May 1st, 1:00 PM

Paper Session III-D - Physiological and Genetic Changes to E. Coli Induced by Exposure to lead - a Two Year Study

Ashley M. Cimino
(Student)

Physiological and Genetic Changes to Escherichia coli Induced by Exposure to Lead - A Two Year Study

Ashley M. Cimino
Freshman
Melbourne Central Catholic High

"Physiological and Genetic Changes to Escherichia coli Induced by Exposure to Lead - A Two Year Study"

Introduction

It's been proven that concentrations of lead severely harm people - primarily children. The purpose of this study is to determine whether or not continuous exposure to lead nitrate concentrations of 100 parts per billion (ppb), 200 ppb, or 300 ppb will produce mutation in Escherichia coli DNA over many generations. The hypothesis formulated was that, over time, continuous exposure to lead nitrate concentrations will produce a harmful mutation in Escherichia coli and reduce the amount of square centimeters of bacterial growth.

Lead is a bluish - gray element found underground that can be obtained by mining the ore. Pure lead is very soft and bendible, and it is popular because it can easily be bent and shaped (Kendler, 1993.) More lead is used worldwide than is mined : six-and-a-half million short tons of lead is used worldwide, but only three-and-a-half million short tons is actually recovered (Kimbrough et al., 1994). The remaining three million short tons is obtained by recycling old lead and reusing it.

Lead alloys are important and are used in making many products. The products that involve using the most lead are acid storage batteries, paints and dyes, and rubber products. Lead paint is not used in the home any more for safety reasons, the same way that leaded gasoline is no longer used (Boiling, 1986.) Lead is found in solder, explosives, insecticides, ceramic, glass, and in places where there is danger of radioactivity (Ruff et al., 1993.) In X-ray rooms, nuclear reactors, and other facilities the lead is used as a shield and protection from the radiation exposure.

Lead poisoning causes many disorders, even after all of the lead in the blood, internal organs, and bones has been removed (Roper, 1992.) Lead affects all of the internal organs, which causes problems and complications with health. Children who are failing at school, have behavioral disorders, have a lack of attention. have a lack of the major motor skills, and perform at a lower level than other children may be victims of lead poisoning and if these symptoms continue the child should be screened for poisoning (Committee on Environmental Hazards et al., 1987.) Lead does the most damage to the nervous system. Long after the BPb level has been lowered neurological disorders may be present, often resulting in retardation (Feldman and White, 1992.)

Lead is poisonous and dangerous when too much of it enters the blood stream and/or the bones of humans or animals. The lead can be found in many places, but is most common in the paint of houses built before 1960. Lead is also found in water where the water pipes are old or rusty, drinking liquids that have been stored in ceramic containers that have not been properly glazed,

certain make-up products and jewelry from Mexico and China, the soil, the air, renovation, the presence of paint chips, and urban residence (according to an printout by the Environmental Protection Agency titled “Lead in your drinking water”.)

Deoxyribonucleic acid (DNA) is the backbone of all living things. It holds all of the information needed for the body to function. DNA controls genetics and all of the processes that occur inside the human body, the microorganism, the cell, or any other living thing. DNA is essentially made up of three basic things : a chemical like phosphoric acid called phosphate, a sugar called deoxyribose which is a smaller version of the sugar called glucose, and the four nucleotide bases (Barbour, 1994.) The four bases are adenine, guanine, cytosine, and thymine. DNA is in the form of a double helix, and it’s easy to think of as a ladder that’s been twisted around.

The phosphates (P) and deoxyribose (D) molecules alternate back and forth, joined by chemical bonds, and they (if you think of DNA as a ladder) would form the sides of the ladder. The bases form what would be the rungs of the ladder, and their order determines the genetic code. The bases are attached the deoxyribose. Adenine (A) only bonds with thymine (T), and guanine (G) only binds with cytosine (C). They can be thought as like the positive and negative pulls of a magnet : the bases only have one opposite that they will bond together with (Wilcox, 1988.) If you straightened out the DNA strand and looked at a very, very simplified and shortened version illustrating what I have described, it would look something like this :

```
~ D ~ P ~ D ~ P ~ D ~ P ~ D ~ P ~ D ~ P ~ D ~ P
  |       |       |       |       |       |
  A       T       G       T       C       A
  *       *       *       *       *       *
  T       A       C       A       G       T
  |       |       |       |       |       |
P ~ D ~ P ~ D ~ P ~ D ~ P ~ D ~ P ~ D ~ P ~ D ~
```

Problems arise when mutations occur within the DNA code. Mutations can happen for many different reasons; some of them are yet to be discovered. If a mutation occurs, there is a chance that a particular bodily function won’t be performed right (Travis, 1995.) For example : the process of transcription (where the DNA code is being copied for protein synthesis by a molecule called ribonucleic acid - RNA) is occurring within an individual. The code being transcribed is: AGTCTGGA.

For some reason, the RNA copies the code as : UCAAACCU, instead of UCAGACCU. An adenine has been substituted in the RNA for the guanine that should be there. Now, during translation, either the wrong protein will be produced, or a protein won’t be produced at all! In some cases these mutations can be fatal.

Particular strains of bacteria (such as the non-pathogenic MM294 and JMIOI Escherichia coli strains) are often used in laboratory experiments dealing with DNA due to the fact that the growth is easy to observe; and the bacteria reproduces quickly, and therefore many generations can be observed (Papichak, 1993.) These tiny microbes are very important in our venture to understand the workings of nature, and what goes on within our own bodies.

Procedure

The appropriate lead nitrate concentrations must be made : for the 100 ppb, 0.2 mg of lead nitrate is added to 20 ml of distilled water; for the 200 ppb concentration, 0.4 mg of lead is added to 20 ml of distilled water; and for the 300 ppb concentration, 0.6 mg of lead is added to 20 ml of distilled water.
The bacteria is grown on nutrient agar, and there are eight petri dishes per trial : two controls (labeled “C” and “CC”), two exposed to 100 ppb (labeled the “.2” and “.2.2” plates), two exposed to 200 ppb (labeled the “.4” and “.4.4” plates), and two exposed to the 300 ppb (labeled the “.6” and “.6.6” plates.) Pour the nutrient agar first, then add 1 ml of the appropriate lead concentration to the surface, let it solidify, and then culture the bacteria. Reculture the bacteria onto a new plate with the corresponding lead concentration for each new trial.

Calculate the number of square centimeters the bacteria growth in each plate took up for phenotypic data; and do a growth curve measuring the absorbency level, comparing the Control bacteria to the 300 ppb lead-induced bacteria. After every five trials do a “Lysis by Alkali” standard restriction analysis procedure (DNA Science : Lab 10, Parts A and B) to determine whether or not there are any changes to the DNA.

RESULTS
The Phenotypic Results :

The colony growth counts done on the amount of bacterial growth showed that the average amount of growth in a Control plate was 9 square centimeters. The .2 plates (exposed to lead nitrate concentrations of 100 ppb, .2 mg PbNO3/H2O concentrations) had an average number of 3.5 square centimeters of bacterial growth from Trials #1 - #25. The .4 plates (exposed to lead nitrate concentrations of 200 ppb, .4 mg PbNO3/H2O concentrations) had an average number of 2.9 square centimeters of bacterial growth. The average number of square centimeters of bacterial growth in the .6 plates (exposed to lead nitrate concentrations of 300 ppb, .6 mg PbNO3/H2O concentrations) was 2.5.

There were very pronounced growth decreases apparent when comparing both the Control plates to the lead-induced bacteria, and when comparing the amount of growth in the Trial #1 plates to the amount of growth in the Trial #25 plates. There was a decrease of 4 square centimeters in the average amount of bacterial growth from the Trial #1 “.2” plates to the Trial #25 “.2” plates; there was a growth decrease of 6.6 square centimeters in the average growth of the Trial #1 “.4” plates to the Trial #25 “.4” plates; and there was an average growth decrease of 4.5 square centimeters in the Trial #1 “.6” plates compared to the Trial #25 “.6” plates.

The Growth Curve Results showed that the Control (normal) bacteria and the bacteria that had been exposed to 300 parts per billion Pb/water % had very different growth curves. The .6 bacteria had a much longer lag phase than the norm; by the 7th hour, the Control had reached an absorbency level of 0.75%, where the .6 bacteria remained at a level of 0.02%.

The Number of Square Centimeters of Escherichia Coli Growth in Each Plate

Chart 1-1

	C	CC	.2.	.2.2	.4.	.4.4	.6.	.6.6
TRIAL #1	9.75	9.25	5.75	6	5.5	5.25	5.25	5
TRIAL #2	9.5	9.2	5.5	5.25	5.25	5.25	5	4.75
TRIAL #3	8.75	9	5.25	5	5	4.75	4.75	4.75
TRIAL #4	9.2	9	5.25	4	4.25	4.5	4.25	4
TRIAL #5	9	9.25	5.25	5	4.25	4.25	4	4.25
TRIAL #6	9.25	9.5	4.75	5	4.25	4.2	4	4
TRIAL #7	9.5	9.75	4.75	4.75	4.25	4.25	4	4
TRIAL #8	8.55	8.75	4.75	4.5	4.25	4	3.75	3.75
TRIAL #9	9.75	8.5	4.5	4.5	4.2	4	3.5	3.75
TRIAL #10	8.75	9.25	4	4.2	4	3.75	3.5	3.5
TRIAL #11	9.6	9.5	4	3.75	3.5	3.25	3	3
TRIAL #12	9.75	9.25	3.75	4	3.5	3.7	3.25	3
TRIAL #13	8.99	9	3.75	3.5	3.25	3	2.75	2.6
TRIAL #14	9.25	8.75	3.25	3.2	3	2.75	2.5	2.25
TRIAL #15	9.6	9.5	3	3	2.75	2.25	2	2
TRIAL #16	9.75	9.7	2.75	3	2.25	2	1.75	1.5
TRIAL #17	9.55	9.25	2.5	2.75	1.75	1.5	1	1.25
TRIAL #18	8.75	9	2.5	2.5	1.5	1.75	1.2	1
TRIAL #19	8.5	8.75	2.5	2.25	1.5	1.5	1	1.25
TRIAL #20	9.5	9.25	2.25	2.2	1.25	1.5	0.75	1
TRIAL #21	9.75	9.6	2.25	2.2	1.5	1.2	1	0.8
TRIAL #22	9.25	9.2	2.2	2.2	1.5	1.5	0.75	1
TRIAL #23	9.2	8.75	2	2	1.25	1.25	0.75	1
TRIAL #24	8.75	8.6	2.25	2	1.25	1.2	0.75	0.75
TRIAL #25	8.6	9	1.75	2	1.2	1.25	0.75	0.5

“C and CC” are the Controls. “.2 and .2.2” are the plates exposed to the 100 ppb concentrations. “.4 and .4.4” are the plates exposed to the 200 ppb lead concentrations. “.6 and .6.6” are the plates exposed to the 300 ppb lead nitrate concentrations.

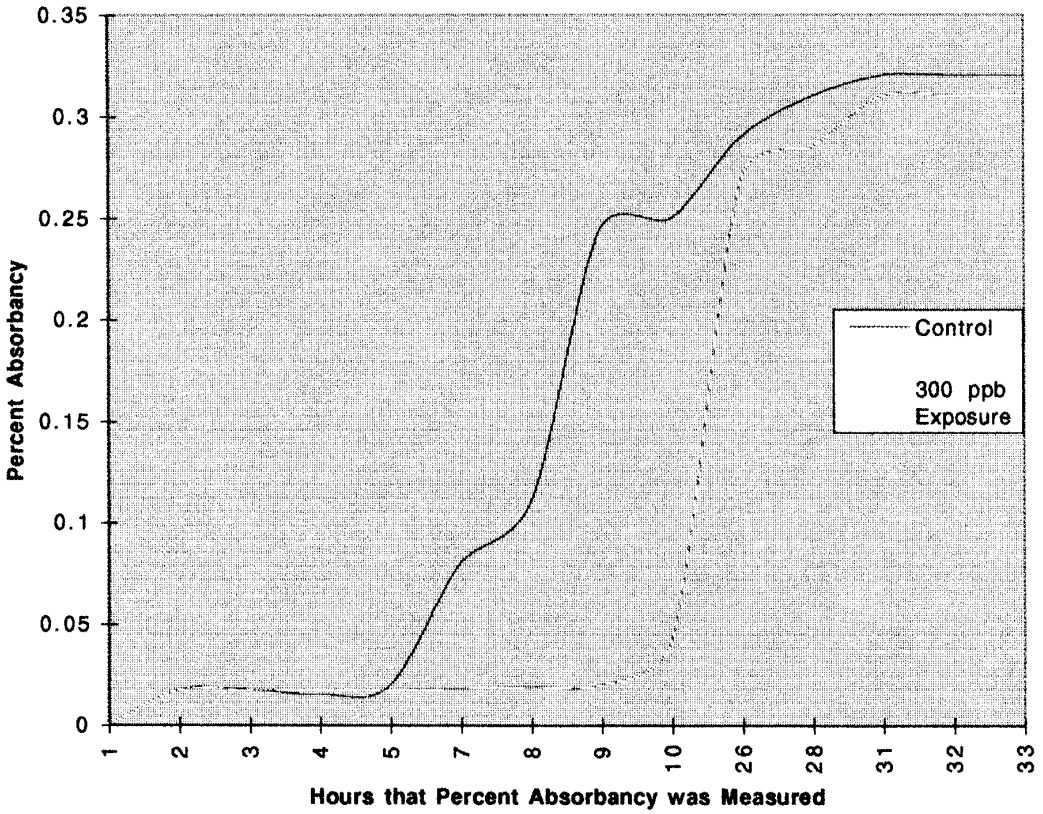
Average Escherichia coli Growth Decreases from the Trial #1 Plates to the Trial #25 Plates

Chart 1-2

	Tr #1: .2	Tr #1 : .4	Tr #1 : .6
Tr#25 : .2	4 sq cm		
Tr#25 : .4		6.6 sq cm	
Tr#25 : .6			4.5 sq cm

Comparison of Growth Curves of the Control *Escherichia coli* and *Escherichia coli* Exposed to 300 ppb as Determined by Using a Spectrophotometer

Graph 1-1



Average *Escherichia coli* Growth in Control Plates and the Lead-Induced Plates

Chart 1-3

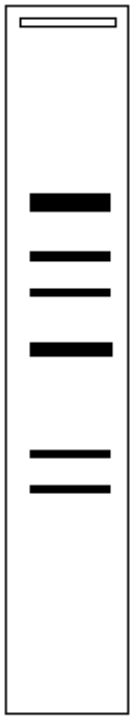
	Average
Control	9 sq cm
.2 plates	3.5 sq cm
.4 plates	2.9 sq cm
.6 plates	2.5 sq cm

λ+MM294+E/B

DNA fragments in all Trial #5, Trial #20,

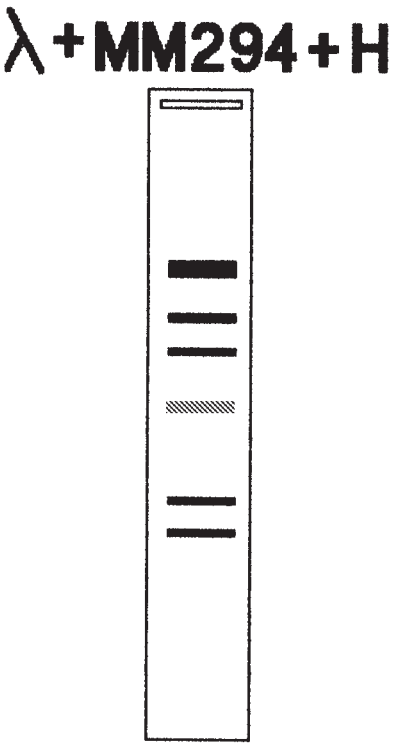
and Trial #25 samples - with the BamHI andEcoRI enzymes. Results in the Trial #10 and #15 samples were due to contamination.

λ+MM294+H

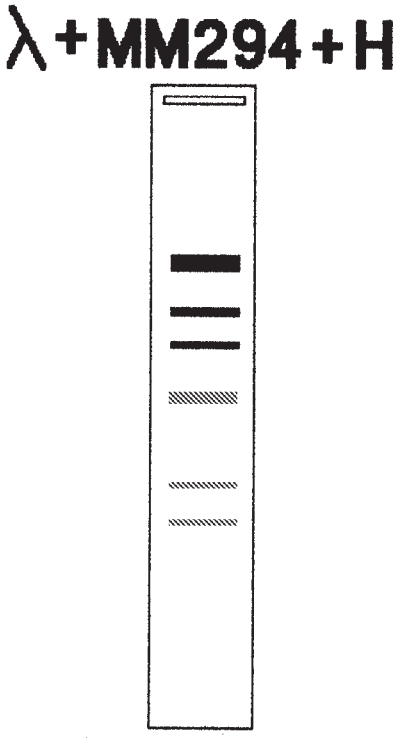


DNA fragments made in the Trial #5,

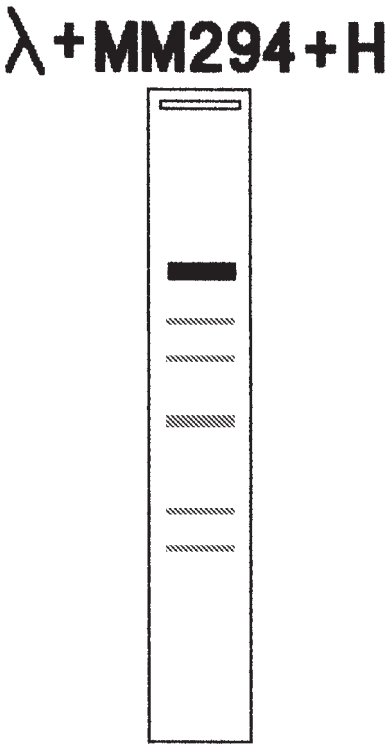
Trial #10, and the .2 and .2.2 samples of Trial #15 - with the HindIII restriction endonuclease.



DNA fragments in the .4, .4.4, .6, and .6.6 samples of Trial #15, and all of Trial #20 - with HindIII. (The light blue band indicates the site of the missing digestion.)



DNA fragments made in the .2, .2.2, and .4 samples of Trial #25 - with HindIII. (The light blue bands indicate the sites of the missing digestions.)



Cuts made in the .4.4, .6, and .6.
Trial #25 samples - with the HindIII.
(The light blue bands indicate the sites
of the missing digestions.)

Genotypic Results :

The restriction enzymes BamHI and EcoRI were used together in the lambda/E. coli DNA samples, and the results obtained were inconsistent. The number of digestions present in the Controls, the Trial #5 samples, the Trial #20 samples, and the Trial #25 samples was nine. The number of digestions made in the Trial #10 and Trial #15 samples varied. In the Trial #10 DNA samples : the .2 sample had 6 digestions; the .2.2 sample had 7 digestions; the .4 sample had 13 digestions; the .4.4 sample had 7 digestions; the .6 sample had 5 digestions made; and the .6.6 sample also only had five digestions made. In the Trial #15 DNA samples : the .2 and .2.2 samples had 6 digestions made; and the .4, .4.4, .6, and .6.6 samples all had 8 cuts present.

The restriction enzyme HindIII was run alone with the lambda /E. coli DNA samples. In the Controls and the Trial #5 samples, the Trial #10 samples, and the .2 and .2.2 samples of Trial #15, 6 digestions were made. In all of the .4, .4.4, .6, and .6.6 samples of Trial #15, and all of the Trial #20 samples there were 5 digestions made (one was missing). In the .2, .2.2, and .4 samples of Trial #25, there were three cuts made (three were missing.) Finally, in the .4.4, .6, and .6.6 samples of Trial #25, there was only 1 digestion made (five of them were missing.)

Conclusion and Future Studies

The phenotypic data showed that there was significant damage done to the lead-induced bacteria in terms of the number of square centimeters of bacterial growth as the lead nitrate concentrations increased. There were definite decreases evident when the amount of Escherichia coli growth in the Control plates was compared to the amount of growth in the bacteria that had been lead-induced; as well as when comparing the amount of growth in the Trial #1 bacteria as opposed to the Trial #25 bacteria (as is seen in the Chart 1-2.) For example, the growth in the Trial #1 .4 was 5.5 square centimeters and had diminished to 1.2 square centimeters of bacterial growth by Trial #25. The Growth Curve indicated that the lead - induced bacteria had a much slower growth rate at the beginning of the curve, as is apparent in Graph 2-1. One can conclude that there is genetic damage taking place due to the fact that there must be a mutation for the growth to have diminished at such a drastic rate. At Trial #17 the number of square centimeters of bacterial growth started to level off, which suggests that the bacteria is starting to build up a tolerance to the particular level of lead that it's been exposed to.

The genotypic data shows that the most obvious changes to the DNA have taken place in the two middle trials that had been tested for genetic damage, Trial #10 and Trial #15. The fluctuations in the number of digestions made in the DNA samples are illustrated in Graph 3-1, and it's apparent that the Trial #15 samples all had a number of digestions below the norm. The Trial #10 samples all had fewer digestions than the normal number (which was nine), except for the .4 sample, which had thirteen digestions. This suggests that either the trials were contaminated or there was a procedural error, or that the lead didn't affect the higher trials because they had become immune or tolerant of the lead levels. This data simply suggests the possibility of genetic mutation

There are many avenues that could be taken to further this research. Trials could be done using various restriction endonuclei (including Hin dIII) to determine whether or not they cause digestions in the DNA that normally wouldn't occur. Trials beyond twenty-five could be studied to determine if the bacteria really has built up a tolerance to the lead levels , or if the leveling off of the bacterial growth is just a temperate stagnation in the decreasing of the numbers. Further trials could be performed to substantiate the possibility of genetic damage.

Expansion possibilities include using imaging techniques that are more specific, such as the Southern Blot technique, to examine the DNA more closely. Recent studies have also been done on testing the amount of lead found in patient's urine and linking that to lead poisoning. Another aspect of this research would be to study whether or not noticeable damage occurs both phenotypically and genotypically in kidney cells.

References

Boling, Rick. "Heavy Metal : Weighing the Dangers of Lead in Your Dinnerware". November/December 1986 :pp. 27 - 32.

Barbour, John. "Discovering the DNA Map." Gazette - Mail. January 9, 1994 : pp 1B +.

Committee on Environ. Hazards. “Statement on Childhood Lead Poisoning”. AJ of P.
March 1987 : pp. 457 - 464.

EPA. “Lead in Your Drinking Water.”
June 1993.

Feldman, Robert G., MD; White, Roberta F., PhD, “Lead Neurotoxicity and Disorders of Learning”. Journal of Child Neurology.
Boston, 1992 : pp. 354-358.

Kendler, Barry S., PhD. “Lead: An Element Of Danger”. Carolina Tips.
1993 : pp. 1 - 3.

Kimbrough, Renate D., MD; LeVois, Maurice, PhD; Webb, David R., MS. “Management of Children With Slightly Elevated Blood Lead Levels”. American Academy of Pediatrics.
1994 : pp. 187-188.

“Lead Toxicity in the 21st Century: Will We Still Be Treating It?” Academy of Pediatrics.
Boston, 1992 : pp. 678-680.

Ledly, Fred D.,M.D. “Somatic gene therapy for human disease : Background and prospects. Parts 1 - 4.” The Journal of Pediatrics.
January 1987 : Volume 110, No. 1, pp 1- 7.

Lipkin, R. “Identifying DNA by the speed of electrons”.
February 25, 1995 : p 117

“Lowdown On Lead Poisoning”. Essence.
January 1991 : p. 99.

Maston, Angela; et al. Heredity : The Code of Life.
Englewood Cliffs, New Jersey : Prentice Halls, 1994.

McKusick, Victor A., M.D. “The Morbid Anatomy of the Human Genome: A Review of the Gene Mapping in Clinical Medicine - Part I.”
1986 : Vol 65, No. 1, pp 1 - 31.

Merz, Beverly. “The Genetic Revolution : Designer Genes”. American Health.
March 1993 : pp 46 - 54.

Micklos, David; Freyer, Greg. DNA Science.
Cold Spirng Harbor : Cold Spring Labratory Press, 1990.

Miller, Walter L. “ Recombinant DNA and the pediatrician”. The Journal of Pediatrics.
July 1981 : Vol. 99, No. 2, pp 1-5.

Nash, J. Madeleine. “Cornering a Killer”. Time.
September 26, 1994 : p. 70.

Papichak, Steve. “Killer Bacteria E. coli 0157:H7 is Old Villain”. Las Vegas Review -. January 31, 1991 : pp. 1 - 3.

Roper, William, M.D. “Lead Poisoning : An Old Problem Impairing a New Generation.” October 1992 : page 1 and 4.

Ruff, Holly A.; et al. “ Declining Blood Lead Levels and Cognitive Changes in Moderately Lead - Poisoned Children.” Journal of American Medical Association. April 7, 1993 : Vol. 269, No. 13, pp. 1641 - 1646.

Travis, John. “Enzymes repair and modify DNA in a surprising way”. Science News. September 16, 1995 : Vol. 148, pp 188-189.

Wilcox, Frank. DNA : The Thread of Life. Minneapolis : Lerner Publications, 1988.