BIOCHEMICAL MEMORY

by

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I. INTRODUCTION

The purpose of this paper is to discuss how a neuron "remembers" that its relationship with other neurons has changed and that this change is a function of input. We are mostly concerned with long-term biochemical memory. Using neurons with the same control mechanism as every other cell, we intend to introduce previous work showing changes due to learning and stimulation (mostly RNA and protein changes) and then incorporate these contributions into a workable hypothesis of memory. We suggest that a reaction which is a function of the action potential (for which precedents are cited) leads to the eventual induction of RNA synthesis by stripping histones. Histones are examined as repressors of RNA synthesis and cell division, and their removal from DNA (i.e., RNA synthesis induction) is discussed. Unnatural repressors of RNA synthesis are also discussed as background. The hypothesis that will finally evolve is then viewed in the light of the chemical studies that have been made on learning and memory.

II. CONTROL MECHANISMS

A neuron is specialized and evolved from the same precursor as every other cell.[1] We may therefore assume that each neuron must work with the same DNA, feedback, and control mechanisms as every

other cell. The neurons of the brain, however, have several advantages over the other cells as far as memory storage is concerned, as follows: (1) the cells do not divide; (2) the cells have a bloodbrain barrier protecting them, keeping out division inducers, hormones, and other substances that could upset brain homeostasis or induce or inhibit adverse synthetic processes (the bloodbrain barrier also keeps out amino acids and limits protein synthesis so that synthesis might be increased for the sake of coding, storage, and utilization of biological information[2]); and (3) the cells are well cared for by the glia, which are the proposed bloodbrain barriers.[3] These stabilizing factors make long-term memory possible because any cell division would disrupt the previous changes occurring between two neurons, and also because biochemical changes should occur only because of the exogenous stimulations, not random firing of cells.

III. CHEMICAL CHANGES DUE TO LEARNING ACTIVITY

A. RNA

The study of changes due to activity and neural excitation will lead to answers about biochemical memory. Hyden conducted much of the early work.[3-8] He noticed that rat brain stimulation due to learning and other events caused a change in the base ratios of RNA. He hypothesized that memory was stored as base sequence changes

(a highly improbable event). In subsequent experiments he found that the changes were due to the production of new RNA and withdrew statements about base sequence changes.

Gaito, who has studied RNA turnover as function of location in the brain and as a function of various activity levels, suggested that although there was no conclusive evidence it is possible that RNA or DNA or both could be the memory molecules.[9,10] He also was the first to suggest that histones might be modified during stimulation. Recently he discussed the role that RNA could have in memory and his final conclusions were that using RNA for any other purpose than for protein synthesis was unlikely, but no single RNA seems to be produced.[11]

Proposals that RNA is used for something other than protein synthesis involve the transfer of RNA from glia to neuron. In particular, Hyden has isolated and stimulated certain groups of Deiter's nuclei and glia, and noticed that RNA quantities in the Deiter's nuclei increased; that of the glia decreased. Hyden later collected evidence that there was no transfer of enzyme between glia and neuron.[6]

This change in RNA quantity could serve as evidence for Landauer's proposal that RNA is transferred from glia to neuron when the electrical gradient of these cells was reversed by an action potential.[12] The RNA would alter the neuron's protein content. This

proposal seems highly unlikely in the light of numerous studies of macromolecule entry into cells, especially if an action potential is to provide the "pores" for entry. Also, if RNA is only transferred and not created, we fail to see how the general brain RNA and protein level increases as observed when RNA supposedly only moved from place to place. Landauer went on to speculate that "information is stored as frequency characteristics of spreading ac potentials which the neuron membranes become tuned to by alteration of the protein structure of the neuron."[12] We must be highly critical of electrical storage mechanisms because the ac potentials are caused by neurons firing, which in turn modify the neuron's "frequency characteristics." This entire process develops into a cycle of instability.

Other workers, taking into account that no particular RNA was being produced, took general RNA extracts from a trained animal and injected it into an untrained animal hoping to transfer memory. Despite reports to the contrary, most workers agree that this process is unlikely or at least not reproducible.[13,14] RNA injection is not a panacea for stupidity.

B. PROTEIN AND SYNAPSE

Early work on memory attributed memory to the modification of synapses, a hypothesis that is gaining popularity. This idea

was augmented by work on the cholinesterases (ChE) whose presence usually parallels in activity and quantity ACh, although this premise may prove to be faulty. One measures ChE level to approximate ACh transmitter levels because the measurement is easier. The ChE level also gives a good indication of the level of protein synthesis.

Krech, Rosenzweig, and Bennett have shown that rats raised in an enriched environment have a higher level of brain cholinesterase than those raised in isolation.[15-17] They conclude that the rats' increased activity is the cause. Aprison and Himwick noted that ChE activity in the frontal cortex and caudate nucleus of the rabbit increased during the early portion of the life span.[18]

Other increasing brain components of interest as a function of age or activity are: chick brain serotonin as a function of age;[19] rat norepinephrine and ChE but not dopamine or serotonin;[20] and other general enzymes of the brain.[18]

Recent experiments by Dutsch on DFP listed below also show synaptic variation for memory.

It is such evidence that in 1962 led to the presentation of two almost simultaneous papers crediting enzyme induction as the memory device. Smith felt that memory in the form of enzymes would be induced by a particular transmitter substance and proceed then to be associated with the synthesis or destruction of that transmitter.[21]

At the same time, Briggs and Kitto proposed the hypothesis "that memory is basically dependent upon neurone pathways maintained by high levels of transmitted substance due to induced biosynthetic enzymes."[22] They felt that memory by base changes in the nucleic acids was improbable because no odd bases are found.

Regarding memory as an interaction between cells, we must look at the speculation of Galambos who holds that memory is implemented by the interaction of glia and neurons.[23, 24] He proposed that memory could be influenced by mechanical, electrical, metabolic, or biochemical factors on growth. He later suggested that astrocytes (glia) could play an active role in neural transmission through gliapses. These circuits through glia (Fig. 1) could be electrical and would have

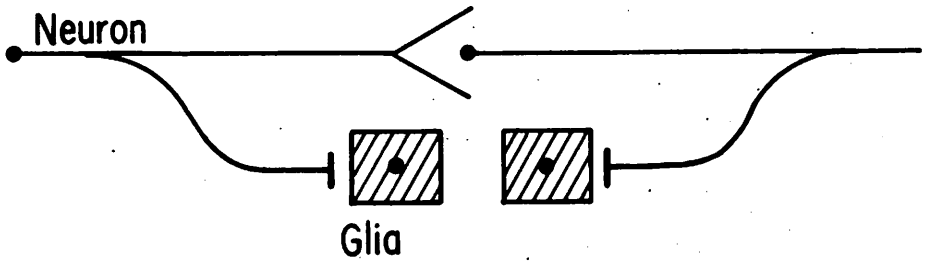


Fig. 1. Gliapse.

been modified by previous electrical history. Besides the problems of electrical modification mentioned earlier, we must also be aware of the great effects that glia show on being stimulated (increase in protein, cytochrome oxidase, and succinoxidase, and decrease in RNA content and respiratory enzyme[3]), some of which run counter to what is necessary to store memory. It may very well be that the glia interact in this "neuronal" fashion; however, this avoids the issue of what actually stores the memory permanently or at least during a human life span.

C. CHANGES AS FUNCTION OF ACTION POTENTIALS

It has long been noted that physical and chemical changes occur as a function of stimulation of an action potential. This section is intended to demonstrate that some phenomena postulated later have some precedents and are not unlikely. J. M. Tobias and others reported that the birefringence and opacity of a frog nerve, as well as its length, change as a function of the action potential.[25-28] The work was rather neglected, possibly because the optical phenomena lagged behind the action potential.

Transmitter substances are released as a function of the action potential. Larrabee has reported that in nerve membrane phosphatidyl-inositol, labeled phosphate turnover doubled because of

stimulation, whereas the phosphate turnover of other lipids remains unchanged from the nonstimulated rate.[29]

Nonnervous system phenomena of this type include contraction and Ca^{++} binding in muscle as a function of stimulation.[30-33] Also, the bioluminescent scintillations in Noctiluca miliaris follow the action potential but lag it slightly.[34] This bioluminescent reaction is the first known to be of an enzyme-substrate reaction caused by an action potential -- perhaps a function of a pH change to which the complex is sensitive in related species.

IV. HISTONES, DNA, AND RNA

Since RNA may somehow be involved we must examine how its synthesis is repressed and induced. Also, since stability is so important for memory, we must examine how RNA can turn over at such a great rate without eventually leading to the production of DNA and cell division.

A. REPRESSION OF RNA SYNTHESIS

Conventional definitions attribute the works "repression" and "induction" to DNA usage for RNA production, whereas "inhibition" and "activation" are associated with enzymes. These definitions are important in understanding the following.

It has been estimated that DNA of a mammalian cell can code 7×10^6 proteins of M. W. 50,000.[35] The cells demonstrate, however, only about 500 proteins, most of them useful. For this reason, we must discuss the way in which readout is repressed and induced.

The addition of adding the basic lysine- and argine-containing proteins (called histones) to DNA material causes a precipitation of a complex of the two, indicating that histones bound to DNA have a lower energy level than free histones and free DNA. It has been shown by Bonner[36, 37] that such a system of deoxynuclear histone inhibits the rate of RNA synthesis as shown in Table 1.

Table 1

Effect of Histone on DNA as Primer in RNA Synthesis

(After Bonner)[36]

System	RNA synthesized/10 min μμm nucleotide incorporated
Enzyme alone	720
Enzyme + DNA	1,440
Enzyme + reconstituted deoxynucleohistone (Histone: DNA ratio 1.04:1)	576
Enzyme + DNA + histone	0

It was Stedman who first proposed that histones control the expression of genetic material. Since then,much supporting evidence has been presented to show that RNA synthesis is inhibited by histones.[37]

It has been found that histones and protamines cover most of DNA and, in fact, the weight ratio of histone to DNA is about 1:1.[38] Histone inhibition is unspecific, and different DNA base sequences are associated with similar histones.[39] Because of this fact, it is necessary to discover how histones dictate the certain combination of proteins present in a cell at any time.

We note that since histones are not found in bacteria, it has been inferred that histones are not the repressors of RNA synthesis. This need not be the case; since bacteria and cells do not seem to have evolved from the same stock, bacteria may have other repressor devices. Perhaps actinomycin D is one of them. It is not lethal to the bacteria it is found in, but then mammalian histones are not lethal to mammals.

X-ray studies of DNA-bound histone show histones at intervals of 37 Å, which is approximately the pitch of DNA. It was thereby concluded that histones lie in the DNA grove so that they block the steps which code RNA and keep the DNA together. RNA synthesis is thereby effectively blocked.

A propular picture of histone inhibition shows the positive nitrogenous amines attached to the DNA's sugar phosphate chains which are rather negative. This picture is not necessarily incorrect but may be incomplete.

Allfrey et al. have shown that for RNA synthesis the arginine-rich histones are strong repressors but lysine-rich histones are weak repressors.[40] Hnilica and Billen found the opposite to be true for DNA synthesis.[41] Lysine-rich histones inhibit DNA synthesis strongly, whereas arginine-rich histones were weaker inhibitors of DNA synthesis (Fig. 2).

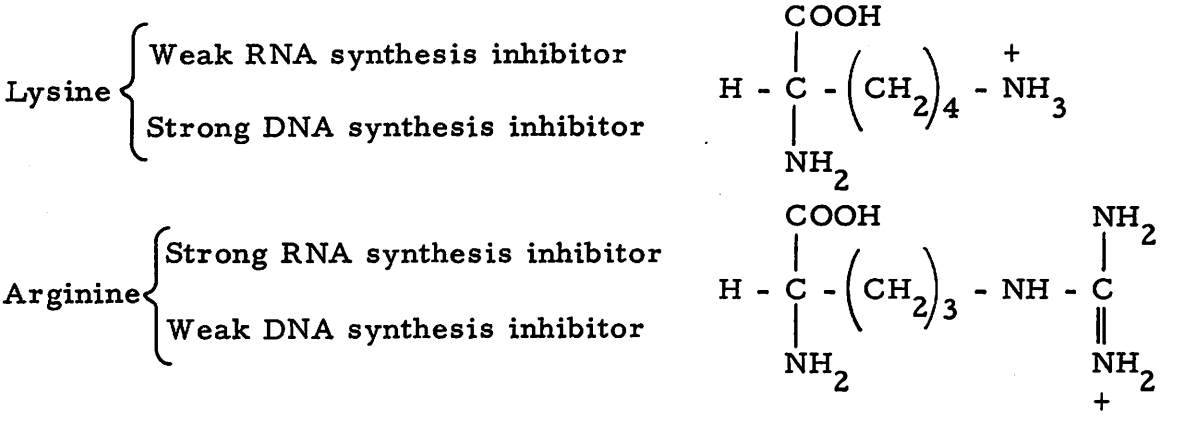


Fig. 2. Structures of lysine and arginine.

Histones could be attached to the DNA in the same manner as RNA bases are coded. The flat amino base of arginine may form a structure with DNA like the flat nitrogenous bases of RNA, thereby strongly inhibiting RNA synthesis. The lysine bases may hold the DNA together and thereby strongly inhibit DNA synthesis. The work of Allfrey et al.[40] and then Hnilica and Billen[41] showed that the lysine and arginine have different inhibiting properties and that such properties could be exploited by the cell. For example, one may consider mitosis, which we wish to prevent in neurons concerned with memory, by maintaining the DNA bound with histones, but paradoxically that would also prevent the RNA synthesis desired.

B. MITOSIS

One explanation of histone action during mitosis is that increasing histone synthesis inhibits RNA synthesis and stimulates DNA synthesis. At a high point, all nucleic acid synthesis would stop and the cell would divide.[42]

We want to expand this idea and propose something slightly different. First, note that Flamm and Birnsteil found using C^{14} that lysine histones are produced at the nucleolus.[43] The increase of arginine histone synthesis might inhibit all RNA synthesis and displace lysine histones so that DNA synthesis occurs. The nucleolus then

emits lysine-rich histones, which stops further DNA synthesis and causes the nucleolus to disappear. The lysine-rich histones displace some arginine histones, leading to the resumption of RNA synthesis. This RNA then leads to the reappearance of the nucleolus at late telophase. These views are further supported by the observations that histones are released to the cytoplasm during mitosis but return in late telophase and early metaphase.[44] This release to the cytoplasm may be a displacement. Thus we have shown that it is possible to control mitosis by varying the ratio of the two types of histones.

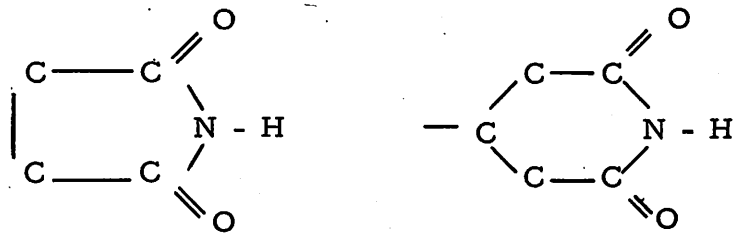
The significance of the above to memory is twofold. First, if the nucleic acids, histone configurations, etc., are storing memory, we can see that mitosis would obliterate any past records. Second, we see that it is possible to strip the arginine RNA inhibitors and leave the lysine histones to prevent DNA synthesis but allow RNA synthesis. Incidentally, this picture correlates with the constant presence of the nucleolus in neurons; the RNA found there,[8] after all, is a necessity since histones are proteins.

C. "UNNATURAL" REPRESSORS

The "unnatural" repressors of RNA synthesis are important because they are some of the main tools for studying memory. Actinomycin D usually inhibits RNA synthesis at the DNA. It seems

that actinomycin D inhibits guanine incorporation. A large section of actinomycin D indicated in Fig. 3 is flat and could cause the inhibition in much the same manner as a histone. Brachet observes that actinomycin D and histone have similar effects on the cleavage and gastrulation of amphibian eggs.[45]

Other "unnatural" repressors are too numerous to mention but we can generalize that they are mainly purine or pyrimidine analogs, they are always flat, and some of the best have the following configuration somewhere in the molecule (e.g., thalidamide).



The characteristic flatness and the amino groups present could cause inhibition of RNA synthesis as in the case of arginine-rich histones. There may be a low-energy state on DNA into which these flat nitrogenous bases fall. Some, like the bases of RNA, have ribose phosphase "handles" by which they are joined together by RNA polymerase and pulled out through utilization of ATP. Others, like the histones, must have some mechanism for removal; perhaps the polypeptide chains help, or cells could not survive. Some small "unnatural" repressors have no way of being pulled from the low-energy states and are lethal to cells.

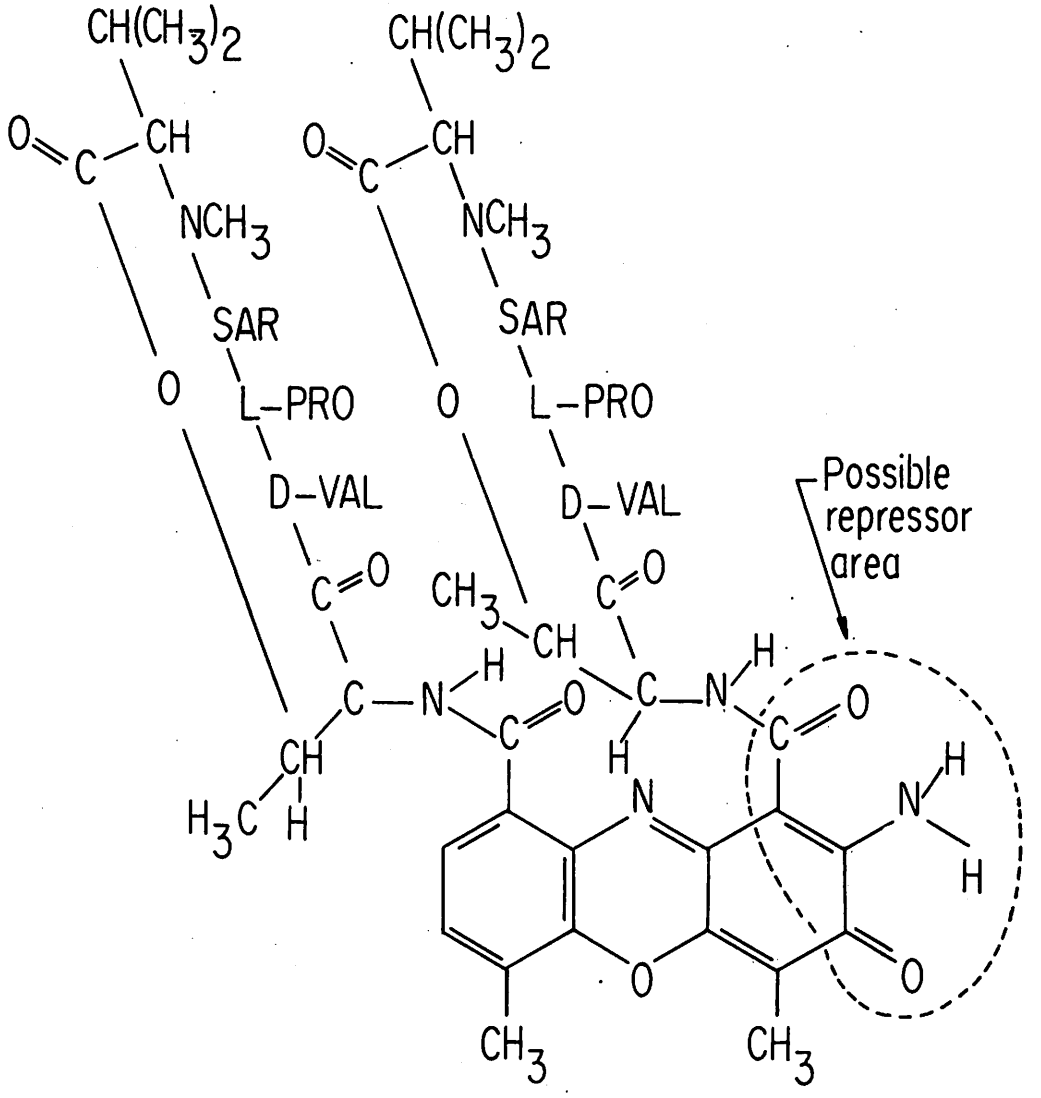


Fig. 3. Actinomycin D.

D. CELLULAR CONTROL MECHANISMS

Our next concern is to find out what control the chromosome has over itself and, since communications between cells occurs via the membranes, what control the membrane has over the the chromosome. The biggest gap in our knowledge about the cell is what control the membrane has in inducing RNA synthesis. We must find out what strips the arginine-rich histone so that RNA synthesis can proceed. Jacob and Monod received the Nobel Prize for their work on the induction of β-Galactosidase formation in E. coli.[46, 47] Although this system is bacterial, it is reasonable to assume that similar systems exist in other organisms.

Since the terminology is quite different from the previous inducer-repressor vs inhibitor-activator systems, we shall first attempt to define these terms.

Types of genes in use

1. Structural gene - the gene producing a protein to be used for something other than as an inducer or repressor.
2. Operator - a segment of DNA demarcating the beginning of the structual gene.

3. Regulator genes

   a. Suppressor gene - produces repressors of RNA synthesis. (Should be named repressor gene.)

   b. Repressor gene - leads to production of inducers; so named because it is thought to repress the action of the operator. (Should be named inducer gene.)

Table 2 shows the findings of Jacob and Monod which led to their theorizing about one particular RNA's production, that of β-Galactosidase. They theorized that a suppressor gene (repressor-producing gene) is responsible for repression of RNA synthesis, but on induction of the repressor gene (inducer-producing gene) an inducer would be formed which gains control over the operator. This action in turn leads to the production of structural RNA.

Since we have postulated that the repressor in nonbacterial organisms is the histone in a low-energy state, we must also postulate that the inducer is the device that strips the histone or at least causes the removal of arginine histones. This inducer could appear in various ways:

1. the inducer can enter the cell from the outside;
2. the inducer can be an enzyme produced by the cell which acts directly on the proper structural gene or operator;

Table 2

Mutations Affecting β-Galactosidase Formation E. Coli

(After Dixon and Webb)[48]

Mutation	Site of mutation	Effect	Result
None	---	Operator blocked by repressor; repressor removed by combination with inducer	Enzyme formed only in presence of inducer
z^-	Part of structural gene forming active center	Structure of active center altered	No active enzyme. Inactive protein only in presence of inducer
0^o	Operator part of structural gene	Operator inactive, even when not combined with repressor; no messenger RNA formed	No enzyme formed, even in presence of inducer
0^c	Operator part of structural gene	Operator active, unable to combine with repressor	Enzyme formed, even in absence of inducer
i^-	Regulator gene	No repressor formed	Enzyme formed, even in absence of inducer
i^s	Regulator gene	Modified repressor formed, unable to combine with inducer	No enzyme formed, even in presence of inducer

3. the inducer can be a self-produced enzyme which needs an activator;
4. the inducer can be a self-produced enzyme which needs to be separated from an inhibitor;
5. the inducer could be the product or substrate of an enzyme reaction; and
6. induction could occur by displacement of the repressors due to an overabundance of another flat nitrogenous base.

Not enough work has been done to rule out any of these cases. Jacob and Monod found the third kind of system, and there is some evidence for the other five, but it is difficult to sort out the confusion of terminology.

After asking where the inducer comes from, we must ask how it functions. If we have a genetic material completely inactivated by histones, we must supply energy to remove the histones from the meaningful DNA. The mechanism for this is not known exactly. Actinomycin D can be removed from DNA by an excess of certain amino acids, dicarboxylic acid, crotic acid, and adenine. It can also be competitively removed by pantothenate. Actinomycin A and C, which have a similar effect, can be removed by excess of pyridoxine (a pyridine precursor), peptone, p-aminobenzoic acid, tyrosine and phenylalanine.[49] Perhaps some similar compound exists for histones.

One molecule that is quite closely associated with the literature on biochemical memory is cyclic 3'5' AMP.[50] Hechter and Halkerston called it the "alphabet of coding."[51] The 3'5' AMP has been credited with many functions such as C-11β hydroxylation in the adrenal cortex by a mechanism which is independent of glycogen phosphorylation, of NADPH generation, and of endogenous corticosteroid precursors;[52] it was the only nucleotide to increase progesterone synthesis without increasing phosphorylase activity and may or may not be an LH intermediate.[53] It also acts between cholesterol and pregnonolone, which suggests that it may be an ACTH intermediate.[54] Other evidence (1) that 3'5' AMP mediates enzyme secretion in parotid gland preparation caused by epinephrine[55] (an excitatory compound and potent inducer of enzyme synthesis) and (2) that there is a time lag reported before epinephrine mediated by 3'5' AMP has any effect[56] (a time comparable to the time required for protein synthesis), leads us to suggest that instead of all these varied functions, cyclic AMP might have just one. That one is, to play some immediate role in the induction of RNA synthesis.

Since ACTH, glucagon, vasopresson, histamine, serotonin, catecholamines, and thyroid-stimulating hormones all increase cyclic AMP content,[50] we suggest that (since most of these compounds are known to be excitatory) the cyclic AMP is formed as a function of the

action potential. The methyl xanthines (caffeine, theophylline, theobromine) inhibit the enzyme phosphodiesterase, which destroys cyclic AMP[56] and probably is the reason why they are known as stimulants and have some positive effects on memory.

The above is presented to stimulate experimentation on the problem of what control the membrane has over the genetic material. It is unlikely that the transmitter substances which produce a general increased enzyme level do so directly. Probably, as discovered by Jacob and Monod, a repressor gene-produced protein will require an activator. We suggest that this activator will occur as a function of the action potential. To strip histones we must assume that the inducer (enzyme and activator) must be very specific; perhaps there is only one compound that is capable of removing the histones and the inducer enzyme merely finds the operator on the DNA. If such is the case, the activator, once carried to the gene by the repressor gene-produced enzyme, strips the histones.

Although there is no evidence that cyclic AMP is the activator or even in the cycle which leads to the production of activators, it is not too incredible to start looking for the activators among the purines or pyrimidines. As previously mentioned, we know that adenine removes actinomycin D.

Also, in considering the problem mentioned by Gaito that no one RNA seems to be produced for memory but that the general level rises,[11] we must remember that what happens is that the activator's presence raises the general level of RNA. What RNA is producible and used by a cell depends on how that cell is differentiated. Similarly, what determines whether a cell will be depolarized by one of the excitatory chemicals also depends on the cell (i.e., phospholipid ratios of the membrane, protein binding sites, amount of proton excretion, etc.).

Outside influences can have an effect on the enzymes present, by their inducer, repressor, activator, or inhibitor actions. For example, the interferon, a protein that inhibits the multiplication of invading viruses, seems to be directly induced by the virus.[57]

A typical antibody response (another memory problem) might be one in which the invading object causes the production of an inducer from the regulator gene, which also takes an activator from the invading object. The inducer then finds the operon of the structural gene which destroys the foreign object. When the object is destroyed, the activator is removed; so the inducer, although it remains, does not operate the structural gene. It is ready for the next invasion of the object, however, and an immunity results.

Block suggests that histones act to record the cell's environment.[58] This action includes (1) the change in genetic state under the presence of alleles, (2) the gene's sensitivity to position, and (3) "historical effects" which are responses to effects of previous history.

E. MAINTENANCE OF HISTONE-DNA CHANGES

Dingman and Sporn present three criteria to be satisfied by any memory theory.[60]

1. the response must be associated with what is to be remembered;
2. any altered state must persist; and
3. permanent loss of memory should result from the destruction of the altered state.

Since RNA theories do not meet the last two criteria, Dingman and Sporn concluded that the RNA as a storage memory device is at least incomplete.

We must remember that the RNA produced is continually in a state of dynamic equilibrium and is being replaced.[46, 60] The same is usually true for brain proteins.[61] The DNA is the only cell component that does not change.[22] Even the histones are combining and separating with DNA. If we are looking for the one thing we may call

memory, the thing stable enough to last a person's lifetime, we cannot assign a protein, or RNA, or lipid, or histone, for they are constantly being replaced. On the other hand, neither can we assign the distinction to DNA because it is too stable. It could be the histone-DNA configuration. Histones probably evolved to repress RNA synthesis because the cell had a mechanism for prolonged, metastable histone-DNA separation. Such a state is probably observed with the chromosome puff where RNA synthesis is said to occur.

V. HYPOTHESIS ON MEMORY

Memory occurs when a neuron recalls that it has been used before in relation to neighboring neurons. It does so by inducing RNA synthesis with an inducer formed as a function of the action potential. The histones displaced or stipped off the DNA during induction are kept away so RNA is synthesized to replace RNA and proteins which are being catabolized. DNA is the only molecule stable enough to store information for many years. The function of the produced proteins, will be discussed briefly in Sec. VII on Output. A schematic representation of this hypothesis is given in Fig. 4.

VI. CHEMICAL EVIDENCE

The use of drugs to modify the various steps of memory is the most promising way to discover, prove, and modify what memory is.

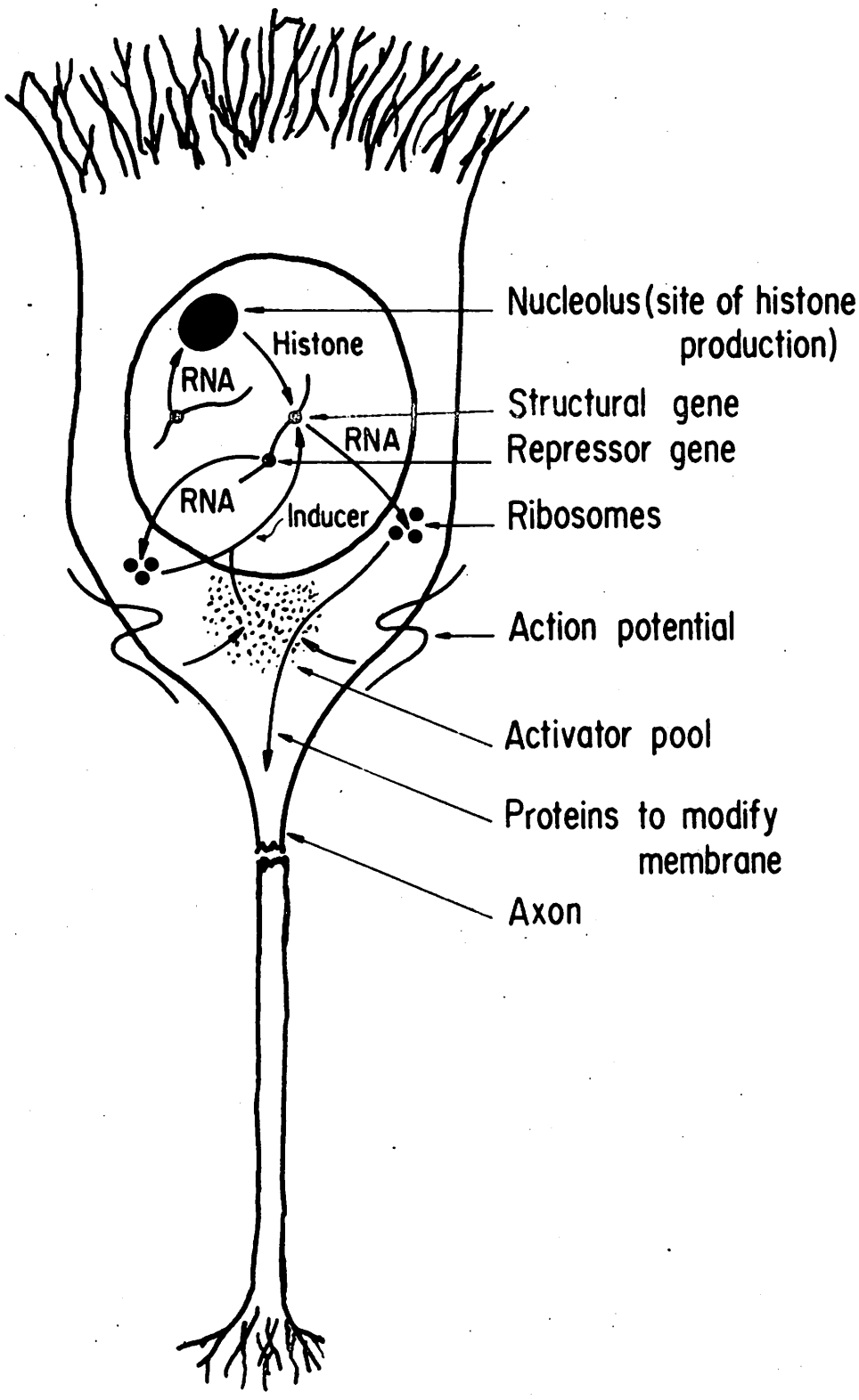


Fig. 4. Summary of memory hypothesis.

Two reviews are recommended in that connection: Deutsch (1962)[62] and McGaugh and Petrinovich (1965).[63]

A. DRUGS ACTING ON RNA AND PROTEIN SYNTHESIS

1. 8-Azaguanine. Dingman and Sporn used 8-Azaguanine (a purine analog which forms nonfunctional RNA) to explore RNA as a memory molecule. After demonstrating that 8-Azaguanine is incorporated into rat-brain RNA, they concluded their findings by saying that 8-Azaguanine depresses learning but has no effect on memory.[64] What they really showed was that 8-Azaguanine disrupts consolidation of what is being learned while the 8-Azaguanine is present, although we cannot be sure that the depression of learning is not due to confusion. They conducted two experiments with water mazes. Using the criterion of fifteen consecutive trials as learned, they then waited 30 minutes while the memory consolidated to inject the 8-Azaguanine and found that at this time it did nothing. Since memory started consolidating after the first trials, the actual wait was more nearly 45 minutes. A training and performance trial was one in which after the rat became familiar with the water mazes, the injections were given 15 minutes before training. The 8-Azaguanine group made many more errors during these "training trials," but the final trials should be considered as "performance" trials for the previous learning. It may then be

concluded that the 8-Azaguanine has a questionable effect on consolidation but that this effect depends on whether or not the drug was present at the time of greatest RNA synthesis induction. Dingman and Sporn did not report any later tests on the rats given 8-Azaguanine during training; that information is essential to the hypothesis presented in this paper.

2. Actinomycin D. Barondes and Jarvik tested actinomycin D on mice which learned not to step between electrified squares to avoid shock.[65] Actinomycin D-injected mice did not differ appreciably from the mannitol-injected controls. The authors point out,however, that failure of actinomycin D to inhibit memory consolidation could be due to (1) the short period of time allowed between training and testing (1 hour) because the actinomycin killed the mice, (2) the possibility that not all parts of the brain were reached by the intercerebrally injected drug, and (3) the fact that not all RNA synthesis was stopped by actinomycin D which was the result of another of these tests. Our objections to these conclusions are that both the chemical studies and training trials were carried out four hours or more after actinomycin D injection. Perhaps that was why RNA continued to be produced as found (four or more hours after injection) by the chemical studies; the effects were probably beginning to decline. We feel that the drugs should be injected closer in time to the training trials because the RNA

synthesis that suffered the greater interference is not that which is associated with those trials. We feel that actinomycin D and perhaps other drugs like thalidomide should undergo further testing. From the hypothesis in this paper, actinomycin D should (assuming that the mechanism which strips histones does not also strip actinomycin D) interfere with memory consolidation but probably not memory to any great extent.

3. Tricyano-amino-propene (tri-a-p). Egyhazi and Hyden found that tri-a-p, a dimer of malanonitrile, speeds RNA synthesis (by activation of RNA polymerase).[5] Later, intraperitoneal tri-a-p was found to facilitate trained rats in a shock avoidance test when given 45 minutes before testing on the second day after training.[66] We feel that this may be a manifestation of the histone-DNA storage mechanism. The tri-a-p increases RNA synthesis at this storage site in the neurons of the pathways as they are reinforced even long after learning. (Note: the same experiment with 8-Azaguanine showed no difference from controls. We feel that this may be due to the dropping of the level of RNA synthesis with time.)

4. Puromycin. Puromycin, an antibiotic that blocks protein synthesis at the ribosomes, has been tested to determine whether protein synthesis is involved with memory. In goldfish, puromycin injected over the cerebrum immediately after training caused a

disruption of consolidation which was interpreted as loss of memory for later tests.[67, 68] Injections 72 hours later had no effect on memory. Other tests with mice were to determine learning ability after protein synthesis had been suppressed by puromycin from 1-7 hours before training.[69] The result showed no depression. Flexner et al. also tested puromycin injected after learning and found no effect on memory. It appears that the injections were not given immediately after training, so we have results similar to the 72-hour wait as above, i.e., no effect on memory. In any event, the experiments failed in one test to find any interference with learning but they neglected time factors. If puromycin interfers with memory, it is most likely to do so on readout of the stored genes' information, i.e., storage is accomplished but readout is not. Puromycin was injected before and after but not during learning where the effect would have shown most. (Another objection to this and other work is that the criterion for "learned" was lacking and sometimes limited to 4 out of 5 correct responses.) In a later work, Flexner et al., in trying to localize memory to various structure of the brain, reversed their earlier findings to say "memory was also completely lost when puromycin was given in bilateral temporal injections."[70] We assume that this was some consolidation observation but no time factors were given, so the data cannot be interpreted. However, the investigators reconfirmed that puromycin has no effect

on memory. Their test was reversal training in a Y maze: three weeks before testing, the mice were trained to enter one arm; 24 hours before puromycin injection, they were trained to enter the other arm. Tests after injection showed that the animals preferred the original arm. Again this shows that readout from the more recent learning is still occurring and that puromycin interferes with this more than the earlier acquired storage readout, whose level probably drops.

B. DRUGS TO EXCITE OR INHIBIT CELLS

1. Steroids. Steroids, which are thought to increase the permeability of lipids so as to facilitate action potentials, are correlated to greater learning rates.[71]

2. Strychnine and a similar compound, 1757 I.S. Strychnine and a similar compound, 1757 I.S. (1-5-diphenyl-3-7-diazadamantan-9-01) has been found by McGaugh and associates to facilitate consolidation upon pretrial and posttrial injection in rats.[72-78] These drugs also improve short-term memory. The dosage in these experiments is critical (and is dependent upon age, strain, etc.). Too much even detracts from normal levels of memory. These observations can be explained in the following way. Memory stored in pathways is "permanentized" through the genetic material. Immediately after use, however, while this long-term biochemical memory is consolidating, it is possible that previous firing has lowered the membrane threshold

of the neurons involved and leads to short-term memory (memory using a path of raised probability of reuse, which we have no reason to suspect as being different from the one involved in long-term memory). Now, it is possible that outside transmitters administered posttrially below a certain dose can fire only the neurons just fired (as strychnine may do) because they are facilitated. This firing increases the probability of RNA synthesis induction. On the other hand, some chemicals may facilitate membrane depolarization without always depolarizing it, which may explain why nicotine facilitates short-term memory but does nothing for long-term memory.[79] Overdoses of the drugs would fire neurons randomly and input would not be a function of what is to be learned.

3. Picrotoxin. Picrotoxin in posttrial intraperitoneal injections into rats was found to facilitate consolidation in the first few trials. Breen and McGaugh concluded, "The data are most consistent with the hypothesis that picrotoxin facilitates learning by enhancing the degree of preservation of neural processes set up by each learning trial."[80]

4. Magnesium Pemoline. Magnesium Pemoline (a central nervous system stimulant) was found to indirectly increase the activity of RNA polymerase.[81] It was found that when this compound is administered orally to rats, they learn faster and retain a jump response longer.[82] The problems with these experiments were (1) the drug was

administered orally before and not during training, (2) the criteria for "trained levels" may not be rigid enough, and (3) when the experiments were repeated with Methyphenidate and Methamphetamine, the controls did not agree with the controls used for the Pemoline experiments. However, if these results are correct, they lend further support to the idea of membrane control over the DNA.

5. Atropine. Atropine has been tested in rats when administed intrapertoneally 60 minutes before training and interfered with the acquisition of new responses and performance of old.[83] We can attribute both these observations to sluggishness on the part of the animal, and we feel that further tests should be made with the injections given during or immediately postrially to learning.

6. Chlorpromazine. Chlorpromazine administered intraperitoneally to rats at different intervals after each daily trial (10 sec, 30 min, 1 hr, 2 hr) showed that only the 10-sec injection had any effect on detracting from memory consolidation.[84]

7. Pentobarbital Sodium. Pentobarbital sodium has been found to depress learning.[85-88] One experiment involved training rats to press a bar. When the training was complete, a shock was given through the bar. At various times thereafter, injections were given through intercranial catheters and testing was conducted 24 hours later. Pentobarbital sodium was effective only when administered within 20 minutes after the shock.[87]

8. Ether. Ether was tested by Pearlman et al. and was found to have no effect after 10 minutes.[87]

9. Pentylenitrazol. Pentylenitrazol (metrazol) (a convulsive agent) was also tested by Pearlman et al. and was found effective up to four days in obliterating memory.[87]

Another form of inducing convulsion is electroconvulsive shock. The effect is directly due to the current and not behavioral convulsion.[89-91] These shocks not only interfere with consolidation but also with memory.[89]

The combination of electroconvulsive shock and reserpine has a greater effect on memory (injections of reserpine intraperitoneally three hours before trials and ECS three hours after trials) than ECS alone.[92] As would be expected, reserpine (a drug that causes depletion of catecholamine and 5 HT stores from nerves but also intereferes with binding sites for these transmitters) had no effect on consolidation because it was injected so long before training.

Since, by the hypothesis presented here, the induction of RNA synthesis depends upon a depolarization, it seems likely that compounds which depolarize the membrane facilitate consolidation while those that inhibit depolarization retard consolidation. Events that fire neurons indiscriminately, such as electroshock or a high dose of excitatory substances, would cause a response not associated with what is to be remembered (one of the criteria established by Dingman and Sporn[59]).

10. Diisopropyl fluorophosphate. Diisopropyl fluorophosphate (DFP) (an anticholinesterase) was injected bilaterally into the hippocampal nuclei of rats 3, 14, and 28 days after maze training; memory was tested one day later. Injection after three days (DFP in peanut oil) had little effect; after 14 days it caused amnesia, and after 28 days it enhanced recall. Deutsch explains these phenomena as an initially nonfunctional synapse which becomes functional after learning. DFP inactivates ChE, thereby causing ACh to collect at postsynaptic sites and facilitate neural firing. Too much ACh, however, blocks neural firing. Deutsch then explains the 14-day amnesia as too much ACh due to the raised presynaptic levels of learning. At 28 days the levels of ACh at the presynaptic sites have declined so that DFP then facilitates recall. The amnesia was reversible after about five days, thus indicating that although the synapse is the main output mechanism, it is not the main storage site.

What finally can be concluded from these pharmacological experiments is how the time factors often dictate the outcome of a drug's effect. If administered too soon, the drugs may be destroyed or washed out so that they have no effect. Drugs administered pretrially may also interfere with the animal's "motivation," motor ability, etc., so we cannot determine whether the effects observed are directly on

consolidation. To be effective, drugs must be administed not later than 30 minutes after the start of training, otherwise memory will have consolidated and most of these chemical agents have little observable effect on memory. Amnesia often results from an interference with the memory process itself and can be evoked for a longer period of time. The time factor of memory itself has not been studied extensively and Deutsch's studies with DFP are among the first. We must be careful to differentiate between consolidation and memory. Table III, summarizing this section, attempts this differentiation.

VII. OUTPUT

In this section, we briefly mention some of the ways in which a protein or polypeptide might be used to raise the probability of a neuron's reuse in a pathway. We may list a few possible mechanisms of change: (1) growth of new synaptic connections; (2) increase of transmitter substances and associated enzymes; (3) change in transport phenomena or excitability of the membrane; (4) change in relationship with glia; and (5) any combination of the above. The most promising of them is an increase in transmitter as recently shown by Deutsch and Leibowitz.[93] These synaptic changes allow neurons to recall the neural pathways which were fired by the original stimulus leading to the firing of the right muscles and glands. Memory exists when the

TABLE III

EFFECTS OF DIFFERENT CHEMICALS AND EVENTS

(See text for explanation)

Compound or event	Ref.	Effect on consolidation (learning)	Effect on consolidated memory	Animal	Tests	Mechanism
8-Azaguanine	64	Depressed	None	Rat	Water Maze	Forms nonfunctional RNA
Actinomycin D	65	None but questionable if active to greatest extent during consolidation	---	Mice	Shocks for stepping between squares	Blocks RNA synthesis like histone
1, 1, 3-Tricyano-3-amino-1-propene	66	---	Improves recall (experiments show this may be due to tests which actually train)	Rat	Shock avoidance	Promotes RNA synthesis by activation RNA polymerase, not by stripping histones
Puromycin	67, 68 69 70	Depressed... None (questionable) Depressed	None None None	Goldfish Mice "	shuttle tank Y-Maze, Hurdle box " " "	Blocks protein synthesis at ribosomes
Strychnine sulphate	72, 74, 76, 78	Speeded-leading to greater memory	---	Rat	various	Excites post synaptic membranes
Drug 1757 I.S. (1-5-diphenyl-3-7 diazadamantan-9-01)	73, 75, 77	Speeded-leading to greater memory	---	Rat	various	Unknown (similar to Strychnine)
Picrotoxin	80	Facilitates consolidation	---	Rat	T Maze	Raises general cerebral excitation
Magnesium Pemoline	82	Enhanced (questionable)	---	Rat	Jump Response	Central nervous system stimulant which stimulates RNA Polymerase (not necessarily directly)
Atropine	83	Slow learning (questionable effect on consolidation)	dulls memory	Rat	Modified T Maze	Depresses cell firing levels
Chlorpromazine	84	Effective if injected immediately after training	None	Rat	Shock box with adjacent compartments	All purpose tranquilizer
Nicotine	71	Enhances short term memory only	---	Mice	Shuttle box	Lowers threshold of membrance without firing
Pentobarbital sodium (Barbiturate)	84 86	Depressed Depressed if given before 20 min.	--- None if given after 20 min.	Rat "	Krech hypothesis apparatus Bar pressing	Depresses neural firing and transmission
Ether	86	Depressed if given before 10 min.	None if given after 10 min.	Rat	Bar pressing	Lipid soluable anesthetic
Pentylenetetrazol (metrazol)	86	Effective for over four days	Effective for so long it might be acting on memory	Rat	Bar pressing	Convulsant
Electroconvulsive shock	89 - 92	Disrupts consolidation	Causes amnesia	Rat	various	---
Disopropyl fluoro phosphate	93	---	Injections-days after training 3 No effect 14 Amnesia (reversible) 28 Improves recall	Rat	Y-Maze	Anti-cholinesterase (See text)

probability of a pathway's reuse is raised. This is done through synaptic change. It is up to the individual neuron, however, to remember that it is part of a pathway and that is done through DNA-histone changes.

VIII. SUMMARY

Drug studies on memory have tended to demonstrate synaptic modification for memory, which requires protein-level increases. Since the firing level of the postsynaptic membrane is a function of the sum of all drugs and transmitters present in the synaptic cleft, pretrial injections of excitatory or inhibitory compounds enhance or inhibit learning, respectively. Such compounds are strychnine, picrotoxin, drug 1757 I.S. and then atropine, chlorpromazine, barbiturates, ether, etc.

Since neurons fired as a function of what is to be learned have a lower threshold of excitation than the neighboring nonfired neurons, and since the induction of RNA synthesis depends on the firing of the action potential, posttrial injections of strychnine, etc., below a certain dose, fire only the neurons just fired, thereby increasing the probability of RNA synthesis induction and therefore enhance memory consolidation. If the dose is increased, it causes the firing of all neurons (convulsions) and thereby induces RNA synthesis, not as a

function of learning, as nonreversible amnesia occurs. Electroconvulsive shock has the same effect. A drug such as nicotine, which facilitates firing of cells but does not actually excite, lowers the probability of a neuron's reuse without inducing RNA synthesis. Nicotine therefore facilitates short-term memory, which uses the same pathways as are used by long-term memory.

DFP experiments have demonstrated synaptic modifications, probably caused by changing protein and RNA content. Puromycin experiments have shown the importance of protein synthesis (which requires RNA synthesis) on memory.

This and other work lead us to propose long-term memory as a modification in transmission capacity between calls caused by what is to be learned and maintained by the induction of RNA synthesis through the removal of histones or a modification of DNA-histone configuration; i.e., a membrane-NA-membrane phenomenon. Short-term memory, on the other hand, is a membrane-threshold change-membrane phenomenon.

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