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**DNA Topoisomerases**

A Chancellor's Scholars  
Senior Research Project

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**Abstract**

Gel electrophoresis was performed on six known mutagens: MMS, EMS, Mtx, Caf, AHA, and Hyc, to test the affect each would have on topo I and topo II enzymes controlling the topological states of DNA. Each compound/DNA/enzyme mixture was prepared three different ways in order to localize the compounds' affect i.e. whether the compound affected the DNA or the enzyme.

Each of the gels was divided into two parts to test the affect of sodium dodecyl sulfata (SDS), used to denature proteins, upon the cleavable complex. SDS is commonly used in the mixtures which halt the enzymatic reactions. In each of the gels the top half contained no SDS while the bottom part was treated with SDS. In both cases identical conditions were used. The result of this was that SDS had no apparent affect on the cleavable complex.

Each of the six mutagens caused enzymatic inactivity in the third mixture; which consisted of adding the compound and enzyme first and then adding DNA. Thus, the compound directly affected the enzyme causing it to be unable to bind to the DNA. Two of the six compounds, caffeine and hycanthone, caused inactivity in the other two mixtures. This however can be explained by the fact that each intercalates the DNA.

**PURPOSE**

The purpose of this experiment is to expose topoisomerase I and II to six known mutagens. Both of these enzymes are found in the body and knowledge of what can affect them or alter their natural functions is necessary to the complete understanding of DNA replication. If these enzymes fail to function properly, then the cells in our body will not be able to replicate themselves. If replication cannot occur, there will be no new celols to replace the old ones once they die out.

The compounds to be used wil be mixed with the DNA and enzymes in three different ways. This will be to localize the affect, if any, the compound has on the DNA. The mixtures will then be placed into a gel and run in an electrophoretic tank, which causes the different strands of DNA to separate from each other.

## INTRODUCTION

### Enzymes

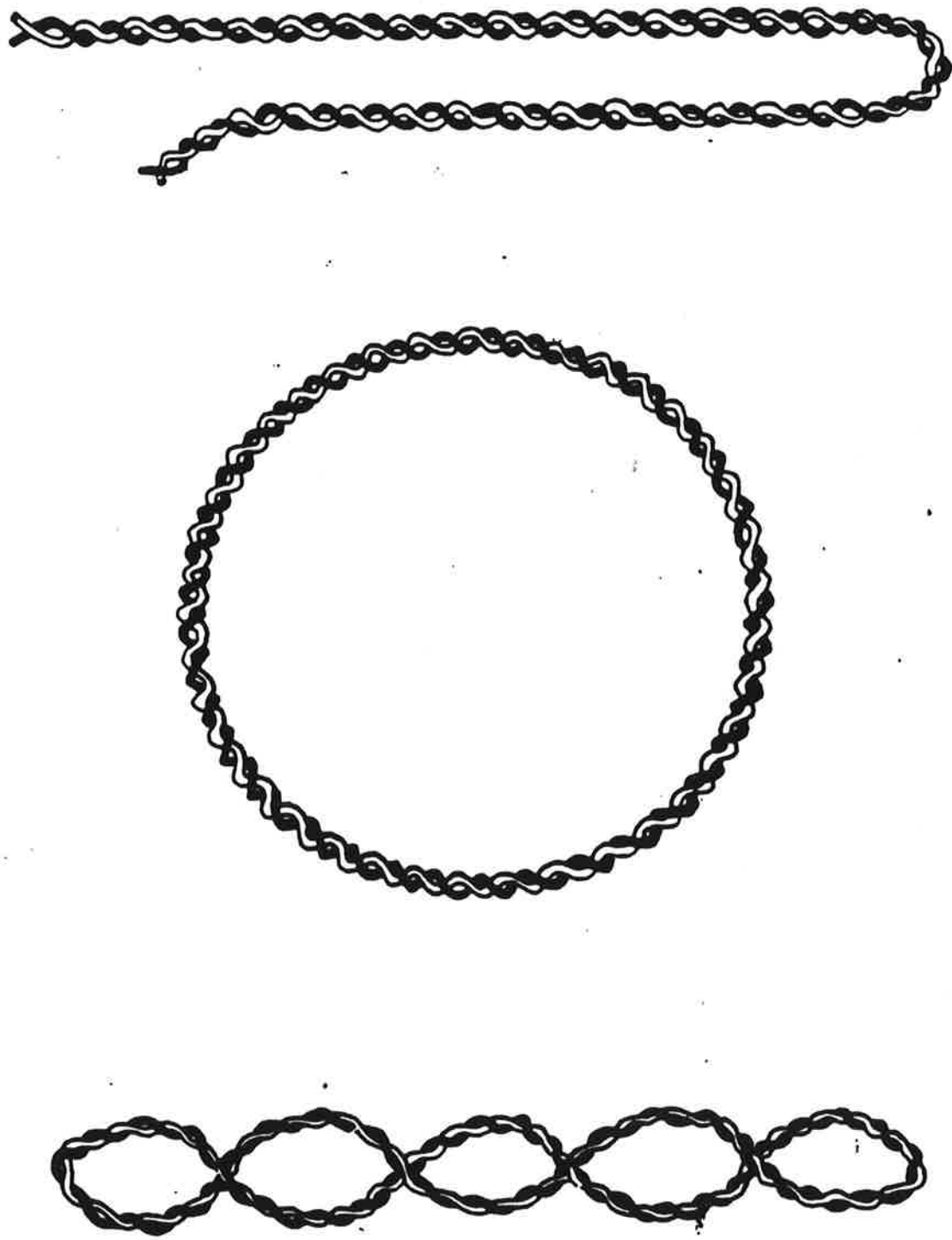
An enzyme is a protein molecule, produced by the body, which helps to catalyze or initiate chemical reactions within the body. The enzyme itself, however, is unaltered by the reaction. Therefore, each enzyme can be reused a vast number of times. Enzymes are specific in nature. That is, each one only reacts with specific molecules. Enzymes help to speed up a chemical reaction by lowering the activation energy of that particular reaction. The activation energy is the energy which the reactants must overcome in order to form the products. Once this is lowered, the reaction can take place at a faster rate. With the help of an enzyme, the rate of a reaction can be increased thousands of times compared to the rate the reaction would take place if the enzyme was not present.

Deoxyribonucleic acid, commonly called DNA, can be found in the nucleus of a cell. DNA is a double stranded molecule, usually found in the helical form, which is similar to a spiral staircase. DNA is made up of nucleotides which are in turn composed of (1) a nitrogenous base, (2) a sugar, in this case deoxyribose, and (3) a phosphate group. There are four different nitrogenous bases in DNA : adenine, cytosine, guanine, and thymine. These are represented by A, C, G, and T, respectively.

In a DNA chain, the bases pair up with each other in the way of A to T and C to G. This means that if A appears on one strand then on the opposite strand T will appear. The same is true for C and G. These bases are held together between the strands by hydrogen bonding. For each C-G, association there are three hydrogen bonds between them and for each A-T association, there are two (Kronberg, 1980). The nucleotides are held in place within the strand by covalent bonding.

DNA is involved in many processes, such as replication and transcription, and therefore a vast number of enzymes is needed to help in these processes. One specific group of enzymes, which is found in the nucleus with DNA, is called topoisomerases. These enzymes help to maintain the topological state or configuration of DNA.

An isomer of a molecule is one that contains the same chemical make-up but is arranged differently. This is where topoisomerases get their name. They change the topological state of DNA, hence *topo*, and create different shapes or configurations of the DNA, hence *isomerase*. Topoisomerase I (topo I) produces a transient break in one of the strands of the DNA helix and allows another single strand to pass through it (Wang, 1985). This means that it relaxes the supercoiled DNA by introducing positive turns. It causes the DNA to become a covalently closed circle with no twists in it (see figure 1-B).



C

B

A

**Figure 1** Variations in the molecular form of DNA. Smaller linear (C) molecules of DNA often assume a circular form (B), regardless of whether they are double- or single-stranded. Closed circles can become supercoiled (A), usually through enzymatic action.

Topoisomerase II (topo II), also known as DNA gyrase, produces a transient break in complimentary strands of DNA and allows another double strand to pass through it (Wang, 1985). This causes the introduction of negative turns, which causes the circular DNA to revert back to its normal supercoiled helical form (see figure 1-A). Topoisomerase II can be found throughout the entire nucleus of a cell. On the average there are about 200,000 copies of topo II in the nucleus (Glisson and Ross, 1987).

Inhibition of topoisomerase activity may produce a number of problems. An example of this can easily be seen in mitosis. Mitosis is the process in which cells replicate themselves. In order for this to occur DNA must uncoil itself. In other words, topo I must bind to the DNA so that it can relax and become circular DNA. When mitosis has been completed, topo II must attach itself to the DNA so that negative superhelical turns will be induced. Thus the DNA will go back to its supercoiled form. If mitosis does not occur then the cells will not be able to duplicate themselves. If this cannot happen then when a cell dies there will not be another one to take its place. With no cells to replace dying ones, the body would die.

There are many chemicals which are known to affect topoisomerase. It is not clear, however, whether these compounds affect the enzyme or the DNA itself. When the enzyme is added to DNA it binds to it in specific sites. These sites are then called the cleavable complex (see figure 2-A). Since the DNA is cut by these enzymes, it is not known whether the strand is cut while the topoisomerase is bound to it, or whether the strand is cut in the process of removing the enzyme.

Once the compound is added to the DNA it could affect the enzyme or the DNA. The compound could change the enzyme so that it cannot recognize the binding site. That is, it could cause the enzyme to undergo some type of mutation causing damage to it. Thus, the enzyme could not possibly bind to the DNA. The compound could also affect the DNA by intercalating it. This means that the compound will saturate the DNA. The compound will insert between the nitrogenous bases. That is, it will go between the "steps" of the "staircase" causing it to expand. This expansion could cause the unaffected topoisomerase to be unable to recognize the binding site. Thus, the enzyme will not bind to it.

Knowledge of what affects the cleavable complex is pertinent to cancer researchers. If a compound or drug is introduced into the cell and this drug causes inactivity of the enzyme without any other damaging affects, this compound could be used as a form of cancer therapy. By keeping the ends of the DNA from joining back together, the cell will not be able to replicate itself. Without these enzymes to help in replication the cell will die. It has been proven that in yeast cells the enzymes' involvement in mitosis is necessary for the cell to survive (Holm *et al*, 1985).

## Electrophoresis

Electrophoresis is a technique used to separate and identify fragments of DNA. DNA is loaded into an agarose gel and an electric current is applied. This current causes the sample to run down the gel based on its charge at a specific time. The migration rate of a sample of DNA depends on several factors. The concentration of agarose in the gel is one of these factors.

The fragments migrate at different rates through different concentrations of agarose. The more agarose contained in the gel the more dense or stiff the gel. So, consequently, the more dense the gel the slower large fragments of DNA will move. Therefore, only small fragments of DNA should be used in an agarose gel which contains a high concentration of agarose. Conversely, large fragments of DNA should be run in a gel which contains low concentrations of agarose. If small fragments were run on a gel of low agarose concentration, they would probably migrate completely off of the gel.

Another factor influencing the migration rate of the DNA is the form which the DNA assumes. In a gel, small fragments of DNA migrate further than large fragments. Thus, linear DNA will migrate further than circular DNA and supercoiled DNA will migrate further than linear DNA. Both of these statements are true because of the surface area. Circular DNA has a larger surface area than the supercoiled and is consequently larger; therefore the supercoiled DNA will migrate further. This will be shown in the viability gel.

The amount of current applied to the gel and the time the current is applied will also affect the migration rate of the DNA fragments. The fragments have an optimum voltage range at which they will separate accurately. At lower voltages, the distances traveled are proportional to the amount of voltage applied. However, the higher the voltage gets, the mobility of the DNA starts to decrease. The time that current is applied to a gel really does not have a pronounced affect on the DNA fragments, because the distances which the fragments travel is proportional to the amount of time for which they are run (Maniatis *et al*, 1982).

## MATERIALS/METHODS

**Test compounds.** Ethyl methane sulfonate (EMS) and methyl methane sulfonate (MMS) were obtained from Eastman Kodak Company, Rochester, NY; caffeine from Sigma Chemical Company; and Methotrexate (Mtx) from ICN Pharmaceuticals, Cleveland, Ohio. Hycanthone methanesulfonate (hyc) was a generous gift from Sterling Winthrop Research Institute, Rensselaer, NY.

2-Amino-N<sup>6</sup> hydroxyadenine (AHA) was generously provided by Drs. D. M. DeMarini and M. M. Moore, respectively ( Genetic Toxicology Division, US EPA, Research Triangle Park, NC.



Of these compounds, two are known intercalators. They are hycanthon methanesulfonate and caffeine.

#### Dilutions of compounds.

AHA 10,000ng/ml dil 1:2.5=4000ng/ml dil 1:40 into Rxn= 100ng/ml  
 EMS 1200mg/ml dil 1:48=25mg/ml dil 1:40 into Rxn= 625µg/ml  
 MMS 1200mg/ml dil 1:10 dil 1:300=400µg/ml dil 1:40 into Rxn=10µg/ml  
 Hyc 1mg/ml dil 1:2.5= 400µg/ml dil 1:40 into Rxn=10µg/ml  
 MTX 50µg/ml dil 1:2.5= 20µg/ml dil 1:40 into Rxn=0.5µg/ml  
 CAF 160mg/ml dil 1:20 into Rxn =8mg/ml  
 mAMSA control

**Enzymes.** Topoisomerases I and II were both obtained from Bethesda Research Laboratories. These enzymes were stored in a freezer at -20°C until used. Topoisomerase I was purified from calf thymus DNA. Topoisomerase II was purified from *Micrococcus luteus* which is a specific type of bacteria.

**Plasmid DNA.** Plasmid DNA is DNA obtained from a bacterial cell. The specific bacterial cell used here is the pBR322 cell. The DNA was isolated from the cell via CsCl EtBr dye-buoyant gradient centrifugation. A 1350 base pair, which is a single pair of nucleotides, insert of mouse skeletal alpha actin cDNA was added to the original pBR322 DNA. This DNA is now referred to as p91 DNA. cDNA better known as complementary DNA is DNA that is synthesized from a strand of RNA using an enzyme known as reverse transcriptase.

When protein synthesis occurs, DNA is converted into RNA (ribonucleic acid). There are certain parts of the DNA that do not code for anything in particular. These are called introns. Before the RNA leaves the nucleus these introns are excised; leaving only the RNA that codes for proteins. These introns are cut out by an enzyme called RNA maturase (Klug and Cummings, 1986). If reverse transcriptase is used on this RNA, it will cause a strand of DNA to be formed. This DNA will not have any of the excised introns in it. Therefore it is not a complete strand of DNA, yet it is complementary to the RNA to be used in protein synthesis. Hence the name cDNA.

The actin refers to the fact that this particular part of the DNA codes for the actin filament found in all muscle fibers.

**DNA/Enzyme/Compound Mixtures.** There were three different mixtures made for this experiment. The first involved mixing the DNA and the enzyme and incubation at 37°C for 30 minutes. Then, the test compound was added to the mixture and incubated at 37°C for an additional 30 minutes. This particular treatment was done to see if the compound affected the cleavable complex, which is formed when the enzyme binds to the DNA.

The second mixture consisted of the compound and the DNA and incubation at 37°C for 30 minutes. The topoisomerase was then added to the mixture and incubated at 37°C for an additional 30 minutes. This was done to see if the compound affected the DNA so that the enzyme could not bind to it.

The final mixture consisted of mixing the topoisomerase and the test compound together and incubation at 37°C for 30 minutes. Then, the DNA was added to the mixture and incubated at 37°C for an additional 30 minutes. This was done to see if the compound affected the enzyme, causing it not to bind.

#### CONCENTRATION CHART

<u>Compound</u>	<u>Amount</u> µg/ml	<u>Amount of</u> <u>DNA</u>	<u>Amount of Enzyme</u>	
			<u>Topo I</u>	<u>Topo II</u>
EMS	620	40 ng	one unit	one unit
MMS	10	40 ng	one unit	one unit
Hyc	10	40 ng	one unit	one unit
Caffeine	8000	40 ng	one unit	one unit
AHA	0.10	40 ng	one unit	one unit
Mtx	0.50	40 ng	one unit	one unit

**Stock Solutions.** The buffer used in this experiment was made according to the following protocol:

<u>10x TBE Buffer solution:</u>	Tris (THAM)	108 g
	Boric Acid	55 g
	EDTA(disodium)	9.3 g

The ingredients were added to 800 ml of distilled water and were mixed, using a magnetic stirring rod, until completely dissolved. The pH was then adjusted to 8.3 and the volume was

brought to one liter using distilled water. This solution was then dispensed into 500 ml bottles and autoclaved for twenty minutes.

### Gel Electrophoresis

The buffer solution used in this part consists of diluted 10X buffer. 100 ml of 10X buffer solution was added to 900 ml of distilled water. 300 ml of this solution is placed into a 500 ml flask. To this flask an amount of agarose has to be added. The amount added depends directly on the desired stiffness of the gel. In my particular case, a 0.7% or 1.0% gel was used. So consequently, 2.1 g or 3.0 g of agarose were needed, respectively. A magnetic stirring rod was placed into the flask and it was put onto a hot plate and allowed to boil. The remaining 700 ml of the buffer solution is then poured into the electrophoretic tank. While the flask is heating, the plastic base for the gel was taped on both ends, in order to keep the gel from leaking. A plastic comb was installed into the base; this will make the wells into which the samples are placed. The base has to be balanced as well. This insures that the gel will be uniform in thickness.

Once the gel solution is boiling vigorously and the agarose is completely dissolved, the flask was placed into a container of cool water for about five minute, so when the gel is poured the intense heat will not warp the plastic base. Once the five minutes were up, the solution was poured into the gel base and allowed to solidify. Solidification usually takes about an hour.

While the gel was solidifying, another supply of buffer solution was made up and poured into the tank. The tank usually requires about two liters of buffer solution to fill it completely. The tape was taken off of the gel base, once solidification has occurred. The gel base can then be lowered into the tank. The buffer solution should cover the gel completely. The comb, which should still be in the gel, is not taken out until the gel is ready to be loaded. The amount of the DNA mixture to be loaded onto each well is 25 $\mu$ l of the original sample.

Once loading has taken place, the top of the electrophoretic tank was placed on it and the power supply turned on. The correct voltage should be selected, usually about 45 volts is used for an overnight run ( Maniatis *et al*, 1982).

### Picture of Gel

The next day, the gel was transferred to a shallow container. About 25 $\mu$ l of ethidium bromide was added in order to make each particular band visible. The gel remained soaking in the ethidium

bromide for about 30 minutes. The ethidium bromide intercalates the DNA and when it is hit with a particular wavelength of ultraviolet light, the ethidium bromide glows and each band can be seen.

After the 30 minutes had elapsed, the gel was destained with distilled water. This washes away the excess ethidium bromide. A picture was then taken of the gel using a Fotodyne FCR-10 camera and Polaroid 667 film. However before was done, the gel was transferred to the ultraviolet illuminator. Once there, the camera fits over the top of the gel (Maniatis *et al*, 1982).

NOTE: The lights must be turned off in order to obtain an accurate picture.

## **RESULTS**

### **Viability Gel**

The first gel was done to test the viability of the enzymes. This gel was a .7% agarose gel that was left to run overnight at 57 volts. In this gel, lanes 1,2,4,11, and 13-20 were left blank.

The third and twelfth lanes of this gel contain lambda DNA that has been cut with hind III. Bacteriophage lambda, or lambda as it is more commonly called, is a virus which has double stranded DNA that is 50 kilobases long (Maniatis *et al.*, 1982). Hind III is a restriction enzyme that recognizes a particular sequence of nucleotides in double strand of DNA. Once the particular site has been found, the enzyme produces a break in the DNA at that site. Hind III cuts the lambda DNA into eight distinctive parts of known lengths. These lengths are 23.1kb,9.4kb, 6.6kb, 4.4kb, 2.3kb, 2.0kb, .512kb, and .125kb long (kb stands for kilobase which is one thousand base pairs of nucleotides). Lambda is usually put into the gel to use as a reference point because the length of each fragment is known. Going from top to bottom of these two lanes, the lengths of lambda are 23.1, 9.4, 6.6, 4.4, 2.3, 2.0 . About three quarters of the way down a faint band can be seen. This is the .512kb fragment. The smallest fragment of the lambda DNA, .125kb long, is not present on the gel because it is too small. When the current is applied long enough, this band runs completely off of the gel.

The fifth lane of the gel is untreated DNA which should consist of 100 % supercoiled DNA. It is in fact however about 90% supercoiled and 10% relaxed circle. The reason for the circular DNA is that part of the DNA has become nicked (see figure 2-B ). If the DNA is nicked one of the strands is allowed to swivel around the other causing a unwinding of the DNA to the relaxed circular form. Proceeding down the lane the first band that is encountered is a light band which is the relaxed circular DNA. This form of the DNA runs about the same distance that a seven kilobase fragment would. The next band which is more intense is the supercoiled DNA. Since, ethidium bromide

intercalates the DNA, the place with the greatest intensity would be the place with the most DNA. On this gel, supercoiled DNA runs about the same distance that a three kilobase fragment would.

Lanes six, seven, and nine of this gel contain DNA that has been treated with topoisomerase I. This enzyme causes supercoiled DNA to relax or be converted to open circles. Topoisomerase I also causes the formation of DNA duplexes which are open circular DNA rings hooked together (see figure 3). The very intense bands in both lanes around the seven kb region and corresponding to the band in lane five represent the open circular DNA. The light-colored bands below these, starting about the 4.6 kb region and going up to the circular DNA represent the different conformations that the DNA assumes while it is being converted to open circles. Given enough time, the entire amount of DNA would go to open circles and no conformation bands would be present. The faint bands above the open circular DNA are DNA duplexes or concatomers. The first band going down the gel, at about the 24 kb region, is a three ring duplex while the second band, at about the 12 kb region, is a two ring DNA duplex.

The next lane, lane eight, contains DNA which has been cut with hind III. Hind III is a restriction enzyme which means that this particular enzyme recognizes a specific nucleotide sequence in the DNA and cuts it. Hind III however produces only a single cut in this type of DNA. Thus, the DNA in lane eight is linear DNA. Linear DNA migrates to about the same position as would a five kb fragment of DNA.

Lane ten of the gel consists of DNA which has been treated with topo I. The reaction was then halted and topo II was added. Topoisomerase II is an enzyme which induces supercoiling of the DNA. The band located at about the three kb region represents supercoiled DNA and corresponds to the band in lane five. This proves that the enzymes are viable and ready for use.

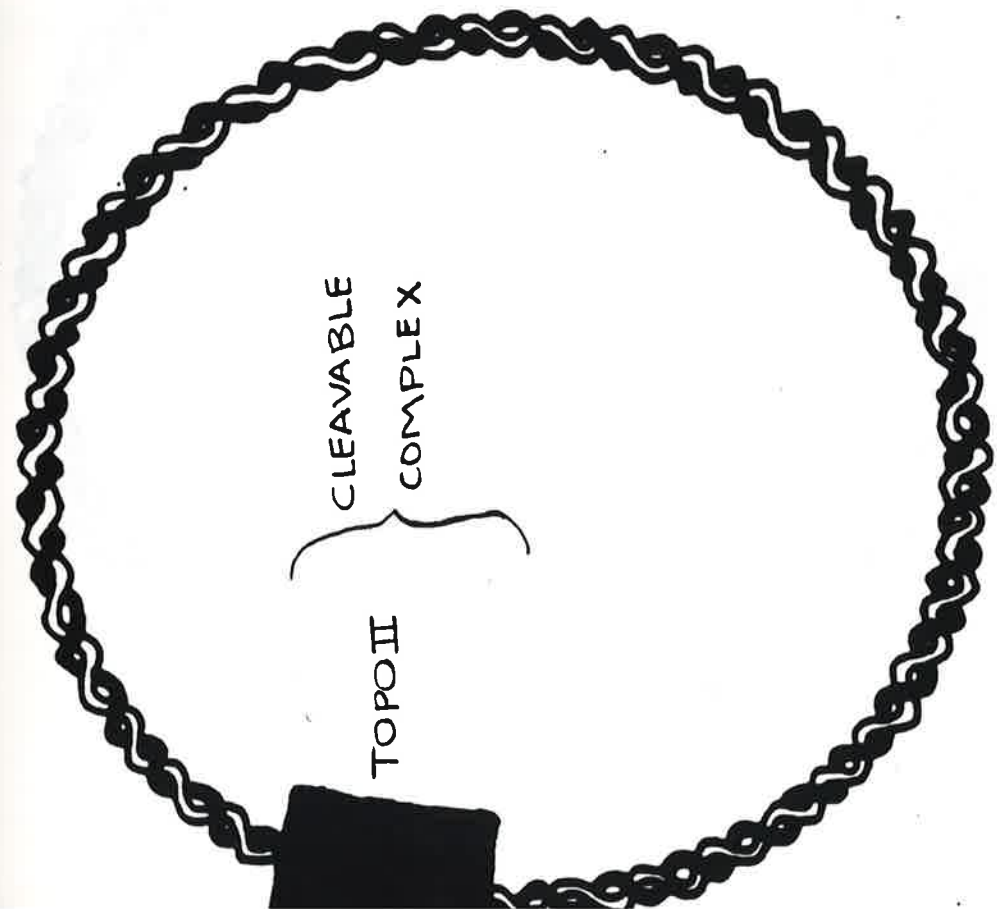
### Topo I Gels

In lanes 1-6 and 20 of the topoisomerase I gels are controls. In lane one and twenty is lambda. Six of the eight fragments of lambda can be seen clearly, The other two have run complete off the gel. Lane two contains untreated supercoiled DNA. Lane three consists of hind III cut DNA and is consequently linear. Lane four is open circular DNA. Lane five is supercoiled DNA which has been treated with topo I. Lane six is supercoiled DNA which has been treated with topo I and m-AMSA which is a compound that does not affect topo I. The relevancy of each of these controls has been explained earlier.

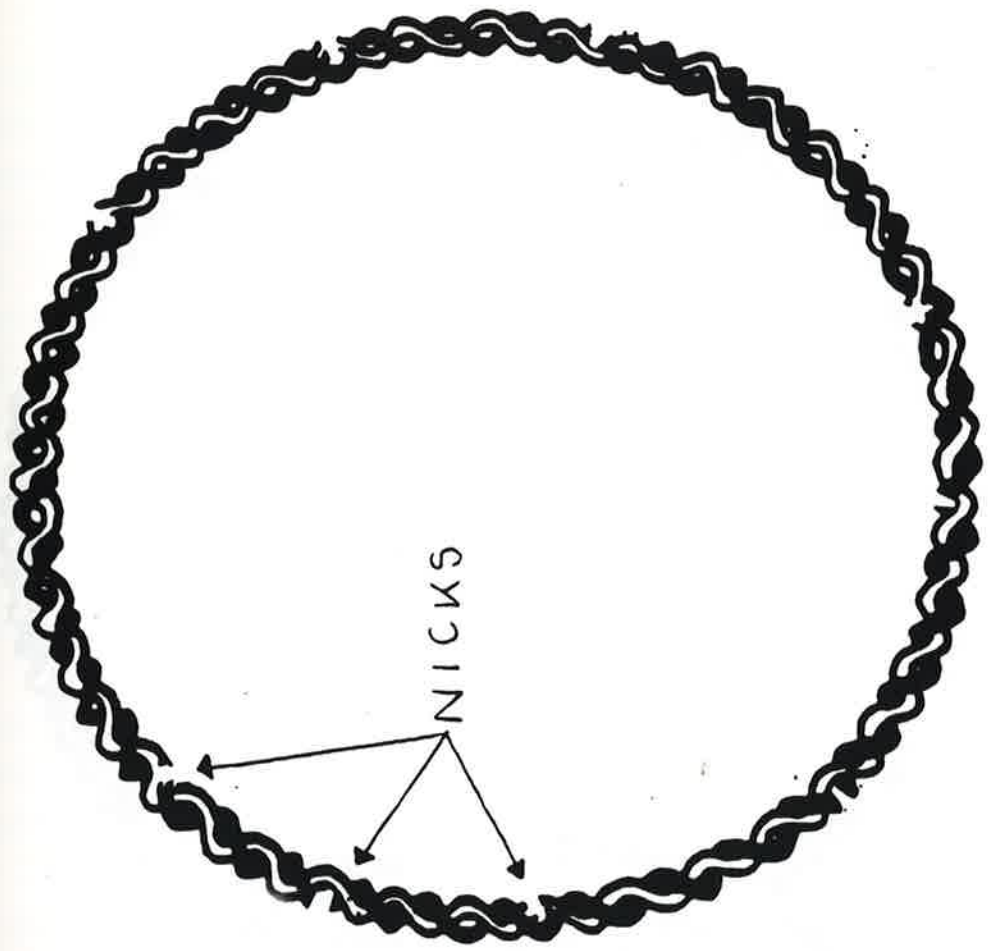
Each compound was treated as previously mentioned and loaded onto the gel. The voltage applied to this gel was 35 volts and the gel ran for 15 hours. The gel however was split into two parts. To the lower part of the gel, sodium dodecyl sulfate (SDS) was added.

CIRCULAR DNA

NICKED DNA



A



B

Figure 2

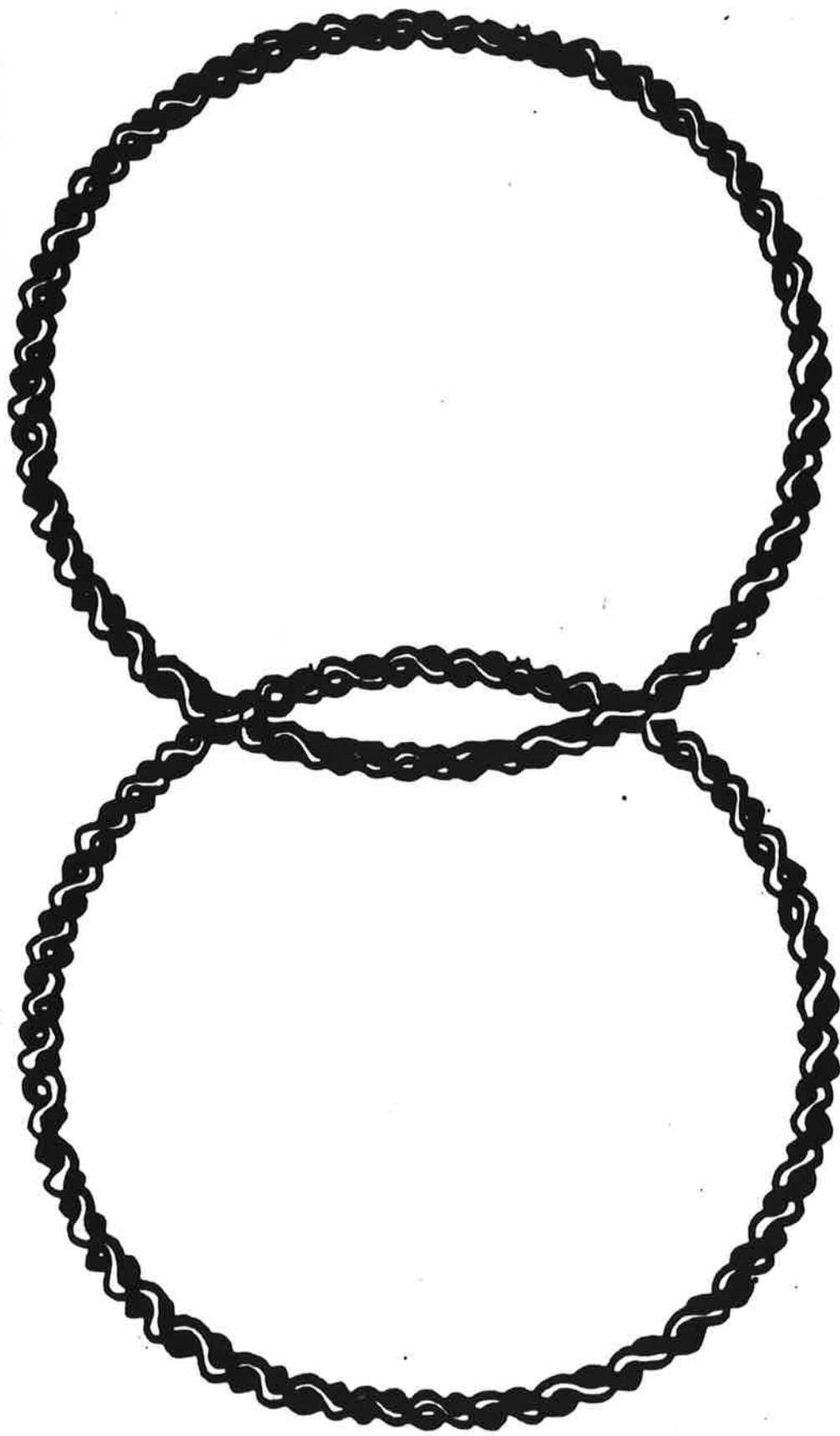


Figure 3

SDS was added to see if it affected the cleavable complex causing it to split apart or if the enzyme was actually causing the breaks. In previous experiments of this type, SDS was always added to terminate the enzymatic reactions. It was unclear whether this had any adverse effect on the cleavable complex or not. The top half of the gel contained no SDS.

Of the six compounds added to the topo I in the various ways, all of them affected the activity of the enzyme. Particularly in the third type of mixture (lanes 10, 14, and 18 of each gel). This mixture consisted of adding the compound and the enzyme together and incubating for 15 minutes. Then the DNA was added to this mixture and incubated for an additional hour. This shows that the compound directly affects the enzyme and alters it enough so it cannot bind to the DNA. The inactivity of the enzyme is shown by the presence of a band at about the three kb location, which is the distance that supercoiled DNA migrates, showing that all of the DNA was not converted to open circles. Thus, it is supercoiled DNA.

Hycanthone methane sulfonate and caffeine affected the activity of the enzyme in the other two mixtures as well. The second mixture consisted of adding the compound and the DNA together and then the enzyme. The inactivity for this reaction can be explained by the fact that both hycanthone and caffeine intercalate DNA, while the other four compounds do not.

The SDS appeared to have no apparent affect on the cleavable complex. This was shown by the fact that the same results were obtained for each part of the gel.

### Topo II gels

The topo II gels also contained the same controls as the topo I gels except that instead of supercoiled and topo I in lanes five and six there were open circular DNA and topo II. In lane five of the second topo II gel the topo I enzyme was left out. It is known however that m-AMSA causes a breakage in the cleavable complex of topo II (DeMarini et al, 1987).

All of the compounds affected the activity of topo II in the third mixture which consisted of adding the compound to the enzyme and then adding the DNA. The inactivity of the enzyme can be seen by the absence of a band of DNA at the three kb region. This means that none of the circular DNA was converted to supercoiled DNA.

Hycanthone and caffeine affected the enzymatic activity in the other two mixtures as well. As previously stated this can be explained by the fact that both compounds intercalate the DNA and keep the enzyme from binding to it. This explains the inactivity in the second mixture.

The SDS again had no apparent affect on the enzyme. All of the places of inactivity in the upper half of the gel were present in the bottom half.



### Conclusion

Topoisomerase I and II are enzymes which control the topological states, different conformations which the DNA can assume, of DNA. When topo I binds to a single strand of DNA it produces a transient break and allows the supercoiled DNA to swivel about the other strand. This causes the DNA to change its supercoiled form into a covalently closed circular form. The binding of topoisomerase II to the DNA forms what is known as the cleavable complex. This enzyme causes a transient break in both of the strands of DNA. This allows the DNA to revert to its original conformation, which is supercoiled DNA.

The understanding of these enzymes and what affects them can be crucial to cancer research. A compound or drug which affects the cleavable complex and causes no other damage to the body could be used to halt the growth of cancerous cells. If the cleavable complex is not allowed to join back together the cell will die. If a cancerous cell dies, it cannot replicate itself. Thus, the cancer could be eliminated. Although these six mutagens affected the activity of the topoisomerases, a clearer understanding of which non-mutagens affect the cleavable complex is needed. Given enough time, non-mutagens would have been tested and mammalian topo II would have been used.

**ACKNOWLEDGMENTS**

If I were to thank everyone who contributed to this paper; it would well exceed 50 pages. However, I would like to try to thank a few. First, I greatly appreciate everyone who proofed this; without your help, mistakes would run rampant throughout. I would also like to thank Dr. Charles Jenkins who helped me get into the Chancellor's Scholars Program to begin with. I would like to thank Dr David Kuo for putting up with my constant delays. I greatly appreciate everyone who works in the genetic toxicology lab at Burroughs Wellcome: Dr. Donald Clive, Roberta Krehl, Patty Poorman-Allen, Ganelle McGee, and Erie Graves, without their help this project would not have been possible. Lastly, I cannot express enough thanks and appreciation to Paul Glover who guided me through this entire project. From him I learned almost everything , except the secrets, a molecular biologist has to know.

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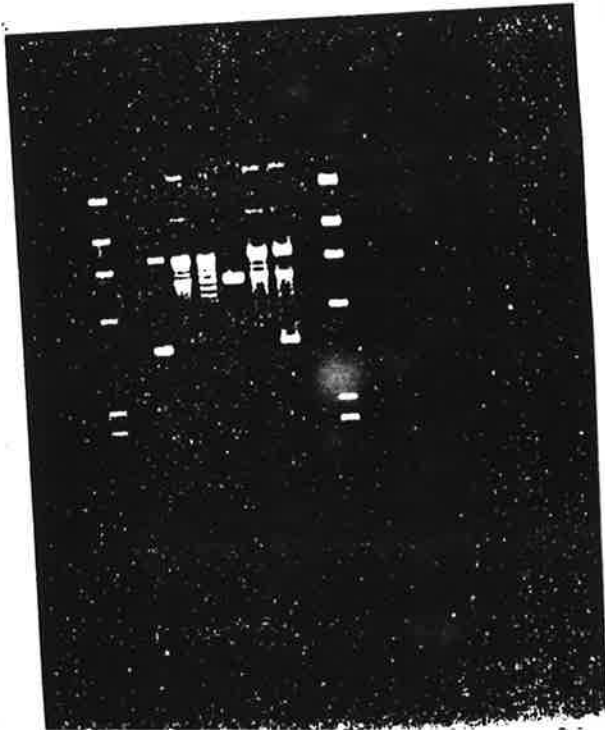
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**APPENDIX**

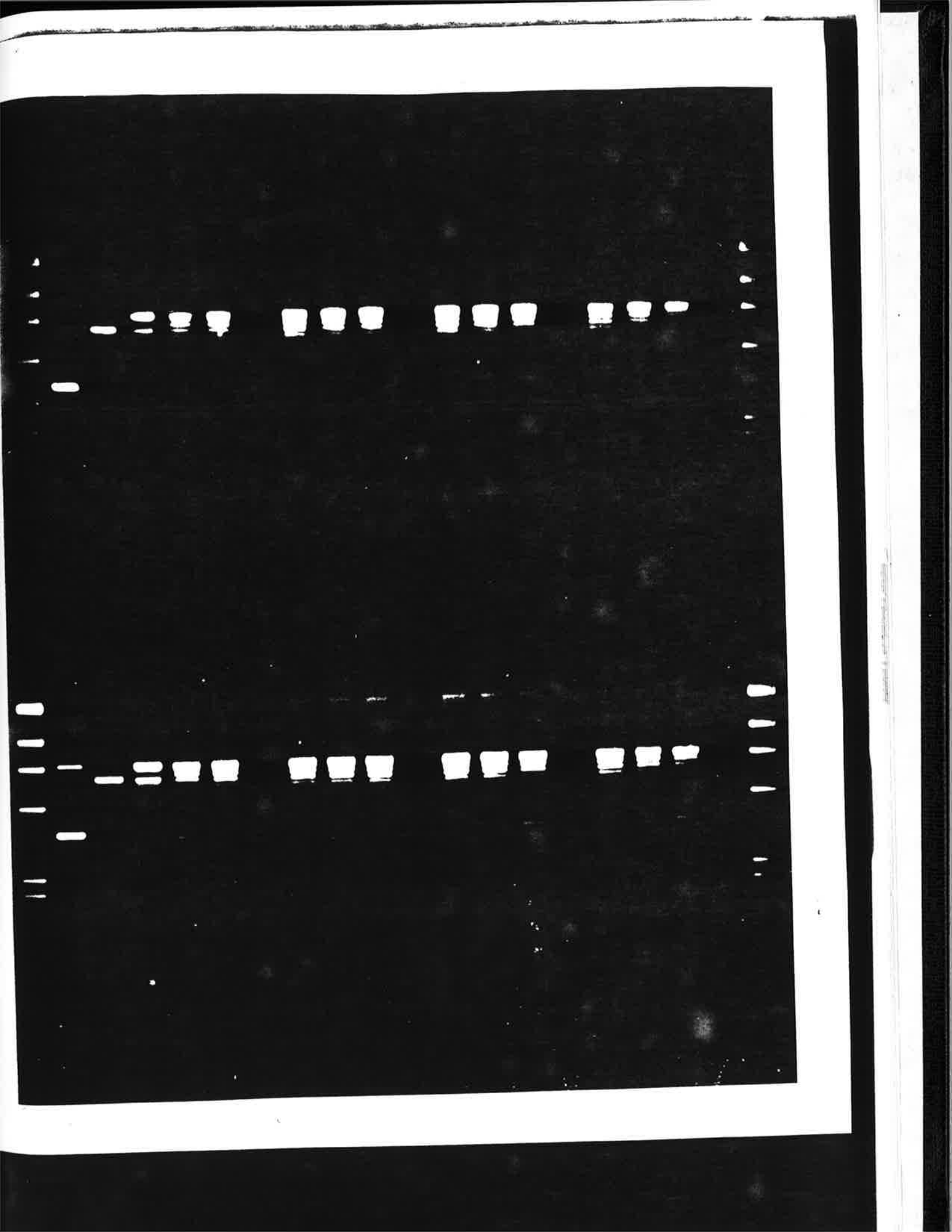
# GEL SHEET

DATE	GEL	BUFFER	CONDITIONS
10-31-89	0.7% Agarose	TBE	57 volts
LANE	DNA	ENZYME	
1			
2			
3	$\lambda$ Hind III		
4			
5	untreated DNA		
6	DNA + Topo I		
7	DNA + Topo I		
8	Linear		
9	DNA + Topo I		
10	DNA + Topo I $\rightarrow$ Topo II		
11			
12	$\lambda$ Hind III		
13			
14			
15			
16			
17			
18			
19			
20			



# GEL SHEET

DATE	GEL	BUFFER	CONDITIONS
11-2-89	0.7% Topo I	TBE	35 volts - 15 hours
LANE	DNA	ENZYME	
1	$\lambda$ (Lambda)		
2	Supercoiled (S.C)		
3	Linear		
4	Open Circle		
5	S.C + Topo I		
6	S.C. + Topo I + m-AMSA		
7	Blank		
8	ENZ + DNA $\rightarrow$ Cpd	AHA	
9	Cpd + DNA $\rightarrow$ ENZ	AHA	
10	Cpd + ENZ $\rightarrow$ DNA	AHA	
11	Blank		
12	ENZ + DNA $\rightarrow$ Cpd	EMS	
13	Cpd + DNA $\rightarrow$ ENZ	EMS	
14	Cpd + ENZ $\rightarrow$ DNA	EMS	
15	Blank		
16	ENZ + DNA $\rightarrow$ Cpd	MMS	
17	Cpd + DNA $\rightarrow$ ENZ	MMS	
18	Cpd + ENZ $\rightarrow$ DNA	MMS	
19	Blank		
20	$\lambda$ (Lambda)		



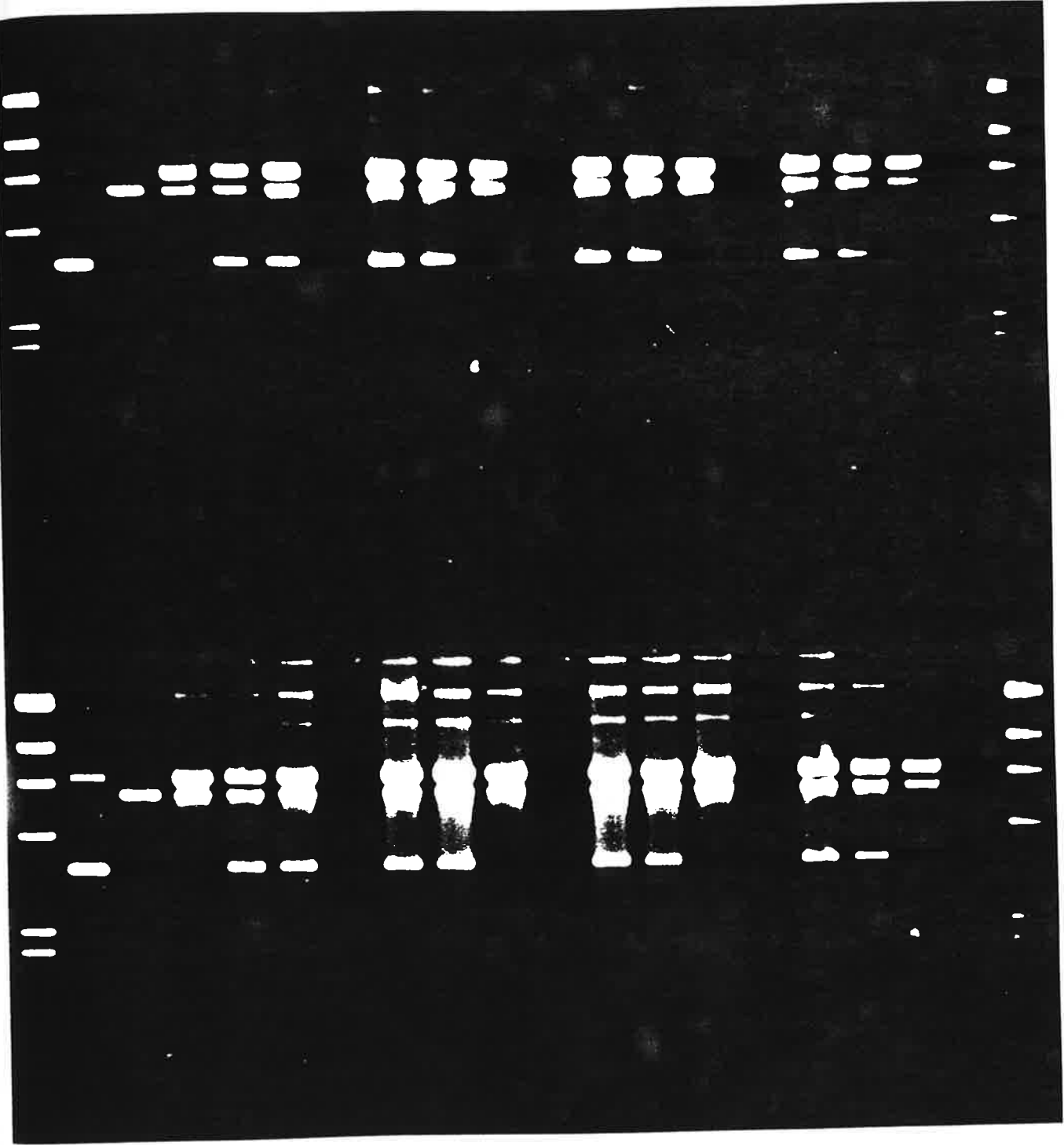


# GEL SHEET

DATE	GEL	BUFFER	CONDITIONS
11-2-89	0.7% Topo II	TBE	35 volts - 15 hours <sup>8</sup>
LANE	DNA	ENZYME	
1	$\lambda$ (Lambda)		
2	Supercoiled		
3	Linear		
4	Open Circle (O.C.)		
5	O.C. + Topo II		
6	O.C. + Topo II + m-AMSA		
7	Blank		
8	Enz + DNA $\rightarrow$ Cpd	AHA	
9	Cpd + DNA $\rightarrow$ Enz	AHA	
10	Cpd + Enz $\rightarrow$ DNA	AHA	
11	Blank		
12	Enz + DNA $\rightarrow$ Cpd	EMS	
13	Cpd + DNA $\rightarrow$ Enz	EMS	
14	Cpd + Enz $\rightarrow$ DNA	EMS	
15	Blank		
16	Enz + DNA $\rightarrow$ Cpd	MMS	
17	Cpd + DNA $\rightarrow$ Enz	MMS	
18	Cpd + Enz $\rightarrow$ DNA	MMS	
19	Blank		
20	$\lambda$ (Lambda)		

#2

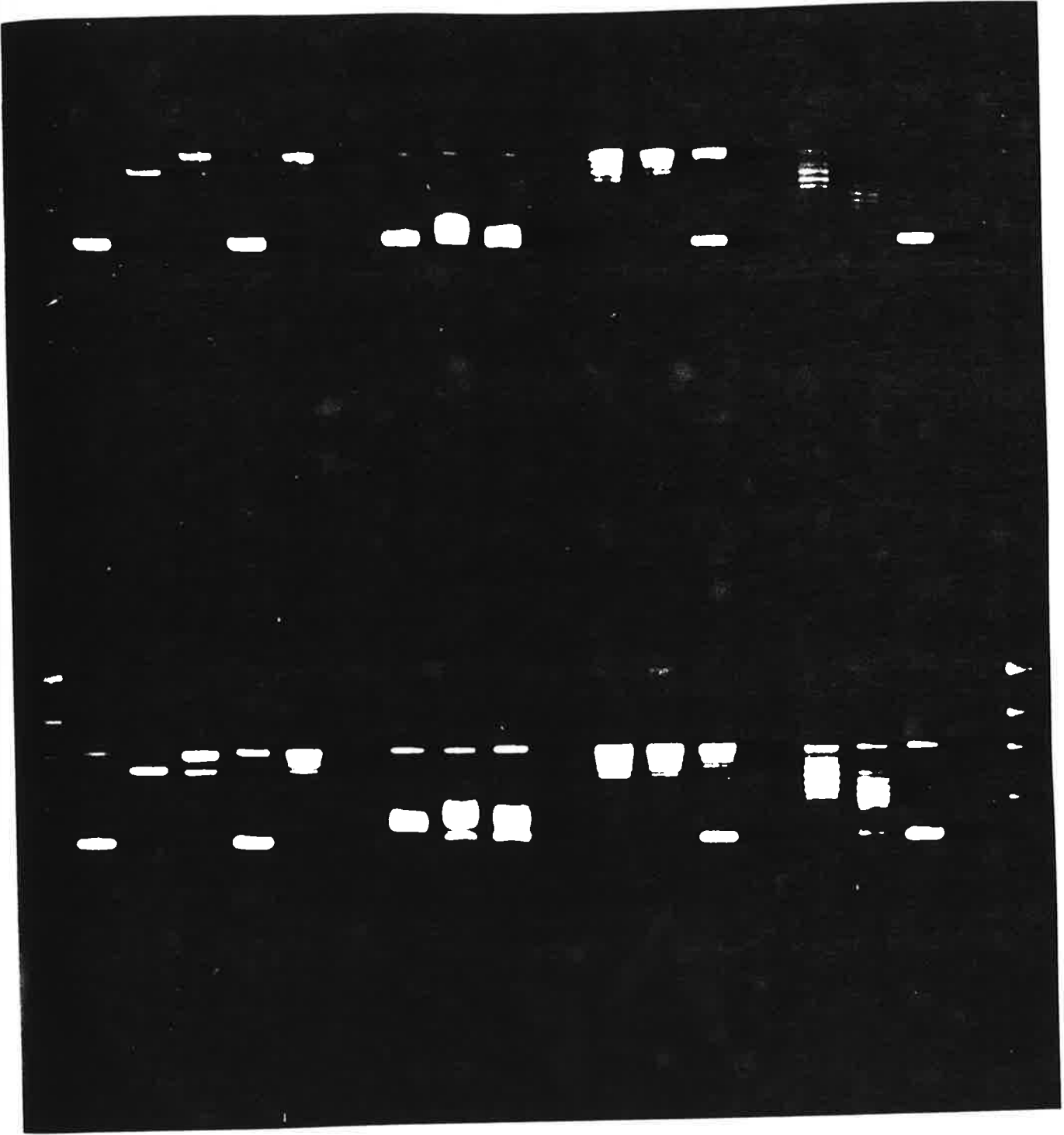
8



# GEL SHEET

DATE	GEL	BUFFER	CONDITIONS
11-7-89	0.7% Topo I	TBE	35 volts - 15 hours
LANE	DNA	ENZYME	
1	$\lambda$ (Lambda)		
2	Supercoiled (S.C.)		
3	Linear		
4	Open Circle		
5	S.C. + Topo I		
6	S.C. + Topo I + m-AMSA		
7	Blank		
8	Enz + DNA $\rightarrow$ Cpd	Hycanthone	
9	Cpd + DNA $\rightarrow$ Enz	Hycanthone	
10	Cpd + Enz $\rightarrow$ DNA	Hycanthone	
11	Blank		
12	Enz + DNA $\rightarrow$ Cpd	Methotrexate	
13	Cpd + DNA $\rightarrow$ Enz	Methotrexate	
14	Cpd + Enz $\rightarrow$ DNA	Methotrexate	
15	Blank		
16	Enz + DNA $\rightarrow$ Cpd	Caffeine	
17	Cpd + DNA $\rightarrow$ Enz	Caffeine	
18	Cpd + Enz $\rightarrow$ DNA	Caffeine	
19	Blank		
20	$\lambda$ (Lambda)		

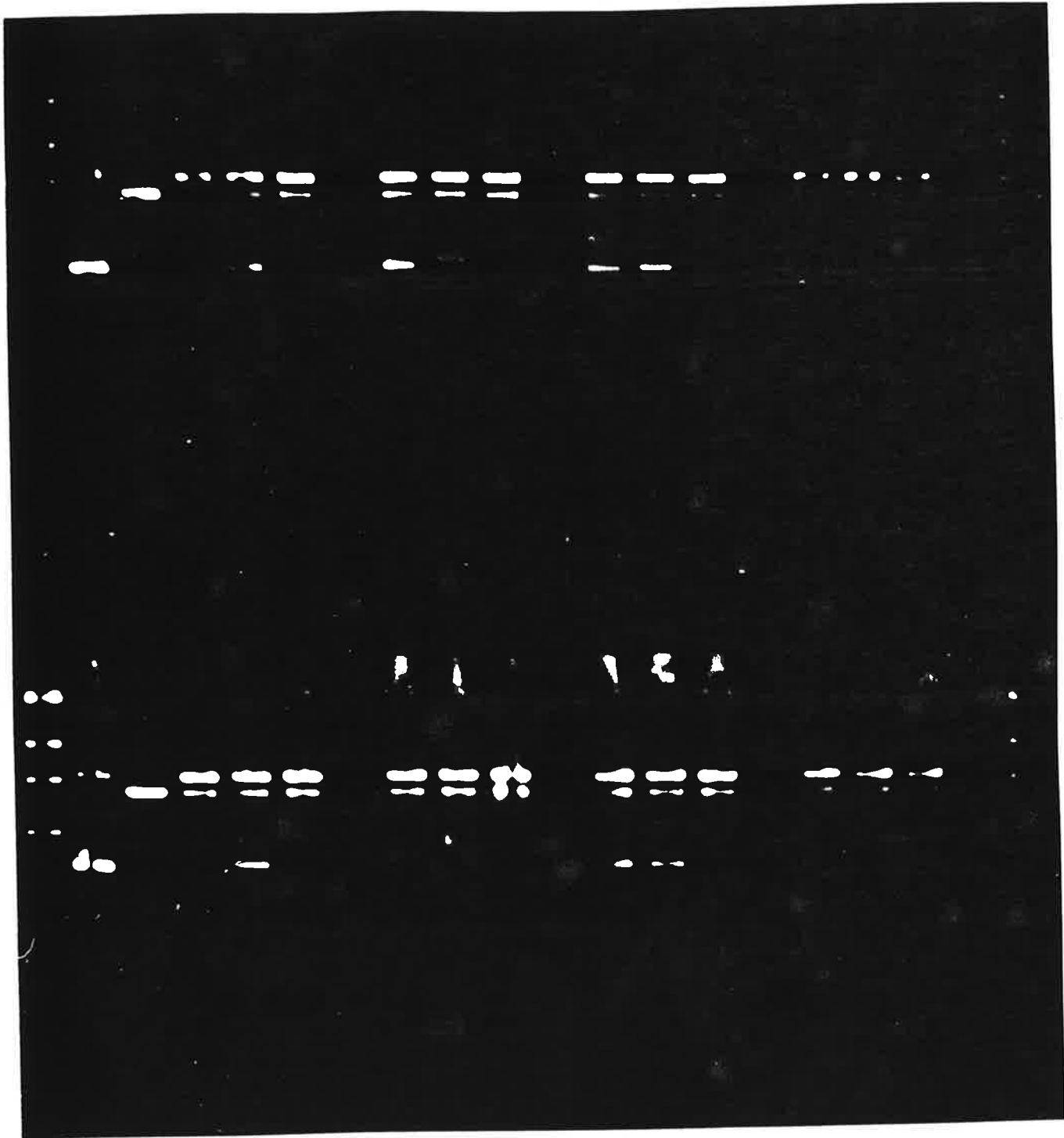
#3



# GEL SHEET

DATE	GEL	BUFFER	CONDITIONS
11-7-89	0.7% Topo II	TBE	35 volts - 15 hours
LANE	DNA	ENZYME	
1	$\lambda$ (lambda)		
2	Supercoiled		
3	Linear		
4	Open Circle (o.c.)		
5	O.C. + Topo II		
6	OC + Topo II + m-ANS		
7	Blank		
8	Enz + DNA $\rightarrow$ Cpd	Hycanthone	
9	Cpd + DNA $\rightarrow$ Enz	Hycanthone	
10	Cpd + Enz $\rightarrow$ DNA	Hycanthone	
11	Blank		
12	Enz + DNA $\rightarrow$ Cpd	Methotrexate	
13	Cpd + DNA $\rightarrow$ Enz	Methotrexate	
14	Cpd + Enz $\rightarrow$ DNA	Methotrexate	
15	Blank		
16	Enz + DNA $\rightarrow$ Cpd	Caffeine	
17	Cpd + DNA $\rightarrow$ Enz	Caffeine	
18	Cpd + Enz $\rightarrow$ DNA	Caffeine	
19	Blank		
20	$\lambda$ (lambda)		

#4



AHA (0.1 µg/ml)

HYC (10 µg/ml)

	TOPO I	TOPO II
NO SDS		
E+D->C	NO EFFECT	NO EFFECT
C+D->E	NO EFFECT	NO EFFECT
C+E->D	BLOCKED	BLOCKED

NO SDS

	TOPO I	TOPO II
E+D->C	BLOCKED	MARGINAL
C+D->E	BLOCKED*	MARGINAL
C+E->D	BLOCKED	BLOCKED

WITH SDS

	TOPO I	TOPO II
E+D->C	NO EFFECT	NO EFFECT
C+D->E	NO EFFECT	NO EFFECT
C+E->D	BLOCKED	BLOCKED

WITH SDS

	TOPO I	TOPO II
E+D->C	BLOCKED	MARGINAL
C+D->E	BLOCKED*	MARGINAL
C+E->D	BLOCKED*	BLOCKED

EMS (620 µg/ml)

MTX (0.5 µg/ml)

	TOPO I	TOPO II
NO SDS		
E+D->C	NO EFFECT	NO EFFECT
C+D->E	NO EFFECT	NO EFFECT
C+E->D	BLOCKED	BLOCKED

NO SDS

	TOPO I	TOPO II
E+D->C	NO EFFECT	NO EFFECT
C+D->E	NO EFFECT	NO EFFECT
C+E->D	BLOCKED	BLOCKED

WITH SDS

	TOPO I	TOPO II
E+D->C	NO EFFECT	NO EFFECT
C+D->E	NO EFFECT	NO EFFECT
C+E->D	BLOCKED	BLOCKED

WITH SDS

	TOPO I	TOPO II
E+D->C	NO EFFECT	NO EFFECT
C+D->E	NO EFFECT	NO EFFECT
C+E->D	BLOCKED	BLOCKED

MMS (10 µg/ml)

CAF (8.0 mg/ml)

	TOPO I	TOPO II
NO SDS		
E+D->C	NO EFFECT	NO EFFECT
C+D->E	NO EFFECT	NO EFFECT
C+E->D	BLOCKED	BLOCKED

NO SDS

	TOPO I	TOPO II
E+D->C	PARTIAL	PARTIAL
C+D->E	PARTIAL	PARTIAL
C+E->D	BLOCKED	BLOCKED

WITH SDS

	TOPO I	TOPO II
E+D->C	NO EFFECT	NO EFFECT
C+D->E	NO EFFECT	NO EFFECT
C+E->D	BLOCKED	BLOCKED

WITH SDS

	TOPO I	TOPO II
E+D->C	PARTIAL	PARTIAL
C+D->E	PARTIAL	PARTIAL
C+E->D	BLOCKED	BLOCKED