Glaser

Vol. I,

Chapter 7

A Possible Effect of the Magnetic Field Upon the Genetic Code

Madeleine F. Barnothy

College of Pharmacy
University of Illinois

A book on biomagnetism would be incomplete if it did not mention—at least in a qualitative way—a further mechanism and its relation to biomagnetic phenomena. P. O. Löwdin[1] has drawn attention to the fact that the quantum mechanical effect of proton tunneling may show up in some processes in which biological amplification plays a role.

In ordinary chemistry, the tunnel effect has so far been of little importance. In chemical kinetics one usually considers only processes which have sufficient energy to take particles above the potential barrier between two states and the effect of quantum tunneling can be neglected. In the following we will attempt to investigate the influence of a magnetic field upon the tunneling of protons in DNA.

It was Delbrück's[2] idea in 1930 that the immense stability of the hereditary substance in chromosomes over thousands of years may indicate that it is nothing but an immense molecule in a stationary state and that mutations correspond to quantum jumps between isomeric forms of that molecule. Chromosomes consist mainly of three kinds of substances: protein, deoxyribonucleic acid (DNA), and ribonucleic acid (RNA). Proteins are made of 20 different kinds of amino acids strung together in long polypeptide chains, sometimes containing several hundred amino acids. The nucleic acids are also long chain molecules, but made up usually of only four nucleotide bases. DNA is the custodian of the genetic code. A kind of RNA, called the messenger-RNA, picks up the code information from the DNA and carries the message from the nucleus of the cell to the surrounding cytoplasma, where with the help of ribosomes the proteins are mainly synthesized. The coding contained in the DNA molecule determines the sequence of the 20 different amino acids in the protein molecules.

According to the Watson–Crick Model,[3] the DNA molecule consists of two very long chains made up of alternate sugar and phosphate groups. The sugar is always the same, deoxyribose, and is always joined to the

phosphate in the same way
repeating the same sugar–p
several hundred thousand t
to it and these bases are no

Four different types of
adenine and guanine, and t
The three molecules suga
The two sugar–phosphate
strands joined at regular int
bonds between complement
type base is always linked
mentary pyrimidine-type ba
per half-turn.

The hydrogen bond betw
proton shared by two lone
oxygen atoms of the bases
lone electron pairs on the sh
well potential separated by
possible equilibrium position

In quantum mechanics th
nonzero amplitude even in r
of the proton wave function
attached to either one of the
bases. If the double-well is
tively stationary in the lowe
tunneling will occur only r
transmitted intact in millions
well has to be highly asymme

Cell division is preceded
The two strands of the doub
separate. Nucleotide bases
attach to each of the single
strands. When the proper bas
formed; thus two complete DN
as the original, are produce
chains begins as soon as th
that only a short stretch of t
H-bond that has to be broken
length of all DNA molecules i
like 4 cm, which means that
bonds in one chromosome.

If the complementary bas
proton in one direction induc
H-bond between the same bas
the original bases are transfo
occurring at some later time

phosphate in the same way, so that the long chain is perfectly regular, repeating the same sugar–phosphate sequence over and over, sometimes several hundred thousand times. But each sugar has a base attached to it and these bases are not always the same.

Four different types of bases are found in DNA. Two are purines, adenine and guanine, and two are pyrimidines, thymine and cystisine. The three molecules sugar, phosphate, and base form a nucleotide. The two sugar–phosphate chains form a double helix, with the two strands joined at regular intervals, like rungs in a ladder, by hydrogen bonds between complementary pairs of nucleotide bases. A purine-type base is always linked by two or three H-bonds with the complementary pyrimidine-type base. The double helix has five base-pairs per half-turn.

The hydrogen bond between complementary bases is formed by a proton shared by two lone electron pairs situated on the nitrogen or oxygen atoms of the bases. The attractive force exerted by the two lone electron pairs on the shared proton can be represented as a double-well potential separated by a potential barrier. The wells represent possible equilibrium positions of the proton.

In quantum mechanics the proton is a wave packet, which may have nonzero amplitude even in regions classically forbidden. The square of the proton wave function describes the probability that the proton is attached to either one of the lone electron pairs of the complementary bases. If the double-well is asymmetrical, the proton will be comparatively stationary in the lower-lying energy level of the deeper well and tunneling will occur only rarely. The fact that the genetic code is transmitted intact in millions of multiplications implies that the double-well has to be highly asymmetrical.

Cell division is preceded by a replication of the DNA molecules. The two strands of the double helix sever their H-bonds, uncoil, and separate. Nucleotide bases floating loosely within the cell begin to attach to each of the single strands, building two new complementary strands. When the proper bases are joined, two new double helixes are formed; thus two complete DNA molecules, with the same base sequence as the original, are produced. The synthesis of the complementary chains begins as soon as the two original chains start to unwind, so that only a short stretch of the chain is ever really single. For every H-bond that has to be broken, two new ones will be formed. The total length of all DNA molecules in a single chromosome can be something like 4 cm, which means that there must be more than 100 million H-bonds in one chromosome.

If the complementary bases have equal charge, the tunneling of a proton in one direction induces a tunneling of the proton in the second H-bond between the same base pair in the reverse direction, whereby the original bases are transformed into tautomeric bases. A tunneling occurring at some later time in the reverse direction may restore the

---

M. F. Barnothy, "A Possible Effect of the Magnetic Field upon the Genetic Code" in M. F. Barnothy, ed., Biological Effects of Magnetic Fields, 1964. Plenum Press Vol. I

178)

original state. If in a replication the breakup of H-bonds occurs before the original state is restored, we will have, instead of the original bases, tautomeric bases, which can no longer combine with their normal complementary bases. Different complementary bases will be attached, thus changing the base sequence and introducing an error in the code. We can see that a code error will be multiplied in each replication process by a factor of two and, hence, in the nth replication by a factor of 2^n.

Should one of the bases of a pair obtain an extra charge, for example through electron donor–acceptor reactions from surrounding molecules or through the circumstance that a proton tunneling was not accompanied by a reverse tunneling of another proton of the base pair, the change in the charge distribution will greatly alter the shape of the double-well potential. For such so-called ionic tautomeric forms there does not exist a normal nucleotide base which could combine with the positive ionic forms of adenine and thymine; hence "deletion" will occur in the complementary sequence, while the negative ionic forms of these bases lack any specific code and may combine with all four normal bases. Such changes or mutations will, therefore, be irreversible.

L. Szilard[4] has proposed that the elementary step in the process of aging is an aging "hit" which destroys a chromosome of the somatic cell, in the sense that it renders all genes carried by that chromosome inactive. He assumes that aging hits are random events and that the probability that a chromosome of a somatic cell suffers such a hit per unit time remains constant throughout life. On the basis of this theory the frequency with which such random events occur in men is found to be about one chromosome of the haploid chromosomal set of the somatic cells in 12 years. The agent causing the hit is not discussed in detail by Szilard, but the assumption that a chromosome suffers a total loss of function in a single random event is suggestive of an external agent of high energy, that is, a high-energy photon or particle.

P. O. Löwdin proposes that the phenomenon of aging of individual organisms depends essentially on the discussed quantum mechanical tunneling and is in effect a corrosion of genetic messages, which accumulates with age.

The basic difference between these two views is that Szilard assumes that genes are subject to random "aging hits" caused by an external agent, while Löwdin assumes that on account of the tunnel effect a "pure" genetic message does not exist at all and errors occur without "hits" and will be multiplied.

Löwdin furthermore suggests that the occurrence of tumors may depend on the fact that the accumulation of errors has passed a certain limit in a particular direction and upset the balance between enhancing and controling enzymes in the growth cycle. One should expect, as is the case, that this will happen more often at advanced age.

From the point of view of biomagnetism we should try to see what effect an external magnetic field could have on proton tunneling in the DNA molecule. The magnetic field may affect the spin orientation of the proton, a possibility already suggested by Löwdin. A second possibility would be that the proton, endowed with a magnetic moment, experiences in an inhomogeneous field an accelerating force which enhances or retards its tunneling in one direction.

The third, in our opinion the most probable, effect of the magnetic field would be that, as in the Zeeman effect, it changes or splits the energy levels of the nucleotide bases. This would change the depth of the potential wells and hence change the tunneling probability, thus increasing or decreasing the stability of the genetic code.

The synthesis of DNA occupies only about 5% of the full time of the growth and division cycle of a cell. We can speculate, therefore, that only alterations in the tunneling probability prevailing during this short time interval will produce an effect. This circumstance could be the subject of an experimental verification. In synchronized cell division we should expect that a magnetic field has the same effect whether applied continuously or only for a small fraction of the generation time, should this fraction coincide with the DNA replication period; but no effect should be observed if the application of the field does not coincide with the DNA replication period.

It should be mentioned that the polypeptide chains of proteins are also linked together by H-bonds in which proton tunneling may occur. It is true that in this case a change in the protein will not be multiplied through replication, as in the case of DNA, and one proton tunneling will hardly entail any observable consequences. But if the external magnetic field simultaneously causes the proton in a large number of protein molecules to prefer a position invariance to its normal position and these protein molecules happen to be enzymes, then the enzyme balance could become upset enough to lead to observable effects.

The mathematical treatment of proton tunneling and the computation of the change of the energy levels due to the magnetic field is complicated. There are at least two double-wells to be considered, a proton tunneling will entail a change in the charge distribution, the motion of the proton will polarize the electron cloud, etc. At the present stage we cannot attempt a quantitative computation, but will rather consider if some experimental evidence indicates that the magnetic field renders the genetic code more stabile or more labile.

I would like to discuss briefly here two experiments, the results of which could be interpreted as an effect of the magnetic field on the genetic code.

J. M. Barnothy[5] has performed some experiments which indicate an influence of treatment in a magnetic field on aging and on the occurrence of spontaneous cancers. In one experiment he subjected 10 female C3H-strain virgin mice 70 days old for 4 weeks to a homogeneous field

of 4200 Oe strength. Five were kept in identical dummy magnets and 25 in standard plastic cages during this time. After removal from the field and dummy magnets, respectively, the activity of the mice (10 magnet, 5 dummy, 15 control) was continuously recorded from the age of 320 days to 509 days, at which time 50% of the treated and 60% of the controls were dead or had developed spontaneous cancers. The magnet and dummy cages used for treatment were 3 in. in diameter and $1\frac{1}{2}$ in. high, with food and water supply. The pole caps were simulated on the dummy cages with 1-in.-thick brass discs (brass has a heat conductivity, as well as radiation length for cosmic ray showers, similar to that of iron).

The activity measurements were made in standard plastic cages $11 \times 7 \times 5$ in. high, divided by a wall into two equal compartments, with water in one and food in the other compartment. At a height of 3 in. the dividing wall had an opening through which the mice could cross from one compartment to the other. While crossing they tripped a microswitch, which electrically recorded the crossing. An average of 100 to 200 crossings was recorded per day and animal, the majority of the crossings occurring during the early morning hours. The cages were cleaned once a week; the first 3 hr thereafter was not used for activity recording, since the new bedding incited all animals to a temporarily higher activity.

The experiments were started with six activity cages with five mice per cage. Since initially he had ten more control mice than used in the activity experiment, this reserve group was kept in two cages which had similar dividing walls, but was without electric switches. Whenever a mouse which was kept in a dummy cage during treatment or one of the controls (kept during treatment in standard cages) died in one of the activity cages or developed cancer, it was replaced from the reserve group. Whenever one mouse of the group treated in the magnet died or developed cancer, the number of mice per cage was also reduced in all other five activity cages. Hence the number of mice per activity cage was gradually decreased from five to two mice per cage.

Table I lists the observed activities from the age of 320 days to 647 days. The data are reported in six age periods in such a way that within each period the number of mice per activity cage remained the same. Columns 2, 3, and 4 show the covered age period, the number of recorded days, and the number of subintervals (N) within each period. Columns 5, 6, and 7 list for magnet, control, and dummy mice, respectively, the number of used activity cages times mice per cage and the average crossings per day and mouse.

Since it might be surmised that the increased activity of the magnet group could be related to a younger biological age, my husband checked for any difference in their ability to reproduce. At the age of 509 days, two male mice were daily alternated between the six activity cages for a period of 2 weeks. One magnet and three control mice became preg-

TABLE I

No.	Age period	Days	N	Magnet	Control	Dummy
I	320-361	41	6	2 × 5 168	2 × 5 171	1 × 5 122
II	361-404	43	5	2 × 5 184	3 × 5 136	1 × 5 144
III	404-444	40	6	2 × 4 192	3 × 4 143	1 × 4 171
IV	444-488	44	5	2 × 3 164	3 × 3 118	1 × 3 127
V	488-509	21	3	2 × 2 198	3 × 2 121	1 × 2 126
VI	600-647	47	6	1 × 2 72	3 × 2 92	— —

nant and gave birth to very small litters. The offspring were generally weak and died shortly after birth. The frequency of pregnancies does not show any difference between treated and untreated mice. Due to this interlude, the activity data between the ages of 509 and 600 days were not used in the present paper and the activity recording was resumed 3 months later (period VI).

Table II lists the difference between the activity of the treated and of the untreated mice for the six age periods together with their standard errors, the degree of freedom, the t-value, and the probability level. As we can see, up to the age of 361 days the activity of the treated mice did not differ from the activity of the untreated mice. However, a significantly greater activity is manifested during the age periods from II to V. After the age of 600 days, however, the treated group shows a significantly lower activity.

The mean standard deviation between the weekly average activities of the cages was 11% for the magnet and 18% for the untreated and was independent of the number of mice per cage. We may note that this corresponds to an individual standard deviation of 28% in the weekly average activity of the C3H-strain mice under the given conditions.

The mean activity of the magnetically treated mice from the age of 361 days to 509 days was $(36.3 \pm 4.5)\%$ higher than that of the untreated mice and $(28.2 \pm 6.4)\%$ higher than that of the dummy mice alone.

TABLE II

Activity Difference of Treated and Untreated Mice

	Effect	Degree of freedom	t-value	Probability level
I	12.7 ± 11.6	5	1.09	
II	47.5 ± 11.0	4	4.30	1:78
III	42.5 ± 15.3	5	2.78	1:26
IV	42.6 ± 4.1	4	10.40	1:2000
V	74.8 ± 8.6	2	8.70	1:75
VI	−19.9 ± 4.2	5	4.75	1:180

In the later course of the experiments the weekly food consumption (Rockland Mouse Diet pellets) was also recorded. Between the ages of 430 and 509 days the food consumption was (26 ± 1)% lower for the treated group. This finding, together with the higher activity, would suggest a better energy metabolism of the treated mice.

The cause of these two effects, higher activity and lower food consumption, is perhaps indicated by the picture in Figure 1, which shows one of the treated and one of the dummy mice at an age of 400 days. The appearance of the treated mouse is far younger, its fur is smooth, and no wrinkles are seen. The difference in appearance and in movements at this age was so striking that when the treated were mixed with the untreated, any person even unfamiliar with mice could correctly separate them. During the next 3 months the difference in appearance gradually decreased and eventually vanished.

In the spirit of the discussed theory this experiment would indicate that magnetic treatment has a stabilizing effect on the genetic code.

In a second experiment my husband treated virgin female mice of the same strain and for the same duration, but began treatment at an age of 270 days. No difference between treated and control groups, either in appearance, in activity, or in food consumption, was observed

Fig. 1. C3H-strain female mice at an age of 400 days. (a) Treated 11 months earlier for 4 weeks in a magnetic field of 4200 Oe; (b) control (dummy) of the same age. Reprinted from Medical Physics, Vol. 3, p. 63, O. Glasser, ed., Year Book Medical Publishers, Inc.

up to their highest age. This experiment would indicate that treatment at a full-grown age, contrary to treatment during youth, does not have an effect upon the accumulation and total number of genetic code errors. It is tempting to explain this difference through the circumstance that during the vigorous growing stage of a biological organism, when the rate of mitosis and therefore the replication rate of DNA molecules is high, an error—but in the same way the nonoccurrence of an error—will be greatly amplified and thus animals treated in their youth in a magnetic field reach their old age with a far lower number of genetic errors. A treatment during advanced age, when the errors are more or less incorporated and the replication rate is lower, will be less effective.

C3H-strain females have a very high incidence of spontaneous mammary gland carcinomas. Of the ten mice treated in their youth in the above-discussed first experiment, four, that is 40%, had an observable tumor at the time of their death. Of the 30 controls, 22 (73%) had palpable tumors at the time of death. The difference, although significant only on a 5% confidence level, again suggests a stabilizing effect of the magnetic field on the genetic code.

Further experiments which I would like to mention in this connection were made by I. Sumegi, J. M. Barnothy, and M. F. Barnothy.[6] We kept young 80-day-old virgin female mice for 35 days in a homogeneous field of 4200 Oe (the cages and magnet are described in Part II, Ch. 3) and sacrificed them 200 days after removal from the field. Sections were prepared from nine main organs and inspected by I. Sümegi of the Pathological Department of the Karolinska Institutet in Sweden. In this experiment, 20 mice were treated in the magnets, 20 were kept in dummy magnets, and 20 in standard large plastic cages. Abnormalities were found in the spleen, liver, adrenal, and bone marrow.

The changes found in the spleen correspond to reactive reticulosis, manifested in the increase in the number of reticular cells in the pulpa and the presence of large number of megakaryocytes. The circumstance that of the inspected 16 dummy mice eight showed the same abnormality to a greater or lesser degree would suggest that this might be a more general organismic reaction to a stress factor and a consequence of some irritation of the reticulo-endothelium. Since all 16 of the treated animals inspected showed this abnormality to a large degree, the difference in the frequency of occurrence in magnet versus dummy group is significant on a 5% confidence level.

Abnormalities found in the liver indicate that the livers suffered some kind of lesion and the liver is in the process of regeneration. This process is characterized by a greater number of nuclear divisions, increase in the number of cells with two nuclei, and a large number of liver cells with large nuclei. In the latter, the nucleoli are on the periphery of the nucleus. Cytoplasm is less basophil and more red on eosin staining. One sees similar abnormalities in the case of chloroform, trichlorethylene, and amino nucleoside poisonings.[7] It seems

that the lesion caused a disturbance in the protein metabolism and in the oxidative processes in the cytoplasm of liver cells. In consequence of these processes, fatty infiltration is visible in the cells. From the magnet group, 16 out of 18 showed these strong regenerative processes, while from the dummy group only four out of 14 showed similar regenerative processes. The difference in the frequency of occurrence of this effect between magnet and dummy group is significant on a probability level of 1 : 1500.

The abnormalities in the adrenal are very interesting. The zona glomerulosa is slightly narrower and the zona fasciculata considerably narrower, in some instances entirely missing. The cell distribution is disorganized. There was no change in the zona reticularis. In humans such abnormalities occur after some shocks, infections, and in response to some toxic agents. None of the dummy or the standard cage group showed any of these abnormalities. From the magnet group only three out of 18 had normal adrenals. This effect is significant on a probability level of 1 : 4 million.

There is some evidence that glucocorticoids are produced in the zona fasciculata. A loss of the zona fasciculata may lead to a depletion of the carbohydrate stores and thus to a lower resistance against insulin and stress.

In the bone marrow of the magnet group the number of megakaryocytes was found to be lower. Their number per microscope field is

for mice in the standard cages	7.43 ± 0.23		
		dummy - standard	0.12 ± 0.46
for mice in the dummy cages	7.55 ± 0.40		
		magnet - dummy	-2.35 ± 0.42
for mice in the magnet cages	5.20 ± 0.15		

Whereas between standard cage and dummy group there is no difference, the number of megakaryocytes in the bone marrow of the magnet group is 31% lower. This effect is significant on a probability level of 1 to 50,000.

We may now raise the following question: are the found abnormalities consequences of stress effects caused by the magnetic field, or are they due to a change in the genetic code? We should expect that stress effects would show up immediately after treatment and genetic changes only after the lapse of a certain time, when the amplification through the multiplication of the code errors has become manifest. In the next experiment we therefore sacrificed the mice immediately after treatment.

In the livers of the treated mice strong signs of lesions were visible, characterized by centrolobular necrobiosis and appearance of small liver cells with pycnotic nuclei. The number of nuclear divisions was much higher than when the mice were sacrificed 200 days after termination of treatment. This finding could probably mean that the pathological changes in the liver are a consequence of the stress effect of the magnetic field and, as expected, the liver shows gradual recovery.

On the other hand, the adrenals of the magnet group did not show any abnormality and the zona fasciculata was not missing. This could indicate that the observed abnormality in the adrenals could be a genetic code effect.

It should be emphasized that both of the reported experiments (postponement of aging and pathological changes in the adrenal) used here to support the proposed theory can be, in spite of the high statistical significance of the results, considered only as preliminary investigations, requiring many further observations until a definite connection of the magnetic field with the genetic code can be established.

In summary, an effect of the magnetic field upon the tunneling probability can theoretically be expected. Hence, should tunneling be the major cause for alterations in the genetic code, the magnetic field could be a powerful tool in the further investigation of the code.

ACKNOWLEDGMENT

The reported experiments were supported by the Biomagnetic Research Foundation and a Graduate Research Grant of the University of Illinois.

REFERENCES

1. Löwdin, P.O., Rev. Mod. Phys. 35:724, 1963.
2. See Schrödinger, E., What is Life? The Physical Aspects of the Living Cell, Cambridge University Press, 1945.
3. Watson, J.D., and F.H.C. Crick, Nature 171:737, 1953; Proc. Roy. Soc. A233:80, 1954.
4. Szilard, L., Proc. Natl. Acad. Sci. 45:30, 1959; Nature 186:649, 1960.
5. Barnothy, J.M., Medical Physics, Vol. 3, p. 61, 1960, O. Glasser, ed., Year Book Medical Publishers, Inc.
6. Sümegi, I., J.M. Barnothy, and M.F. Barnothy, Biophys. Soc. Abstr. WC 10, 1964.
7. Kylin, B., H. Reichard, I. Sumegi, and S. Yllner, "Hepatoxicity of Inhaled Trichlorethylene, Tetrachlorocthylene, and Chloroform," Acta Pharm. et Toxicol. Scand. 20:16, 1963.