic facilitation of nervous conduction. Whatever be the structure of the dynamic network related to memory, there is accumulative evidence that biological information storage has a chemical molecular basis.

2. STAGES OF MEMORY PROCESSING

The consideration which requires a molecular or subcellular record of memory is based on the following observation: According to von Neumann's estimate, the number of bits of information in the human brain is much larger than 10^{15}. The average number of neurons is $\simeq 10^{10}$, and it is assumed that the number of links which every nerve cell can make with other neurons is 10^3. Thus, if the bits of information are located in single cells and in their connections the number of locations would not suffice. The other alternatives are either subcellular storage in molecules or structures, or storage in the form of reverberating circuits.

To maintain 10^{15} bits of information in the human brain permanently in the form of reverberating circuits would require such a tremendous expenditure of energy that this concept is highly implausible. However, short-lived circuits, corresponding to learning experience, are conceivable. Already Lorente de Nó (1938) and Hebb (1949) suggested that the labile phase of the short-term memory is connected with reverberating circuits in the nervous system. This hypothesis seems to be supported by the more recent work of Verzeano and Negishi (1960) who recorded using microelectrodes, typical sequences of discharges in the thalamic region. But even if memory is a network phenomenon, memory molecules could facilitate its propagation.

A large number of experiments have led to the conclusion that the process of memorizing is multistaged. The stage of short-time memory involves the imprinting of memory and transient storage. The stage of long-term memory seems to follow the short-term stage and comprises the consolidation and fixation of experience. As long as memory does not reach the stage of consolidation, it can be wiped out by an electroconvulsive shock given within approximately half an hour after learning. As shown by Cerf and Otis (1957) in their experiments on goldfish, heat and cold narcosis can also prevent the consolidation of learning the way in a maze. Pearlman and Jarvik (1961) showed that spreading depression with an osmotic shock due to KCl, applied on both sides of the cortex, wipes out the traces of short-lived memory.

Within the duration of short-term memory it is possible to influence the rate of fixation of information in more stable forms. Thus, Lashley found already in 1917 that non toxic doses of strychnine increase learning capacity, whereas caffein decreases it. Tryon (1940) selected two strains of mice: maze-bright and maze-dull mice. Following Lashley's observation, Petrinovich, Bradford and McGaugh (1965) demonstrated that the injection of small amounts of strychnine transform the dull into bright mice. Additional evidence for the enhancement of memorizing by strychnine was provided by Pearlman, Sharpless, and Jarvik (1961). They showed that an electroconvulsive shock applied to rats ten minutes after learning, obliterates the memory record; if, however, the animals were pretreated with strychnine, the electrical shock had no such effect, indicating that with sublethal doses of strychnine, memory can be consolidated within 10 minutes.

One of the first observations that memorizing is influenced by biosynthetic processes is due to Sachs (1961), who showed that the injection of microquantities of potassium into the brains of mice increased their learning capacity. On the other hand, similar administration of small amounts of calcium transformed maze-bright mice into maze-dull ones. These observations are in accord with the findings of Lubin and Ennis (1963) that accumulation of potassium enhances protein synthesis, whereas calcium reduces the biosynthetic process. Similar reasoning led to the recent studies on the relation between specific biopolymer formation and memory.

3. BIOPOLYMER SYNTHESIS AND MEMORY

Brattgard (1952) made the observation that the RNA content of the ganglial cells of the retina increases linearly with visual stimulation. Further, Wilson, Boggan, Zemp, and Glassman (1966) found that learning is accompanied by an increased uptake of tagged phosphorus and uridine. Using a rather sophisticated technique of analyzing quantitatively the RNA contents of single cells, Hydén (1967) and co-workers found a good correlation between the amount of RNA and training. Thus in mice which learned to climb a rope, or in left-handed rats which learned to use their right paws, the amount of brain RNA increased significantly. According to Zemp, Wilson, and Glassman (1967), however, the increase in RNA (in the thalamic region) is not specific to memorizing, but is rather due to the stimulation itself.

The possible relation between memory and RNA content found strong support in the intriguing experiments of McConnell (1962) on planarians. McConnell's results led to the hope that learning could be transferred through memory molecules. Although serious doubts have been expressed about the memory transfer experiments, it was recently recognised that learning behavior is species-dependent and under correct conditions the transfer experiments could be confirmed (Golub, Masiarz, Villars, and McConnell, 1970). It is rather remarkable that tricyano-amino-propene, which increases the rate of RNA formation, also stimulates learning (Hydén, 1967); similarly, strychnine which positively influences learning, enhances also the formation of RNA without affecting DNA synthesis. (Conversely, marihuana inhibits RNA synthesis, but accelerates the formation of DNA.)

The interest in RNA synthesis accompanying memory imprinting was generally related to the belief that the ultimate step in the memory process is the synthesis of specific proteins. Proteins, or their aggregates, were regarded as the long-lived storage records which retain imprints of experience throughout life. Following this direction Flexner and Flexner (1967) were able to demonstrate that injection of pyromycin, which inhibits protein synthesis, obliterates maze training in mice. These experiments were beautifully amplified by Agranoff's (1967) studies on goldfish. Acetoxycycloheximide, which interferes with protein synthesis prevents memory consolidation, while injection of Rabinol, which stimulates protein synthesis, enhances the rate of acquisition of conditioned avoidance tasks.

Recently, Hydén and Lange (1968) have found that learning in mice is accompanied by the synthesis of an acid protein S–100, which is isolated from the hippocampal region. When an antibody against this protein is injected into the brain of trained mice, all the learning acquired during training is obliterated.

As was reported at this meeting, Ungar has isolated a basic peptide of 15 amino acids from the brain of mice trained to avoid darkness. This peptide was purified and synthesized and the synthetic product, called scotophobin, has the same behavioral effect as the peptide isolated from the brains of trained mice.

Nevertheless it is doubtful whether there is a direct correlation between memory and protein synthesis. Acetylcycloheximide, which is a stronger inhibitor of protein synthesis than puromycin, is a much less effective agent in erasing memory. Moreover, Barondes (1965) has recently demonstrated that injection of acetylcycloheximide does not impair learning, although it reduces protein synthesis for hours.

As is well known, the sequence of protein synthesis is based on the *transcription* of the DNA code into m-RNA, which is subsequently *translated* into protein.

$$\text{DNA} \xrightarrow[\uparrow\ \text{Actinomycin D}]{\text{transcription}} \text{RNA} \xrightarrow[\uparrow\ \text{Puromycin}]{\text{translation}} \text{Protein}$$

The translation step can be inhibited by puromycin, while the transcription can be abolished almost entirely by actinomycin D. In 1966, Cohen and Barondes made the following striking observation. When they injected actinomycin D into the brains of mice, up to 95% of the transcription was blocked. No effect on the learning capacity and memory retention of mice was, however, observed for four hours.

It seems that we are forced to conclude that the first step of the recording process is not a *chemical* synthesis, neither of RNA nor of protein, but rather a *physical* change in existing molecules. Only at a later stage, when consolidation and fixation occur, do chemical processes become involved. As was pointed out by Roberts and Flexner (1969), this physical change may comprise changes in conformation or location of macromolecules, induced by concentration gradients of ions or small molecules. Such changes could well alter the permeability

of nerve membranes or release suitable transmitter substances.

4. HYSTERESIS AND MEMORY IMPRINT

The problem now facing us is which physical changes should be considered for the imprint of a pattern of external stimuli on a chemical system. The requirements of a sufficiently fast record with low expenditure of energy, and the storage of the record after the stimulus is over, severely limit the choice of possible physical mechanisms. A long retention of the memory imprint is necessary to allow the induction of further consolidation in permanent structures. It seems that this imprint could be based on equilibrium states which are *a priori* time-independent and stable. But any equilibrium processes are *a priori* excluded, since equilibrium states are independent of path, and therefore memoryless. The only simple mechanism which seems to answer all biophysical requirements is that of hysteresis. As is well known from computer technology, physical memory storage devices utilize hysteresis cycles which, as already recognized by Ludwig Boltzmann a hundred years ago, are endowed with a physical memory.

Most familiar is, of course, the hysteresis loop of magnetization and demagnetization. But in other fields of chemistry and physics, hysteresis phenomena are also quite common; for example, the cycles of adsorption-desorption or of melting and crystallization.

The term hysteresis was proposed by Ewing in 1885; it is taken from the Greek, meaning 'to lag behind'. This fits Everett's (1967) definition of hysteresis: 'A process is said to exhibit hysteresis if, when the direction of change of an independent variable, x, is reversed, a dependent variable, y, fails to retrace the values through which it has passed in the forward process; the dependent variable *lags behind* in its attempt to follow the changes in the independent variable.'

Although irreversible in nature, stable hysteresis loops are time independent under ordinary conditions. The elementary processes of a hysteretic system always imply metastable states and abrupt nonequilibrium transitions; the sharpness of the transition is however spread out to smooth (hysteresis) curves in larger assemblies of elements.

The transition behavior of a single element or domain can be described thermodynamically by a state function $y(x)$ of a van der Waals type, with well developed metastable states. When sufficiently high energy barriers prevent an equilibrium transition between two states A and B, at x_{eq}, the system will undergo a change only after passing through metastable states. The actual phase changes are then unidirectional and irreversible. They occur at different values of x, vary from state A to B at $x(AB)$ and from state B to A at $x(BA)$. Thus, in contradistinction to a state of equilibrium, in a hysteretic system, the internal parameter is not a single-valued function of the external parameter. In the path of transition of A to B, the value of y will depend on the history of tracing: either from A towards B or from B toward A; in other words, hysteretic systems 'remember' the path by which the actual state has been reached.

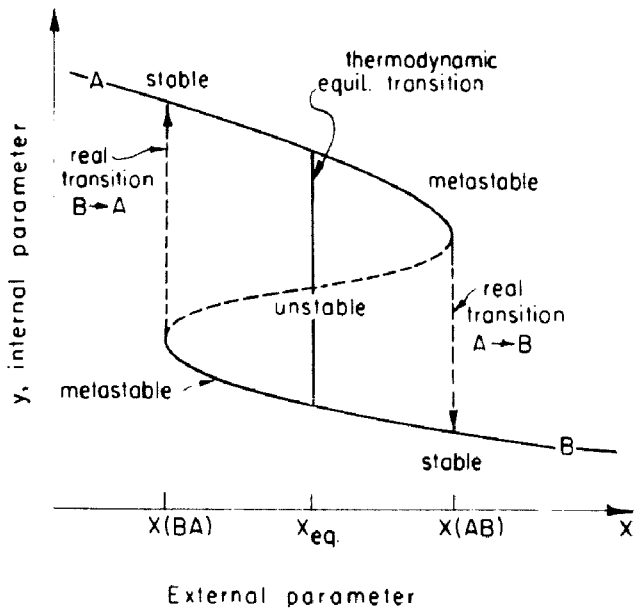

FIGURE 1 Schematic representation of a phase transition. Stable states of A and B; metastable states of A and B; and unstable states in a hysteresis system.

Everett and Whitton (1952) explained with the help of a magneto-mechanical model how the energetic barriers of metastability arise and how they can be circumvented.

The model consists of a thermostated bimetallic strip which serves as a switch in an electric circuit, as shown in Figure 2a. The circuit may be closed by establishing contacts between the two magnets C. Upon increasing the temperature T, the bimetal is bent and at a certain point the magnetic attraction will enhance the bending towards a closure of the contacts, at T_1. Thus a current i is switched on. On

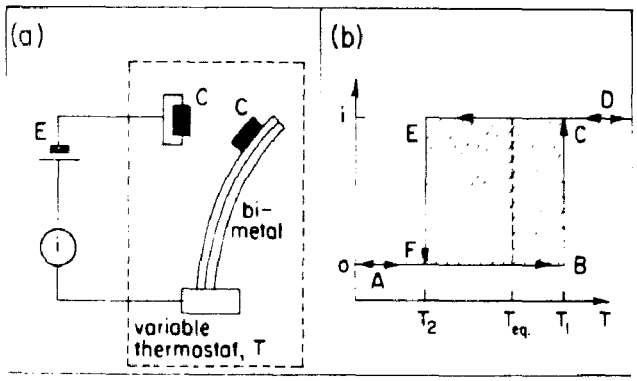

FIGURE 2 The Everett-Whitton model:
a. Magneto-mechanic circuit.
b. Electric current i as a function of temperature T.

decreasing the temperature from values $T > T_1$, the magnetic attraction now prevents the opening of the switch at T_1. Only when T is reduced to T_2, the stress in the bimetal will suffice to overcome the attraction and open the circuit. Thus, as shown in Figure 2b, upon heating the path *ABCD* is followed, whereas upon cooling, the path *DCEFA* is traced; an area of metastability *BCEF* remains as a kind of 'hysteresis loop'. In this example, the energy barriers are provided by the magnets and it is these energy barriers which compel the system to 'go around' and 'switch' at higher and lower temperatures than the equilibrium temperature T_{eq}.

It should be realised that the majority of the objects around us are in metastable rather than in perfect ideal equilibrium states. Indeed, it is only the *unstable* state which cannot persist, while metastable states are commonplace. Moreover, the time independence of metastable states permits their description by thermodynamic state functions, in the same way as for equilibrium states.

5. MACROMOLECULAR CONFORMATION AND MEMORY RECORD

It was suggested already in 1964 that the record of biological information could utilize a hysteretic mechanism (Katchalsky, Oplatka and Litan, 1964). The idea was born when inspecting the finding of Cox, Jones, Marsh and Peacocke (1956), who discovered in the potentiometric acid-base titration of RNA the first example of molecular hysteresis. Recently, we have verified the hysteretic behavior by a spectrophotometric study of RNA in 0.1 N NaCl solution, which gave reproducible time-independent hysteresis loops (Revzin, Neumann, and Katchalsky, 1972). The result of an acid-base titration of RNA is reproduced in Figure 3, which also shows that scanning curves may be obtained (Cox, 1968) which are similar to those known from magnetization-demagnetization cycles. If, as shown in Figure 3, the acid titration along curve *A* is stopped at point *P*, and followed by an addition of base up to point *M*, an intermediate curve within the main loop can be traced. Subsequent addition of acid brings us back to the boundary curves of the loop.

The existence of scanning curves characterizes the RNA macromolecule as a multidomain system. The domains (or elements) are structured nucleotide stretches of different length and stability forming 'microcrystalline' regions distributed along a single chain molecule. The conformation of these domains, their size and their position in the macromolecule, are topological variables of the molecular memory system (Neumann and Katchalsky, 1970). The analysis of the scanning curves reflecting the passage through intermediate states reveals the distribution of the domains and leads to the establishment of a 'memory function' (Everett, 1967).

If we consider the domain subunits as the symbols of a script, then the scanning process implies the possibility of decoding the script. The code itself

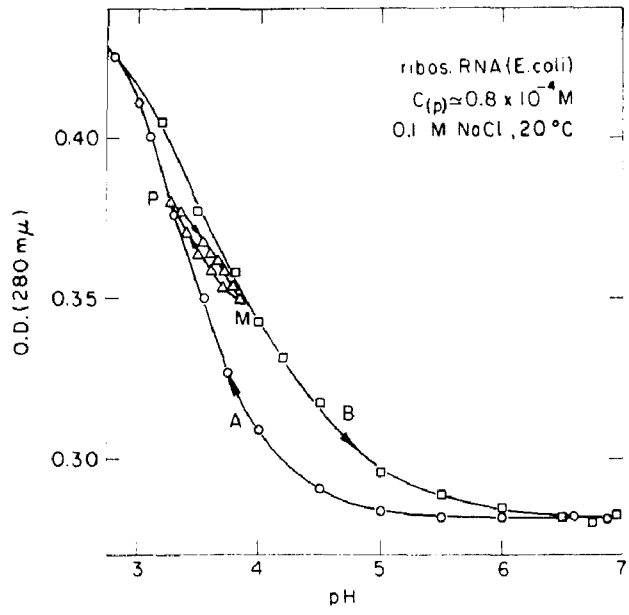

FIGURE 3 Spectrophotometric titration of ribosomal RNA: optical density (O.D.) at 280 mμ as a function of pH. o, acid titration (curve A); □, subsequent base titration (curve B); △, scanning loop; see text (Revzin, Neumann, and Katchalsky, 1972).

comprises a combination of subunits realised physically by a distribution of domain sizes and conformation (Neumann and Katchalsky, 1970).

It was recognized at an earlier date that biopolymers as well as higher organised and more complex structures, should be able to develop metastabilities (Katchalsky *et al.*, 1964). Indeed, Cox obtained in 1963 hysteresis loops in the spectrophotometric acid-base titrations of 1 : 1 mixtures of polyriboadenylate (poly A) and polyribouridylate (poly U). More recently, we have found pronounced metastabilities in membraneous systems and in the catalytic activity of several enzymes, e.g. those isolated from the halobacteria of the Dead Sea (Mevarech, Neumann and Katchalsky, 1972).

Controlled changes in the environment of metastable macromolecules or of subcellular macromolecular organisations such as membranes could therefore induce conformational changes which could serve as reproducible imprints of a memory nature. The imprinting process itself would then be based on the release of a metastable state followed by a sharp nonequilibrium structural change. It is assumed, moreover, that the forces of the electric fields accompanying nerve impulses or the ionic fluxes across the nerve membrane, could 'trigger' such conformational transitions and control their extent.

6. MODEL OF A MOLECULAR MEMORY SYSTEM

In an attempt to estimate the energetics and kinetics of nonequilibrium transitions in metastable biopolymers, we studied the physico-chemical behavior of a model system (Neumann and Katchalsky, 1970). The synthetic polyribonucleotides poly (A) and poly (U) are able to combine and form structures similar to those assumed in natural RNA. Indeed, the acid-base titrations of both RNA and the poly (A)–poly (U) model exhibit hysteresis loops within approximately the same pH range.

In Figure 4 is shown the proton binding curve (α, the degree of proton uptake as a function of pH) of a 1 : 2 mixture of high molecular weight poly (A) and poly (U), between pH 7 and pH 3. The acid titration of a solution containing the triple helical complex poly (A)·2 poly (U) formed at pH 7 is traced along curve *A*. Reversing the direction of pH change at pH 3 by adding alkali, we do not retrace curve *A* but obtain a new curve *B* as the base branch of a hysteresis loop. When following

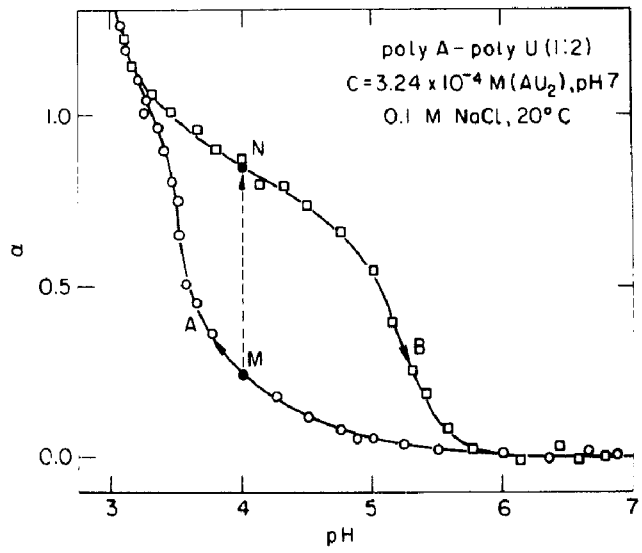

FIGURE 4 Potentiometric titration (α, degree of protonation as a function of pH) of poly (A) and poly (U) mixed in the mole ratio 1 : 2; o, acid titration (curve A); □, base titration (curve B) (Neumann and Katchalsky, 1970). M → N field 'jump' experiment, see text.

the optical density in the ultraviolet range during the acid-base titration, a similar loop is obtained (Neumann and Katchalsky, 1970).

It was possible to demonstrate that the chemical 'transcrystallization' reaction, accompanying the (hysteretic) acid-base titration,

$$2\,[\text{poly (A)}\cdot 2\,\text{poly (U)}]\ ^{(H^+)}$$
$$\text{poly (A)}\cdot\text{poly (A)} + 4\,\text{poly (U)} \qquad (1)$$

can be expressed in terms of ξ, the advancement of the conformational change. In Figure 5 it is demonstrated that ξ(pH) is fairly well independent of the actually observed parameter α or the optical density.

The two contributions to α (as given in Figure 4) are due to α_3, the proton uptake of the adenosine in the triple helix, and α_2, the protonation of the poly (A)·poly (A) double helix. The equation correlating the α's to ξ is

$$\alpha = \xi\alpha_2 + (1-\xi)\alpha_3 \qquad (2)$$

Figure 5 demonstrates clearly that starting from pH 7 and proceeding with an acid titration, the reaction described in Eq. (1) occurs spontaneously at pH -3.5. The ξ versus pH curve of the base titration is smooth and points to a cooperative equilibrium transition. Further evidence exists that our model system possesses *only one* range of metastability; the metastable states comprise protonation equilibria of the partially protonated three stranded helix. The energetic barrier preventing

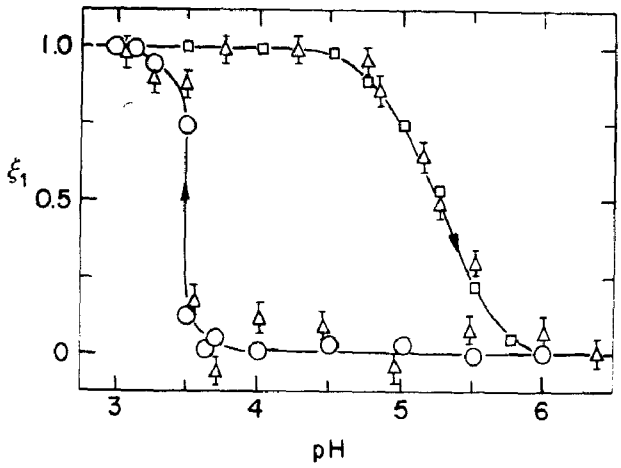

FIGURE 5 Extent ξ of the (hysteresis) structural transition 2 [poly (A)·2 poly (U)] $^{(H^+)}$ poly (A)·poly (A)+4 poly (U) as a function of pH. o, □ potentiometric acid-base titration (Figure 4 and Eq. 2); △, ξ calculated from spectrophotometric data (Neumann and Katchalsky, 1970).

the transition to poly (A)·poly (A) and free poly (U) is comprised of the electric field effect of the negatively charged phosphate residues of the polymer backbones. This effect obviously hinders the nucleation of (mainly electrostatically stabilised) sequences of (A)·(A) base pairs.

The time intervals necessary to 'transcrystallize' the domains along the nonequilibrium transition are of the order of milliseconds, whereas the double helix-to-triple helix conversion along the base branch of the hysteresis loop is a relatively slow process.

The types of secondary structure possible in RNA and in the model system depend on temperature, pH and on the ionic strength of the solution. Since we are dealing with polyelectrolytes, we may use the following relation for the dependence of the degree of ionisation α on pH and on ψ, the electric potential at the site of protonation which is determined by the phosphate residues:

$$\log \frac{1-\alpha}{\alpha} = \mathrm{pH} - pK + F \cdot \psi (2.3\ RT)^{-1} \qquad (3)$$

K is the dissociation constant of the protonated adenosine residue, F the Faraday constant, and RT the (molar) thermal energy.

While Eq. (2) provides the possibility of studying structural changes (ξ) in terms of α, Eq. (3) correlates changes of α with changes either in pH or in ψ. In general, ψ is determined by the intramolecular fields controlled by the degree of protonation and the ionic strength of the solution as well as by external fields to which the molecule may be exposed. Changes in pH at constant ionic strength provide a powerful method to study in a relatively simple way the thermodynamics of hysteresis loops. Thus, the area of the hysteresis loop, $\oint \alpha d\mathrm{pH}$, measures the amount of Gibbs free energy dissipated in one isobaric isothermal titration cycle.

$$\Delta G^{irr} = -2.3\ RT \oint \alpha d\mathrm{pH} \qquad (4)$$

In our model system, we obtained $\Delta G^{irr}(20°C) = -1.4$ kcal/mole A-residue and $\Delta G^{irr}(37°C) = -0.9$ kcal/mole A-residue (Neumann and Katchalsky, 1970). Compared with the energy of stabilization of chemical bonds ($\simeq 50$ kcal/mole) the energy loss in our hysteretic system represents a rather low energy expenditure.

The stability of the partially protonated metastable poly (A)·2 poly (U) is determined by the difference between the actual pH value and pH 3.5, the instability point at 20°C (Figure 5). Hence the Gibbs free energy of stabilization is

$$\Delta G^{st} = -2.3\ RT\ (\mathrm{pH}-3.5) \qquad (5)$$

The general stability requirement is $\Delta G^{st} > RT$, the thermal energy per mole; at 37°C, $RT = 0.62$ kcal/mole.

Inspecting Eq. (3) we see that at constant pH a change in ψ will change α, and therefore ξ. Thus, we can change the ionic strength of the solution, and thereby 'trigger' the release of the metastability (Neumann, Spodheim, and Katchalsky, 1972). More recently, we have discovered that external electric fields having a field strength of about 15–20 kilovolt/cm can directly induce the conformational transition. Using a series of electrical pulses, we have succeeded in penetrating into the hysteresis loop at constant pH, for instance from M towards N, see Figure 4 (Neumann and Katchalsky, 1972). Since a potential change from -70 mV to $+50$ mV during the passage of a nerve impulse would correspond to a field strength of 120 kV/cm, the behavior of polynucleotides fits the expectation for biopolymers to record the pulses in the form of configurational changes.

CONCLUSIONS

There is accumulative evidence that the first step of the memory record is a physical process. A plausible mechanism is based on conformational changes in biopolymers, which produce long-lived metastable states. Such metastable states, incorporated in crystalline domains, can be readily detected in hysteresis phenomena. Since hysteresis was found in the pH-titration of ribosomal RNA,

a detailed study was carried out on model polynucleotide complexes of the poly (A)–poly (U) system, which exhibit hysteresis, and the metastable helical domains of which can accommodate a physical record of experience. It was found that changes in pH, ionic strength and external electrical fields may induce conformational changes. The range of parameters used for the recording, and the energetic requirements for the hysteretic loop, lie within the range of biological interest.

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