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CHEMICAL CARCINOGENESIS

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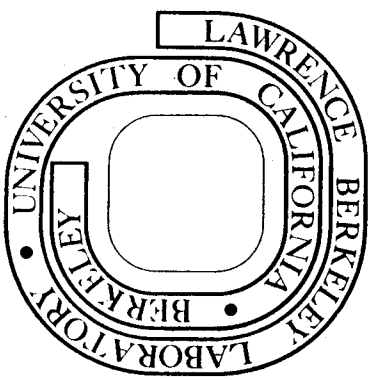

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CHEMICAL CARCINOGENESIS

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ABSTRACT

The first step in the generation of a malignancy seems to be a transformation in the genetic apparatus of a single cell. The ultimate nature of the cancer which appears is a result of the interaction of that change with the control and regulatory apparatus of the whole animal. It appears that the primary cellular change which may be induced by physical, chemical or biological agents (or a combination of them) may be something which is common to all carcinogenesis. The nature of that primary change and how it may result from the action of viruses, chemicals and radiation (or interaction between them) is the subject of this discussion.

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INTRODUCTION

Chemical carcinogenesis is a term which is used to describe the fact that many kinds of natural and synthetic chemicals present in our environment could conceivably be components in the triggering, or genesis, of malignancy. It has been known for over a hundred years that a component of soot is indeed a principal source of certain kinds of cancer, and it has been known for the last twenty years which component of soot is most active in this regard. Since this early recognition, the number of chemicals (natural and synthetic) which have been designated carcinogens has increased enormously, usually by virtue of some kind of epidemiological study or more recently and, more frequently, by deliberate screening programs with animals of various kinds.

The structure of today's discussion is: First, is there any chemical event, or common chemical property, of these chemical materials which has been recognized and is there any common chemical reaction which they perform; and, secondly, is there any common mechanism of achieving the biological consequences which we know these chemicals have.

CHEMICAL CARCINOGENESIS

A good deal of progress has been made during the last two decades in learning more about a whole variety of organic chemical carcinogens, and a rather straightforward view of chemical carcinogens has emerged. These materials do indeed have a property among them which is recognizable, that of electrophilicity in their initial structures. A group of chemicals of this type is shown in Figure 1, and it is possible to see that there are two types of electrophilic chemical carcinogenic reactions, enzymatic and non-enzymatic. We will be principally concerned in this discussion with the enzymatic reactions, but both types have cross-linking characteristics.

Those chemicals in the lower part of Figure 1 are known to function in the same way as the non-enzymatically active chemicals, but they require an enzymatic transformation to produce the electrophilic reagent which will then attack some of the cellular nucleophiles. One of these chemicals, acetylaminofluorene (AFF), is known to go through the identical sequence of oxidations to give hydroxylamine and then the ester of hydroxylamine (either sulfate or acetate) which gives rise to an electrophile by virtue of the loss of the anion, leaving behind a nitronium ion instead of a carbonium ion. The product of that reaction has been definitely established, and it has rather unique characteristics. The methyl group is oxidized to give, eventually, a hydroxylamine, and the same sequence of events occurs with the acetylaminofluorene. The two nitroso compounds can give rise to methylating diazomethane in situ, by virtue of a sequence of oxidations.

The diazomethane, in turn, can methylate the various nucleophiles.

The polycyclic aromatic hydrocarbons (PAHs) have remained a mystery for quite a long time because they are really not reactive molecules. It is only in the last decade that the nature of the activation of PAHs has begun to be understood. In general, they are activated by an oxidation mechanism. Much of today's discussion will focus on how the PAHs are activated and what the reactions in the cellular material are as a result of that activation. That particular type of reaction, therefore, will serve as a model for the nature of the chemistry, biochemistry and biology which are involved in carcinogenesis.

Three different macromolecules which conceivably could be target molecules for any of these electrophilic reactions are shown in Figure 1. We have known for some time that if you treat a cell or cell suspension containing all of these molecules with active (activated) materials, they will be covalently linked to all three components: DNA, RNA and protein. In principle, we do not know which of those targets is the critical one. However, circumstantially, and because of the nature of the biological effects of the chemical carcinogens, it seems almost certain that the important target is the DNA itself since the transformed cells behaves as a mutated cell, i.e., the transformed condition reproduces continuously, which is one of the qualities of tumorogenesis which makes us believe that the DNA is the important target rather than RNA or protein which would not "remember" these events and continuously reproduce the transformations in the cell.

Having recognized that the product of the activated carcinogen AFF with guanosine has been identified (Figure 2), let us examine that product. The AFF has been acetylated after oxidation, followed by loss of acetate anion, leaving the nitronium behind which can

become an electrophile on a collection of DNA bases. It has been shown that the nitronium ion attacks the #8 carbon atom of guanine selectively and produces the type of product shown. These experiments done about half a dozen years ago were the first case where the electrophilic reaction of a chemical carcinogen with DNA components was firmly established.

It is interesting to note that while the unsubstituted fluorene works and the 7-fluorofluorene works, the 7-iodofluorene does not react with DNA components in this fashion. Two consequences of that fact have been recognized. One is that the hydrogen compounds and the fluorine compounds are both carcinogenic, whereas the iodine compound from the reaction is not carcinogenic. The second consequence resulting from biophysical studies is that acetylaminofluorene and the corresponding fluorine compound affect the helical structure of the DNA whereas the iodo compound does not. The argument is that the carcinogenic compounds can intercalate into the DNA molecule to begin the chemical reactions which follow from it. This is a reasonable, circumstantial argument that the first step is intercalation. Part of the reason that this type of molecule is such an important and selective carcinogen is the fact that it can intercalate in the DNA in a rather special place and perform a rather special reaction in that place. This knowledge has been one of the starting points of our work on chemical carcinogenesis.

About five years ago we began the study of the question of why smog, tobacco smoke and, as it turns out, any combustion product of organic material, contain carcinogens and what the individual carcinogens are. It turns out that the principal carcinogen contained in <u>all</u> of the organic combustion products is benzo(a)pyrene, whose structure is shown in Figure 3. To a chemist it does not seem to make sense that

a molecule as non-functional as benzo(a)pyrene (BaP) should be so effective in a biological system. It is a very potent carcinogen and is the principal source of lung cancer, for example, and is present in all organic combustion products. How does the BaP become a reactive material and what are the reaction products?

For the last fifteen years there has been a continuing worldwide study on the reactivity of benzo(a)pyrene. (This work has been done mostly in the United States and England, with some effort in France. However, the French study was dominated by the ideas of a theoretical organic chemist to the effect that the important place and reactive position of the BaP is the 4,5-double bond (K region), and the French researchers spent their time and effort trying to show that the reactive product had something to do with these two positions on the molecule.) The conviction of the activity of the K-region epoxide was not so widespread in England and the United States where people recognized that reactivity could be anywhere on this molecule.

The problem has ultimately been solved by examining the BaP metabolites in animals and in animal cells. There are many products of such a chemical carcinogen, and almost every position on the molecule has been oxidized during the course of the metabolism. Which reaction is the important one, or are they all side reactions which really are not important in relation to carcinogenesis?

This is a typical problem created by a mutagen because of the nature of biological systems which are not simple reproducible chemical systems. The dominant reaction products may have very little to do with the biological consequences of the molecule. It may very

be some trivial step, or side reaction, which is actually the crucial one for the biological consequences of carcinogenesis.

It appears that the 1,3- and 6-positions of the benzo(a)pyrene are the most active with respect to either oxidation reactions or photochemical reactions. Our first efforts in this area were some photochemical experiments using BaP as one of the chemical reagents with a nucleic acid component (N-methylcytosine) as the other reagent to see if the reaction occurred. It was known that when BaP was painted on the skin of an animal, it would produce skin cancer. It was also known that when illuminated it would produce an even more severe skin cancer. The effect of light on this type of a chemical carcinogen is shown in Figure 4, and the isolation of the product showed that the #5 position acted as an electrophile on the N-methylcytosine. This result also gave us a clue that it would be possible to activate the #6 position of BaP. We therefore proposed to activate the #6 position of BaP (or the #1 or #3 positions) by the conjugated bond system which lies between them. This was actually translating photoactivation into chemical activation.

It turned out, however, that this type of reaction is not really important. At various laboratories in the United States and England experiments were performed to extract the metabolites of BaP and test them to see if they are more potent chemical carcinogens than the original material. On the other hand, we have used BaP, activated it with aryl hydrocarbon hydroxylase (AHH) (an enzyme) to try and coupl the BaP with a nucleic acid, or nucleic acid analog, to deduce what has happened in the product. Both approaches have actually converged and have come to the same conclusions within the last year.

Aryl hydrocarbon hydroxylase (AHH) is an enzyme which will oxidize the hydrocarbon (BaP; for example) with a cofactor such as reduced pyridine nucleotide and oxygen; the AHH will epoxidize this hydrocarbon in various positions. All of the products of the reaction have been extracted, and it has been shown that this epoxide has been created by a mixed function oxidase which is present in low levels in most mammalian cells. If the mammalian cells are exposed to an aryl hydrocarbon, the enzyme is induced to much higher levels. The AHH is an iron enzyme, and a good deal of effort has been devoted to learning how the iron enzyme works. Many things can induce the AHH to raise its level in mammalian cells. One of the oxygens of the AHH is involved in making an epoxide and the other is involved in making water with a reducing agent. The epoxides can then undergo a hydration reaction which involves opening the epoxide with water to produce a diol (Figure 5). In general the reaction occurs as follows: A double bond on the AHH reacts with an oxygen atom to form an epoxide; the epoxide, with water, opens to form the diol. This particular sequence of reactions can be accomplished in most mammalian cells.

The AHH can act on all of the positions in the benzo(a)pyrene, but none of the derivatives (except the ones shown) turn out to be better carcinogens than the starting material; this is the crucial fact to remember. We have learned this only recently by using a combination of synthetic and enzymatic techniques.

The sequence of events in these transformations seem to be as follows: The AHH epoxidizes the 7,8-positions of the BaP (or the 9,10-positions). The 7,8-epoxide, upon hydration, gives a 7,8-dihydrodiol; a second epoxidation is even faster (with the first epoxidation it was necessary to epoxidize a partly aromatic double bond). The

The 9,10-double bond is no longer aromatic and it is very rapidly epoxidized (like styrene) and the 7,8-dihydrodiol does not build up. The 7,8-dihydrodiol-9,10-epoxide is the most potent carcinogenic derivative of BaP. In fact, it is important to consider the stereochemistry of that carcinogen. The one formed enzymatically is more carcinogenic.

This result, which was the combination of metabolic and carcinogenic studies, has focused our attention on the (a) ring as the crucial, active position in the benzo(a)pyrene molecule. However, it is still not known what this material is reacting with and how.

Many of the BaP derivatives have been synthesized in our laboratory, but we have not used many of them as stoichiometric reactants with known bits of DNA. We are in the process of doing that type of experiment at the moment. This is actually a combination of synthetic organic chemistry and biochemical enzymology. It is known that the diol is a trans-diol, but, more important is the geometric relation of the epoxide to the 7-hydroxyl. The 9,10-epoxide and the 6-hydroxyl derivatives of BaP are trans to each other, which is the most effective carcinogen.

Using AHH from induced rat livers and a variety of DNA analogs as substrates, we found, before we even treated the complex with the AHH, that only guanine-containing polymers would intercalate the BaP to any extent. Calf thymus DNA will intercalate very little; Poly(A) will not intercalate the BaP product at all; and the polymeric pyrimidines are not successful in this type of experiment. Poly(G) is the most successful polymeric material for this type of a reaction with chemical carcinogen, but this material is only a model. It will be necessary to use genuine DNA for the final consluvie result to this hypothesis.

The results of optical measurements on the model substances and the products of the reactions of diol epoxide and activated BaP with AHH and with Poly(G) and DNA are shown in Figures 6 and 7. The enzymatic hydrolysis of the BaP-Poly(G) product gives an absorption with two rather sharp peaks which are characteristic of 7,8,8,10-tetrahydro benzo(a)pyrene in which the benzene ring is completely hydrogenated, leaving a pyrene nucleus; the absorption spectrum is actually characteristic of pyrene. This was the first clue that the product activated BaP with Poly(G) was a reaction which had destroyed the 7,8- and 9,10-double bonds of the BaP but had left the pyrene aromatic nucleus intact. There are six positions which apparently were not touched.

The fluorescence spectrum of the product was more critical, showing the fact that the chemically hydrolyzed products were similar. The emission spectra of a tetrahydrobenzpyrene is about the same, but has a different relative intensity. This difference is an important component in gathering our information about the ultimate product. The emission band (380) is less intense than the second (400), whereas in a simple tetrahydrobenzpyrene (7,8,9,10-tetrahydrobenzpyrene) the first band is always the most intense in fluorescence. There is one model case, however, in which the first fluorescence (emission) band is less intense than the second, and this occurs with 10-hydroxy-tetrahydrobenzpyrene. In the 10-position there is a substitution other than hydrogen. This example is the only one which shows the first fluorescence emission less intense than the others. That fact indicates that there will ultimately be a tetrahydrobenzpyrene product, but it will also have a substitutent on the #10 position, probably bearing an unshared electron pair.

The fluorescence information tells us that the guanine is on the #9 or #10-position, with two hydroxyls on the 7- and 8-positions of the BaP ring. When you examine the structure of the 7,8-diol-9,10-epoxide of BaP, you can begin to surmise what the reaction product really is. When the 7,8-diol-9,10-epoxide reacts with the guanine of the Poly(G), the obvious place for the epoxide to open is such as to put the carbonium conjugate with the pyrene. This is a stable carbonium ion and we then have an electrophilic reagent. So far, the only model we have is that of the 8-position of the guanine asthe electron-rich position, as evidenced by its reactions with acetylaminofluorene, shown earlier. I have therefore surmised that one pssible product of the reaction between the activated and intercalated benzo(a)pyrene is an electrophilic attack on the #8 carbon atom of guanine. We could then remove the C-8 proton to the epoxy-oxygen, insert the double bond again, with the resulting product being 10-(guanyl)-7,8,9-trihydroxytetrahydrobenzo(a)-pyrene. Alternatively, we could ring-close to give a ring-closed product, because when we treat the ultimate reaction product with toluene sulfonic acid, we do not find a new double bond. If there were a hydroxyl at C-9 and a hydrogen at C-10, we should get a double bond upon treatment with toluene sulfonic acid. An alternative point of C-10 carbonium ion attack would be on the exocyclic nitrogen atom of the guanine, giving a simple C-10 N-substituted C-9 hydroxylated 7,8,9,10-tetra-hydrobenzpyrene derivative.

BIOLOGICAL CONSEQUENCES OF CHEMICAL CARCINOGENESIS

It is now necessary to introduce the concepts that are current in the chemical and viral cancer community in discussing the biological consequences of chemical carcinogenesis. The process of viral transformation is shown diagrammatically in Figure 8. Here the black spots represent pieces of DNA containing information which, when integrated into the chromosomal DNA of the cell, will transform it into a tumor cell. If the virus simply infects, the replication of the virus lyses the cell, and the cell is not transformed. However, some part of the viral genome is integrated into the cellular genome, and the cell can be transformed into a tumor cell. If a cell is transformed, such cells overgrow each other, creating foci (individual cells piled on top of each other). Cell transformation by virus can be assayed by focus-formation, indicating the degree of viral transformation of the original cell culture.

The insertion of the oncogenic information, or the whole viral genome (or some crucial part) which contains the oncogenic information into the chromosomal DNA, occurs through a mechanism as yet unknown. The scheme for cell transformation, including chemical function, is shown in Figure 9. Here it is seen a DNA virus gets inside the cell through the function of various nucleotide hydrolyzing enzymes (endo-nucleases, exonucleases, ligases) which can insert bits of DNA into the chromosomal DNA. The result is chromosomal DNA containing some combination of the viral DNA which gives rise to the transformed cell. (For an RNA virus it is necessary to go through a special enzyme, RNA-dependent DNA polymerase (RDP) to make a DNA copy of that RNA

virus and insert that. Both viruses act by inserting bits of information representing the viral oncogenic information into the chromosome of the cell.)

If we had an RNA virus and it had to go through RDP to get to DNA to be inserted and transformed, and if we could specifically block thie enzyme, we could then prevent cell transformation by that virus. This experiment was done several years ago because there was a drug available which would inhibit that particular reverse transcriptase enzyme. We were able to do thatparticular type of experiment, using synthetic modifications of the drug, rifamycin (the chemical formulas of which are given in Figure 10), and information concerning the effect of these drugs on cell transformation is given in Table 1.

How is this work related to chemical carcinogenesis? I had the idea that the chemical triggers integration of some endogenous information which is in the cell and which is not being expressed. In order to test this hypothesis, we tried to find a system where the chemical alone appears to produce a tumor. We knew, for example, that there were certain strains of rats where a single injection of a chemical carcinogen such as dimethylbenzanthracene (DMBA) produced mammary tumors in nine weeks, killing all the animals; this result occurred each time the experiment was performed. I felt that this resulted indicated that this particular strain of animals carries endogenous oncogenic information which is triggered by the chemical carcinogen. If it was an RNA virus, the rifamycin should prevent or slow down the carcinogenesis, which actually is the case. The results of an experiment of this type are shown in Figure 11.

The converse of this former experiment has been done recently using a strain of rats which does not produce a tumor at all with the carcinogen alone. However, if these rats are given an adenovirus at a suitable time before administration of the chemical carcinogen, then there is tumor formation. Adenovirus alone has a certain rate of tumor production, the chemical alone produces no tumors, but the chemical carcinogen with the adenovirus is much more than additive. This experiment is the opposite of the first type where the animal itself carried the oncogenic information which was not being expressed, presumably in the form of extrachromosomal information or in the form of a putative virus. In the experiments which I have just described, there is a strain of animal which does not respond to the chemical treatment unless it also receives the viral infection.

These same types of experiments can be done in tissue culture where the results are somewhat more reliable than the information obtained from whole animal experiments. The viral transformation of hamster cells following treatment by a chemical carcinogen (4-nitroquinoline oxide, 4-NQNO) has been studied, as an example. The adenovirus alone without the chemical treatment with 4-NQNO produces very few foci; with 4-NQNO treatment and virus, the number of foci increases; eventually the chemical effect on the repair mechanism of the cell is over, and the excess integration ceases. This results indicates a synergism between the chemical and viral transformation, as indicated in Table 2.

How can this synergism be understood? The chemical starts a process of manipulation of the cellular DNA to repair ot; the repair enzymes are operating to replace the defective DNA. If there is

a source of misinformation (oncogenic information) in the cell at the same time, there is a certain probability that the oncogenic information will be integrated into the cellular genome during that repair operation. It turns out that there are many different types of nucleic acid repair enzymes, each one specific for a different type of error, and each one, in some cases, for different base sequence. It is only relatively recently that the variety of clipping enzymes (endonucleases and exonucleases) have become visible in reactions of this type.

I want to suggest that the chemical (carcinogen) produces a distortion of some kind in the DNA which, as a result of that distortion, is subject to the attempt to replace the distorted piece by the variety of repair and replication enzymes which arepresent. If oncogenic information is present, it has a probability to be inserted. The chemical thus enhances the probability of insertion of oncogenic information. The chemical alone is a mutagen, but is not a carcinogen by itself. The carcinogenic result is due to the presence of some other piece of information which the chemical triggers to insert into the cell.

These speculations which I have just discussed are shown diagrammatically in Figure 12. The chemical carcinogen puts a "kink" of some kind in the DNA to start the whole process, leading to an accelerated probability of insertion of the oncogenic information which is there from some other source. You have heard comments to the effect that the test for mutagenicity is a good enough test for carcinogenicity as well, but I do not believe this is the case. All carcinogens are mutagens to be sure, but all mutagens may not be carcinogens because the carcinogenic information is not there. Mutagenesis generally leads to a lethal event. I believe that a single point mutation, which is what the chemicals can induce, cannot introduce enough information to lead to transformation.

The introduction of that much information really means the introduction of a large piece of DNA, or the removal of a large piece of DNA.

Using what we know about the insertion of known bits of DNA which can be done in the test tube (I am presuming that the same kinds of events go on in the cell) from one species of DNA, we can insert them into another species of DNA using suitable clipping enzymes (exonucleases, endonucleases, etc.). When the chemical carcinogen forms a covalent complex with guanine, the double helix must be completely destroyed in that vicinity, warping the geometry of the molecule. The benzo(a)pyrene has been completely intercalated, and when covalent linkages are formed and the guanine is pushed out ofthe double helix, the helical structure is distorted around the point where the carcinogen has formed the covalent link with a suitable base. The distortion will be recognized by a variety of enzymes which must act upon it by some mechanism. The specificity of those enzymes is not yet known. It is also not known whether the enzymes must be induced, or whether the enzymes are always present, and a clonal selection process operates similar to that in which the antigen induces new cells to make a particular antibody.

In any case, some event occurs which leads to the beginning of the removal of this distortion by a restriction enzyme. This may lead to insertion, leaving a gap behind with sticky ends (characteristic pieces of DNA information). The same clipping enzyme (endonuclease, etc.) acting on exogenous information, or at least information which is not chromosomal in the cell, will produce other such sites with complementary ends. It can thus produce a piece from an oncogenic virus with corresponding sticky ends which, in turn, can fill the gap. The breaks can be "sewn up" with the ligase, resulting in the transformed DNA with the oncogenic information inserted. This is not a real "repair" mechanism, at least it is not conceived of in that fashion. How the actual

repair mechanism may work in this case, I do not know. The diagram gives only one concrete example of how information which is not in the chromosome could be taken and inserted into the chromosomal DNA as a result of a chemical action by a particular type of a chemical carcinogen or chemical mutagen.

Most recently in our efforts to define and measure the differences between the normal and transformed cell, we have explored a new method of probing the characteristics of the cell surface, the most easily accessible part of the cell for possible treatment. This new method has not only yielded information on the differences between normal and transformed cells, but also seems very likely to provide a much better, simpler and more quantitative method for measuring the rate of appearance of transformed cells in a cell population.

It is clear that these two objectives -- (a) to define the differences between the normal and transformed cell surface and (b) to be able to measure the kinetics of transformation using the degree of that difference -- are both extremely useful and important. The latter one will, of course, allow us to explore more readily that synergism for which we have only the focus assay to guide us.

The method basically depends upon the availability of a chemical reagent which will react rather specifically with free amino groups, either terminal ends of proteins or other biological molecules, to form a fluorescent product. Neither the reagent itself nor its hydrolysis product is fluorescent. The reagent is not transported across the cell membrane in a reactive form. Therefore, when the cell is treated with this reagent, only the cell surface amino groups become fluorescently visible.

The fluorescent reagent and its reaction are shown in Figure 13, and the results of treating normal and transformed cells with this reagent are shown in Figure 14. Here it is clear that the available amino groups on the cell surface for labeling are somehow reduced by transformation. If the cell membrane is broken and the entire protein population of the cell (both internal and external) is allowed to make contact with the reagent, the difference between the two cell populations is very little, which is shown in Figure 15.

An attempt to determine the nature of the particular proteins which have been deleted from the cell surface upon transformation is shown in the electrophoretogram of Figure 16. One of the potentially fluorescent proteins which is absent from the transformed cell membrane is clearly shown as the missing band in this figure. It seems very likely that this missing protein in the transformed cell membrane is identical with the large external transformation sensitive (LETS) protein which has been described by other methods and whose detailed character and function are yet to be determined.

Finally, we have used two fluorescent stains on the same group of cells. The first is propidium iodide which enters the cell and stains the nucleic acid, the fluorescence intensity of which is a measure of the amount of nucleic acid present in the cell. The other stain, fluorescamine, is used to labeled the cell surface. These doubly labeled fluorescent cells are shown in Figure 17, and with such doubly labeled cells and a flow microfluorometer (an instrument for measuring the fluorescence intensity for individual cells) which can be set to measure at least two different colors of fluorescence, we have been able to distinguish very clearly a population of normal cells from

that of a population of transformed cells. The data showing this capability are exhibited in Figure 18 in which particular populations of each of the cell types are shown in the top two panels. It is clear that the ratio of surface fluorescence to nucleic acid fluorescence is very much smaller for the transformed cells than for the normal cells. From our analysis, the population shown in the various panels of Figure 18 contain transformed cells as follows: (a) 4.5% transformed cells; (b) 34.6% transformed cells; and (c) 82.6% transformed cells.

With this information, we are now prepared not only to explore the nature of this characteristic surface protein which is absent from the transformed cells but also to use this quantitative characteristic of a cell population to measure the carcinogenicity of chemicals by a quantitative determination of their synergisms with suitably transforming virus in a selected cell population.

As this work proceeds, the more precise relationship between a chemical's ability to induce mutations and its ability to induce malignancy can be defined. In fact, it is very likely that this kind of assay for carcinogens will be the quickest and most relevant one for examining chemicals in our environment.

If our synergistic proposal withstands these tests, the obvious confirmatory experiment will be to demonstrate the integration of the oncogenic information from some external viral source induced by the chemical. Plans are underway to accomplish this.

FIGURE CAPTIONS

Figure 1 Electrophilic nature of chemical carcinogens

Figure 2 Products from activated acetylaminofluorene (AFF) and guanosine

Figure 3 Structure of benzo(a)pyrene and benzo(e)pyrene

Figure 4 Photochemical coupling of benzo(a)pyrene to N-methylcytosine

Figure 5 Benzo(a)pyrene activation by microsomal oxidases

Figure 6 Emission spectra of hydrolyzed benzo(a)pyrene-Poly(G)

Figure 7 Fluorescence spectra of benzo(a)pyrene covalently linked to nucleic acid

Figure 8 Transforming infection with SV-40 virus

Figure 9 Scheme for cell transformation, including chemical function

Figure 10 Some synthetic modifications of rifamycin

Figure 11 Prophylactic effect of dimethylbenzyldesmethyl rifampicin against dimethylbenzanthracene and trimethylbenzanthracene in rats

Figure 12 Schematic proposal for collaboration of chemical carcinogen and oncogenic information (Parts I and II)

Figure 13 Reaction of fluorescamine with primary amines

Figure 14 Fluorescamine labeling of intact cells

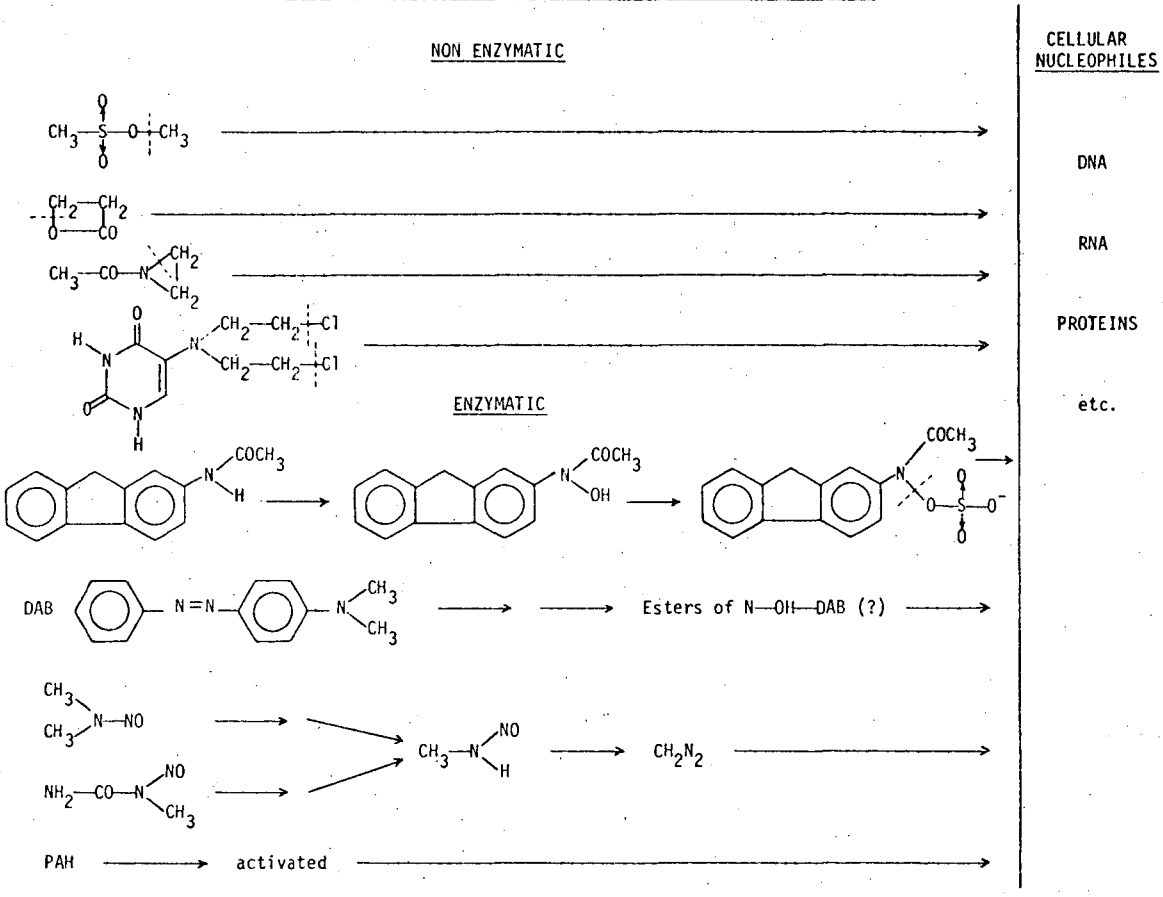
Figure 15 Fluorescamine hbeling of cell sonicates

Figure 16 Electrophoretogram of proteins deleted from cell surface upon transformation

Figure 17 Doubly labeled Balb/3T3 cells (labeled with propidium iodide and fluorescamine)

Figure 18 Detection of transformed cells using fluorescent probes on MSV-infected Balb/3T3 cells

ELECTROPHILIC NATURE OF THE ULTIMATE CHEMICAL CARCINOGENS



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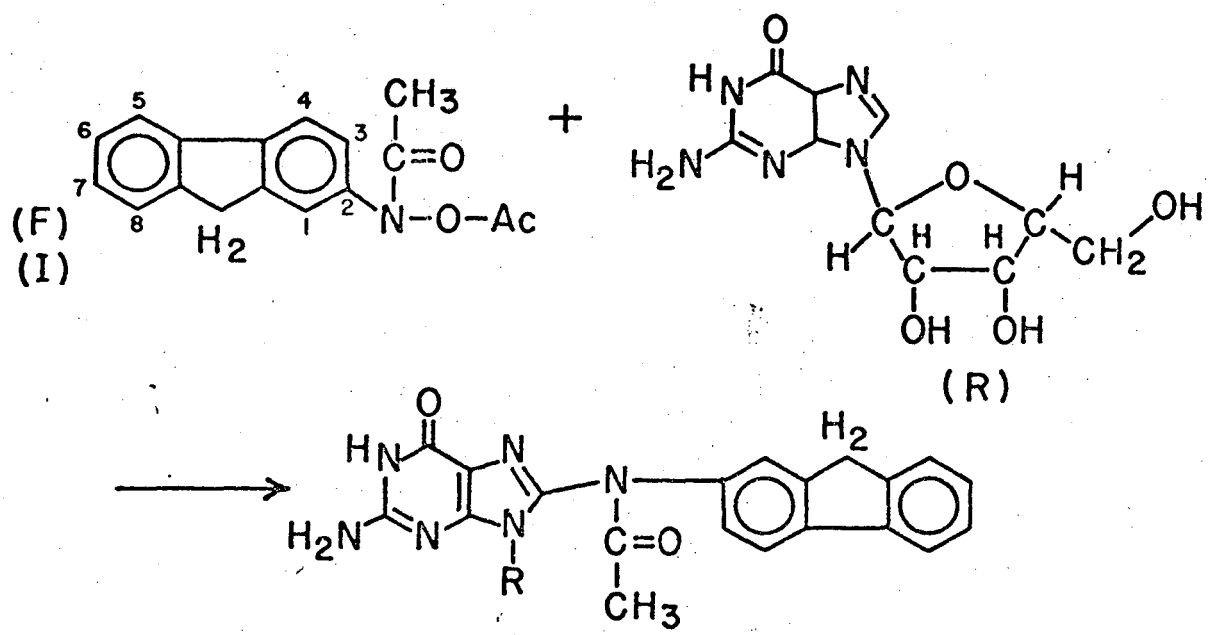

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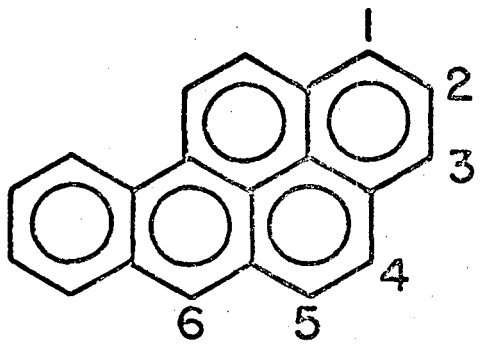

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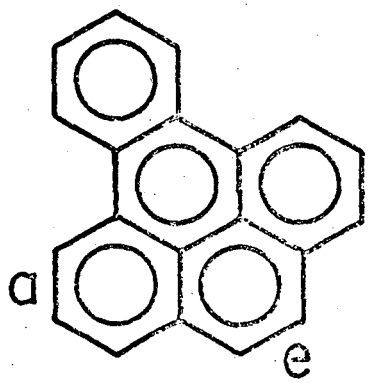

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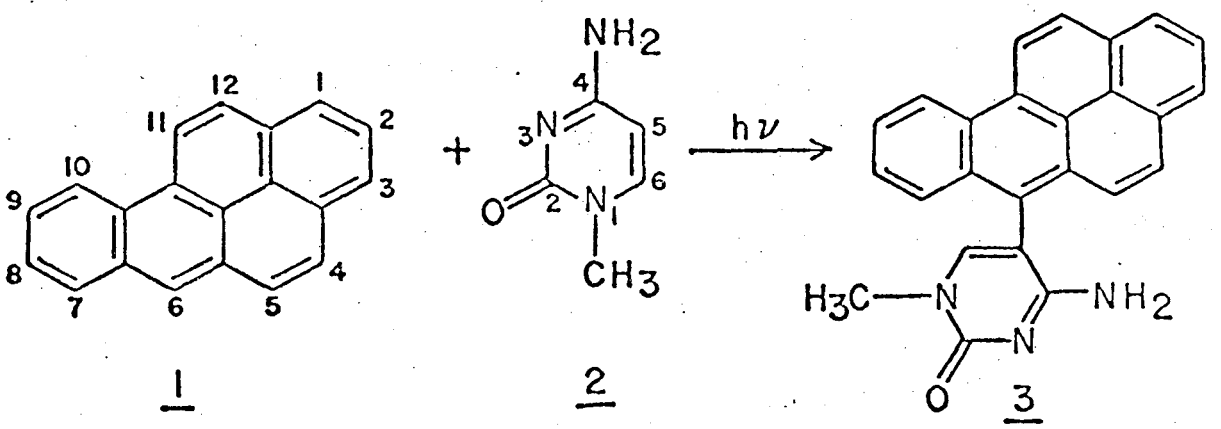
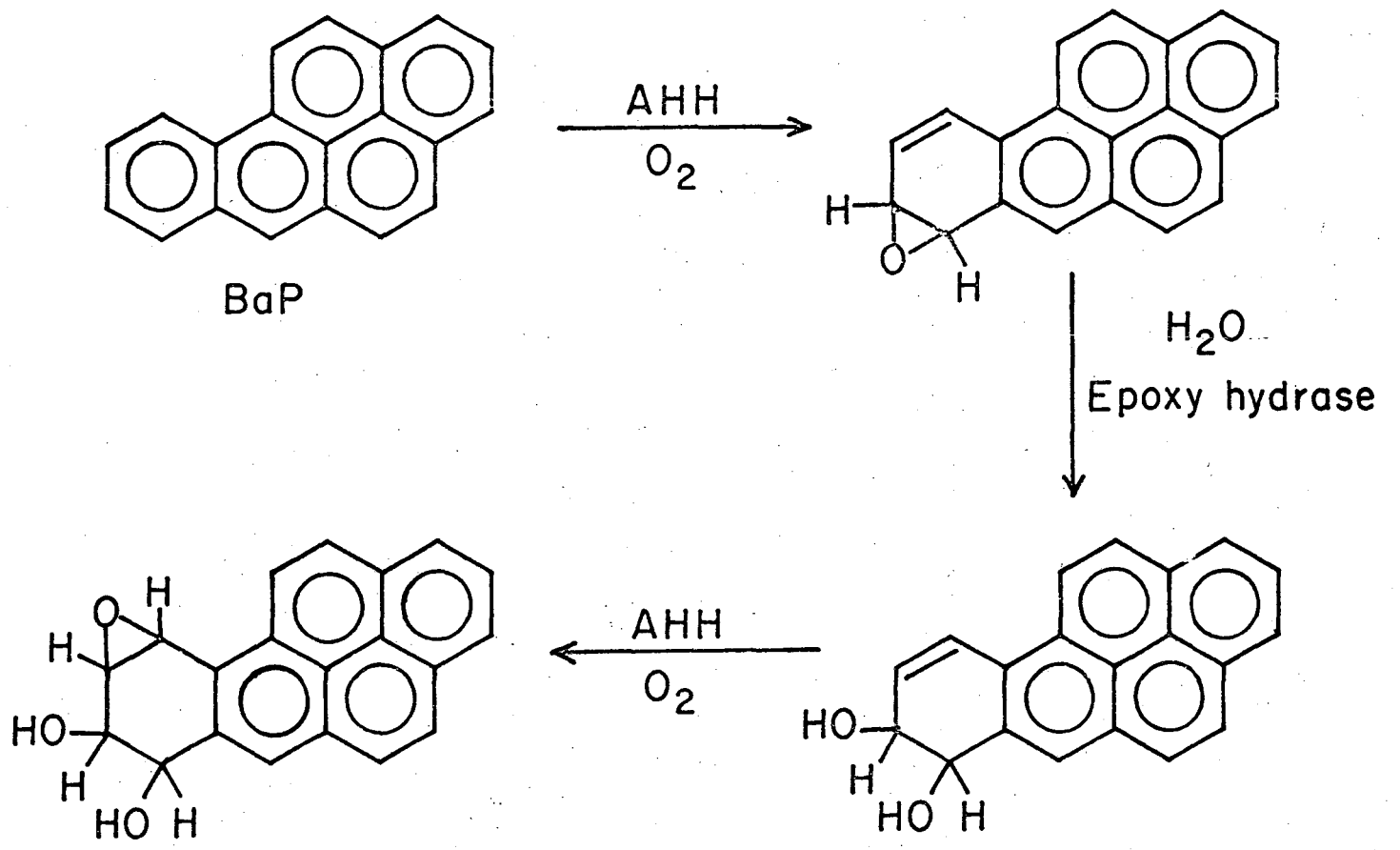
Non-Carcinogenic

XBL 716-5198

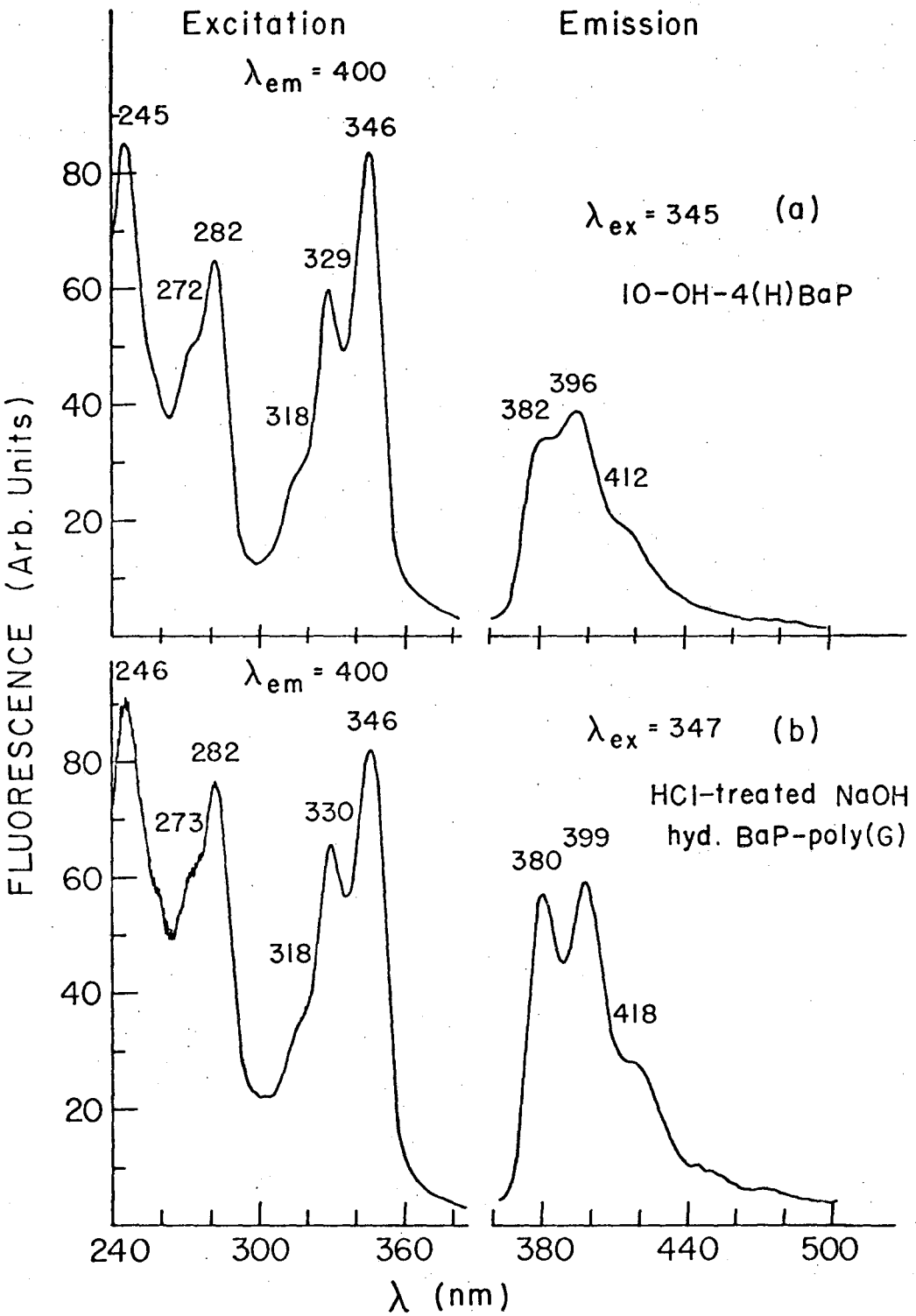


XBL 716-5238

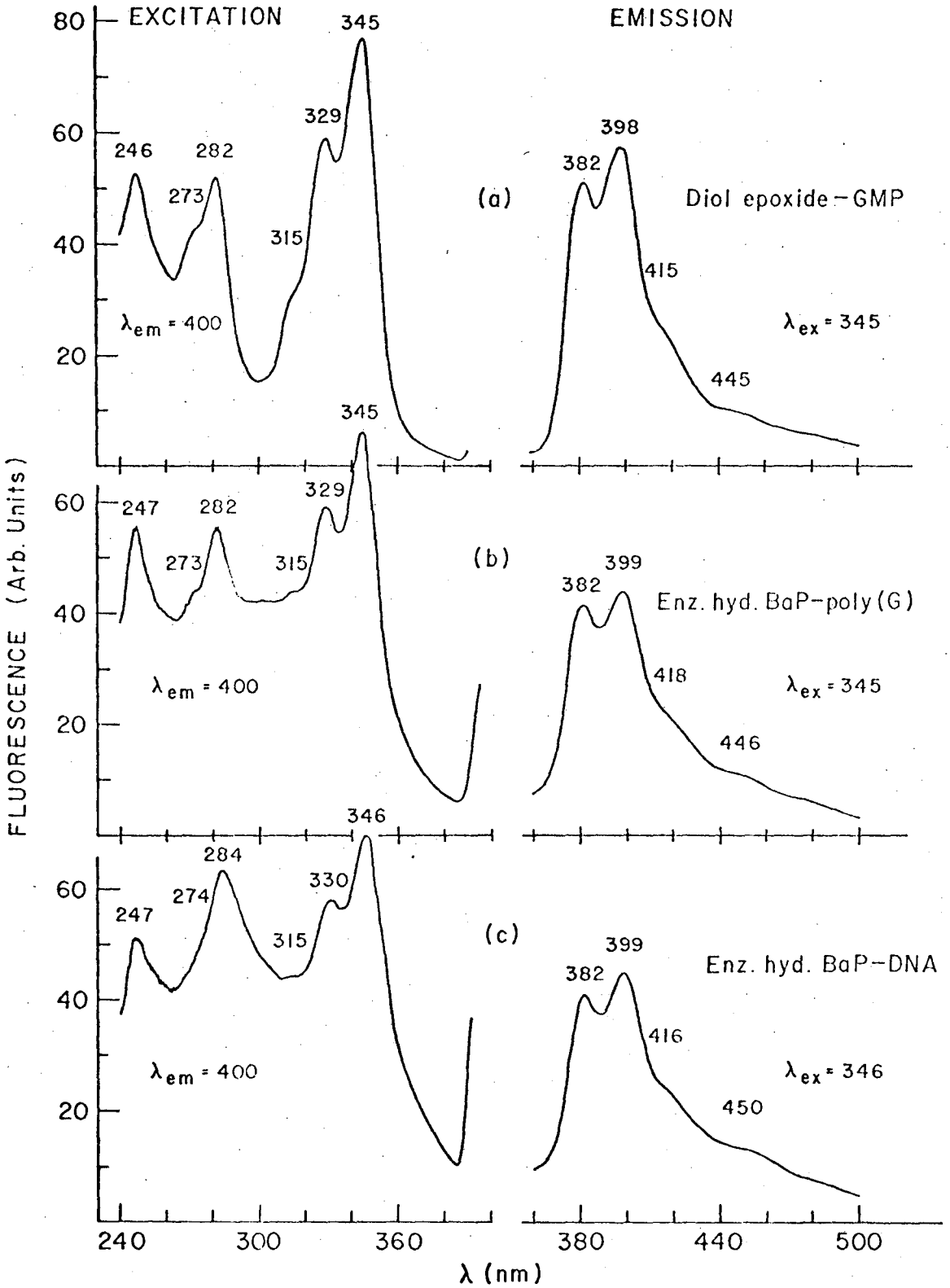
Photochemical coupling of BaP to N-methylcytosine



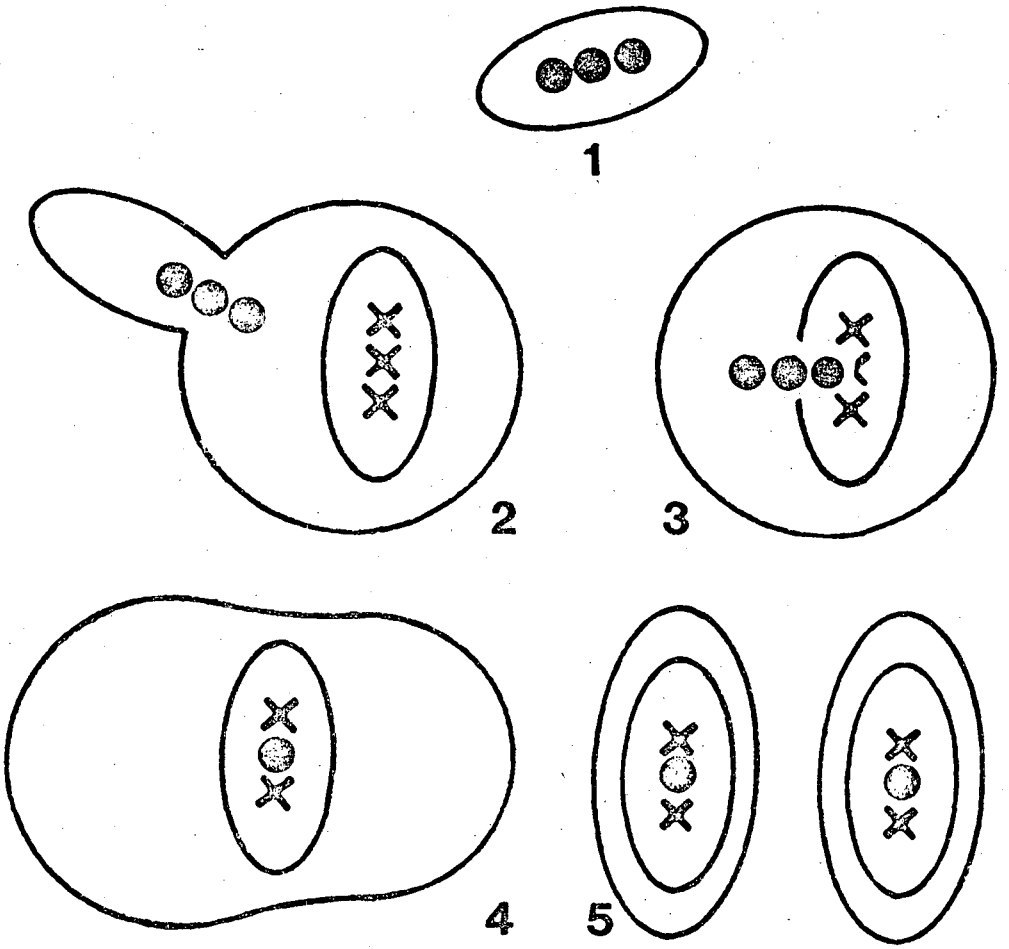
XBL762-5662



XBL7512-8789 A

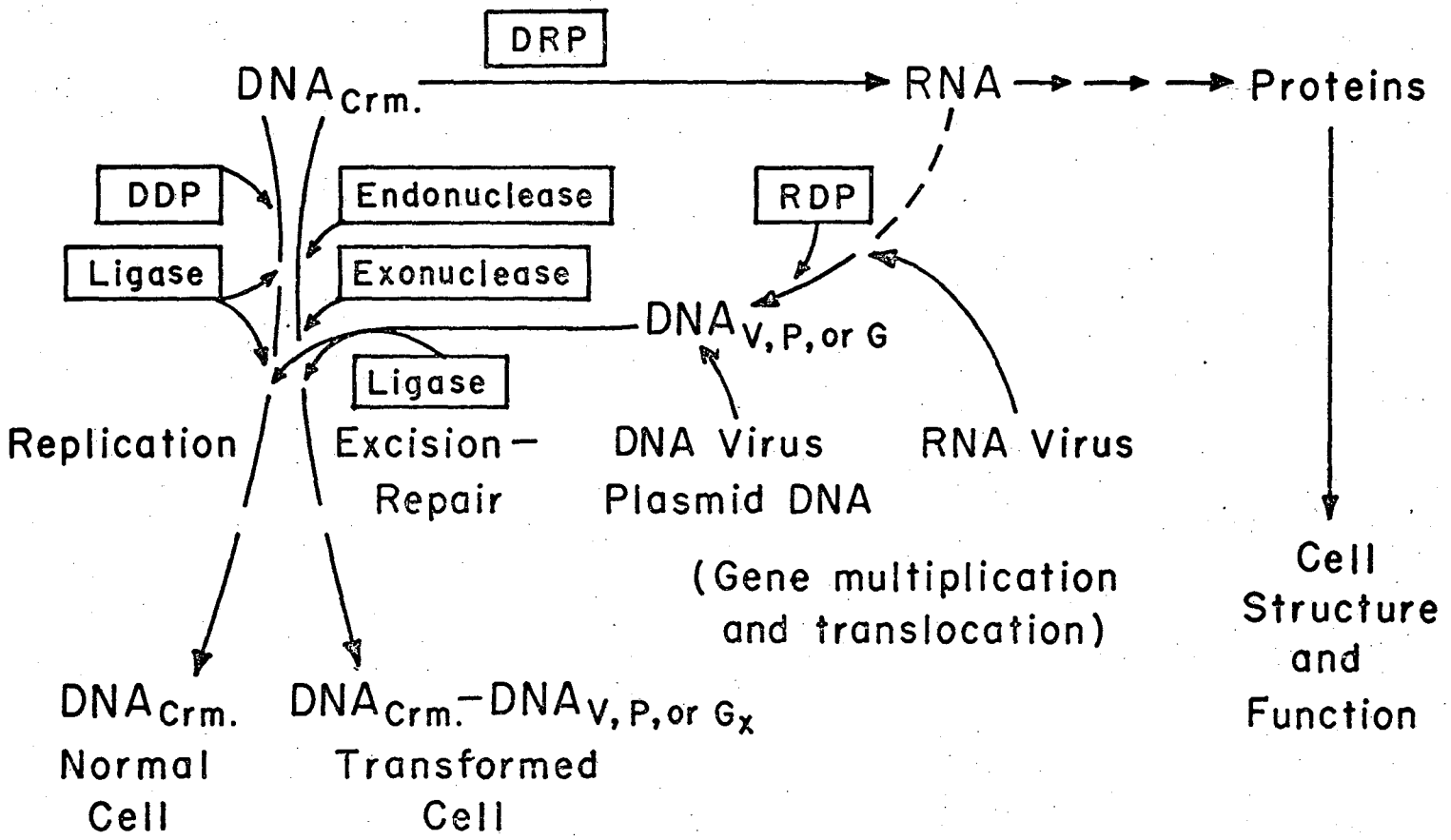

EXCITATION
EMISSION
(a)
Diol epoxide–GMP
(b)
Enz. hyd. BaP–poly(G)
(c)
Enz. hyd. BaP–DNA
λ_{em} = 400
λ_{ex} = 345
λ_{ex} = 346
FLUORESCENCE (Arb. Units)
λ (nm)
246
273
282
315
329
345
382
398
415
445
247
399
418
446
284
274
330
416
450
240
300
360
380
440
500
20
40
60
80

XBL 765-5925

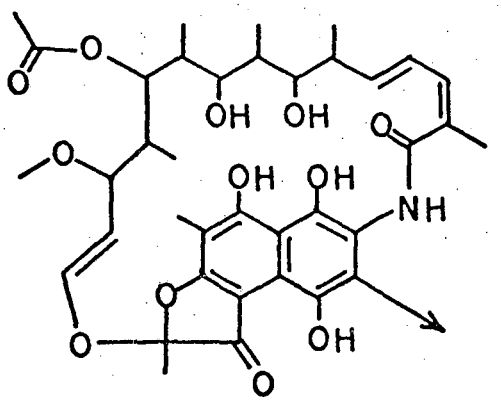


Transforming infection with,
for example, SV40 virus.

XBL7311-4914

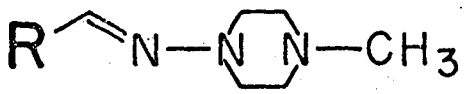


XBL736-4835

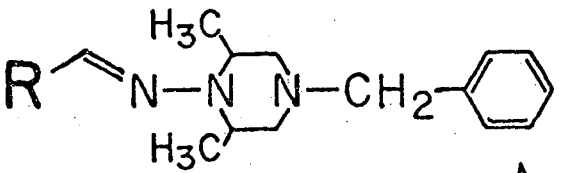


= R

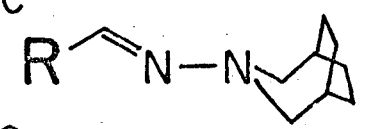
Rifampicin



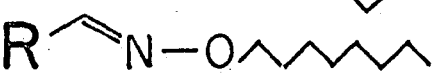
DMB



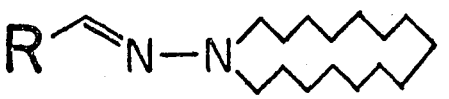
Rifazabicyclo-9



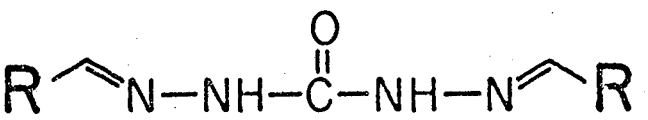
Rifoctyloxime



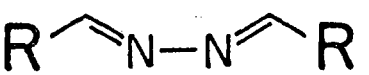
Rifazacyclo-16



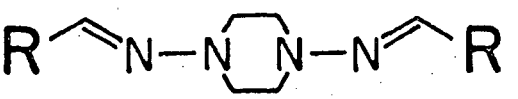
Rifurea



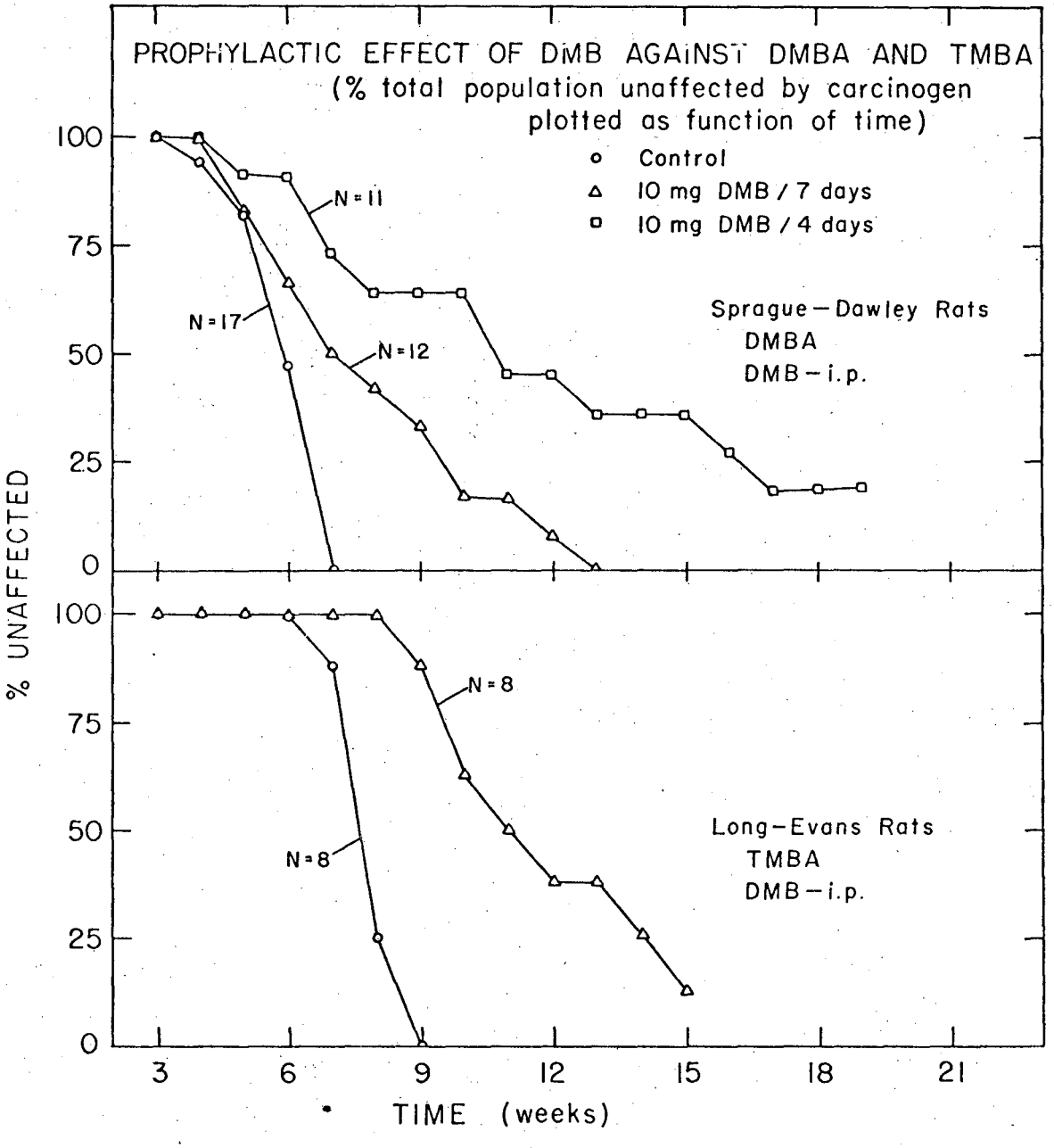
Rifamazine



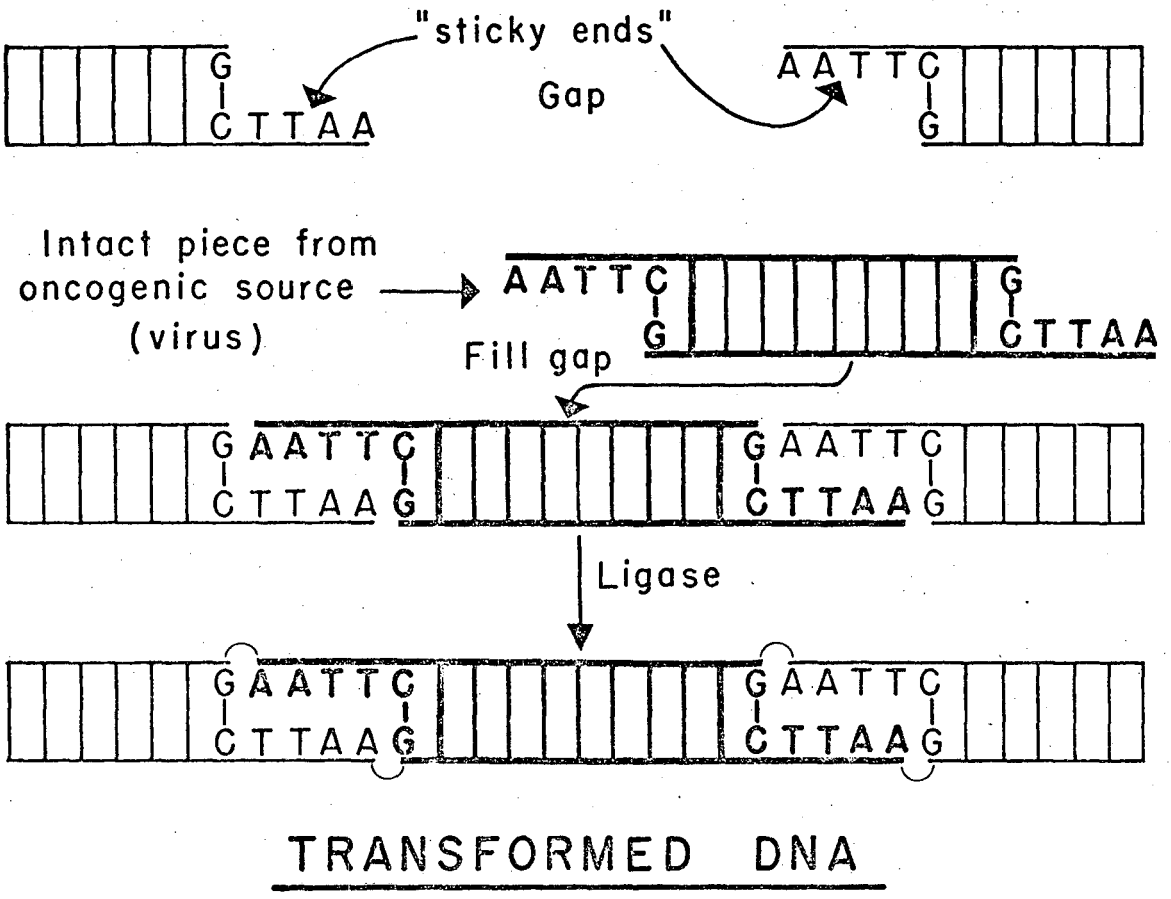
Dirifampin



XBL729-4774 A



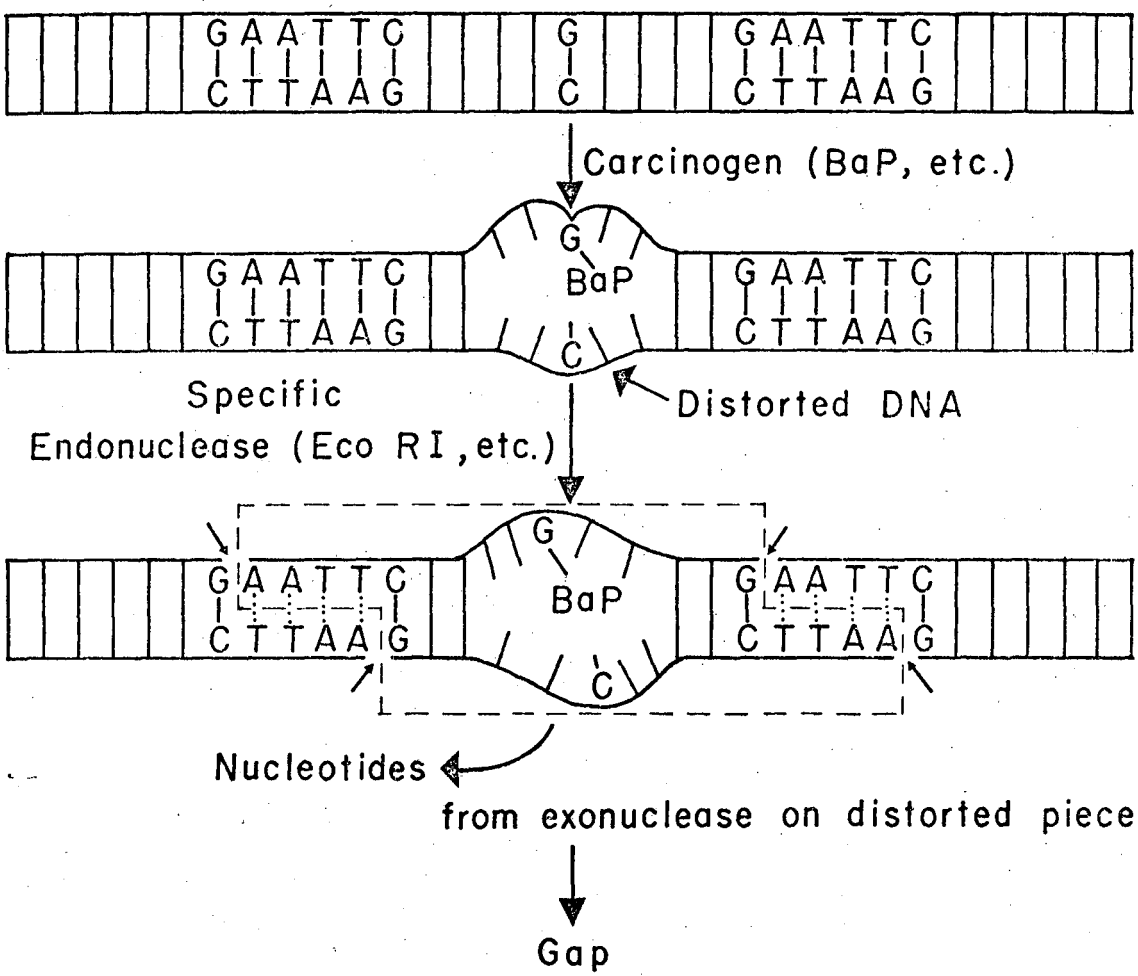
XBL728-4738A



TRANSFORMED DNA

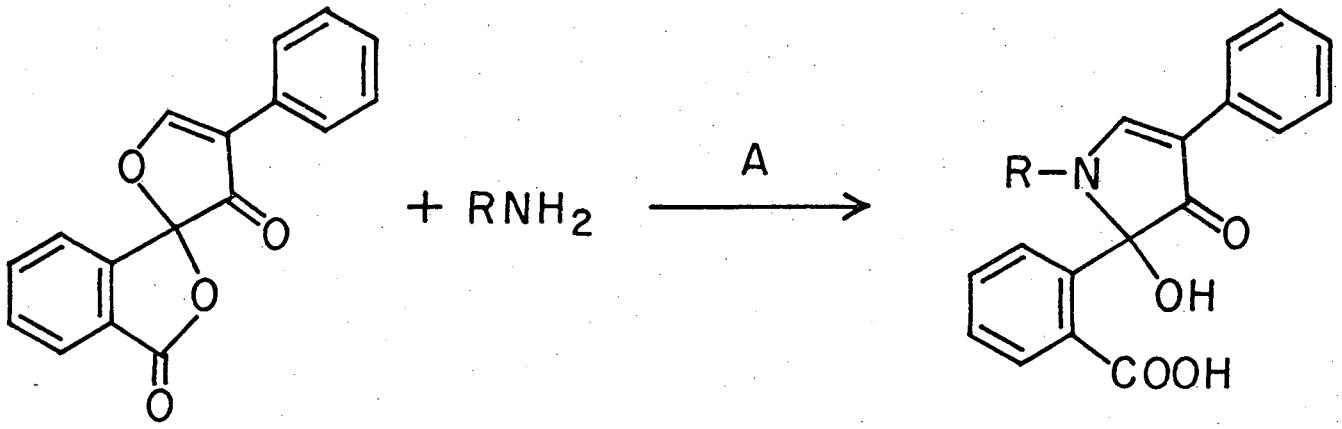
XBL762-5663

CARCINOGEN DNA INTERACTION



XBL762-5663A

REACTION OF FLUORESCAMINE WITH PRIMARY AMINES AND CONCOMITANT HYDROLYSIS OF REAGENT



Fluorescamine
(Non-fluorescent)

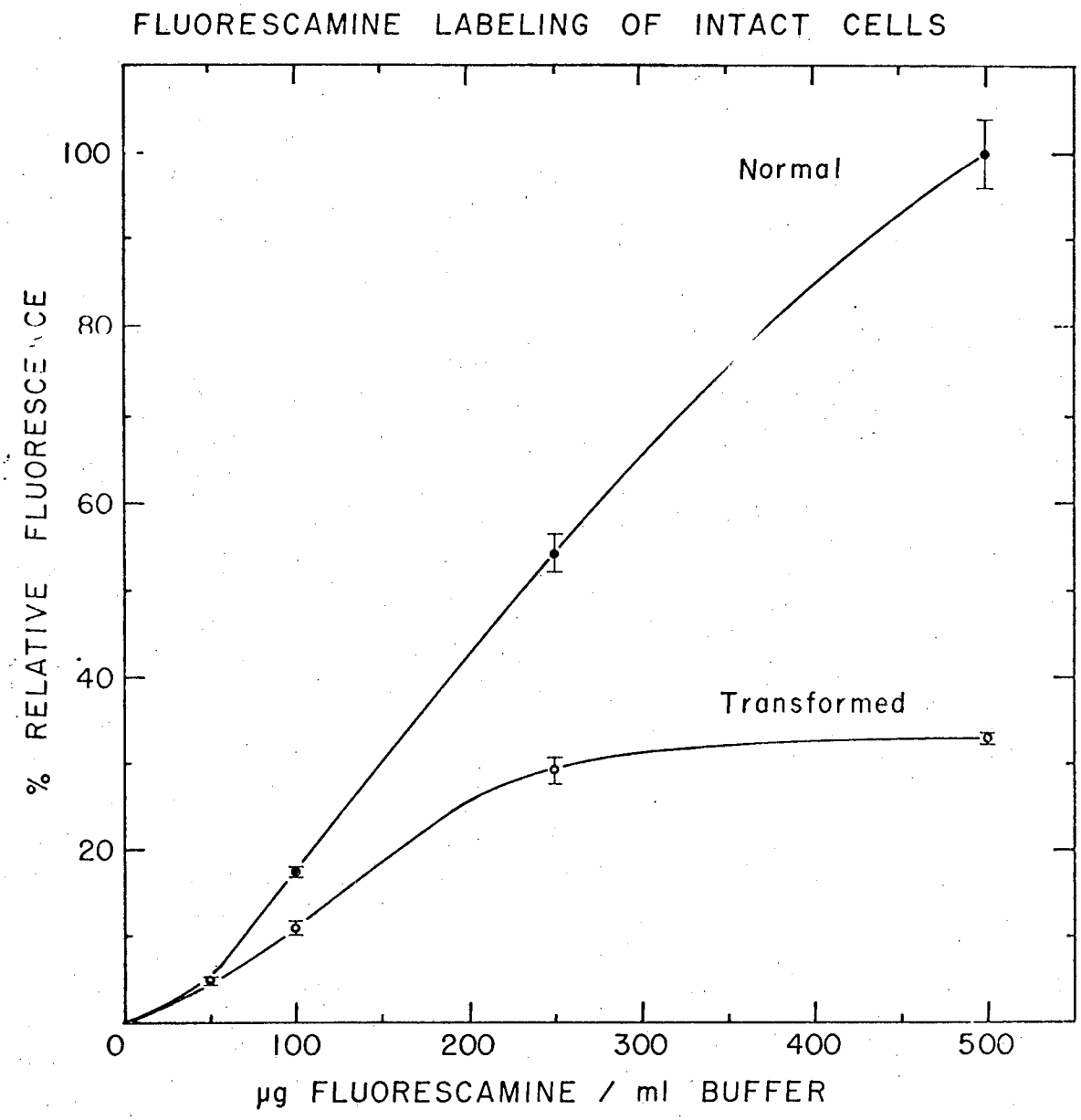
Fluorophor

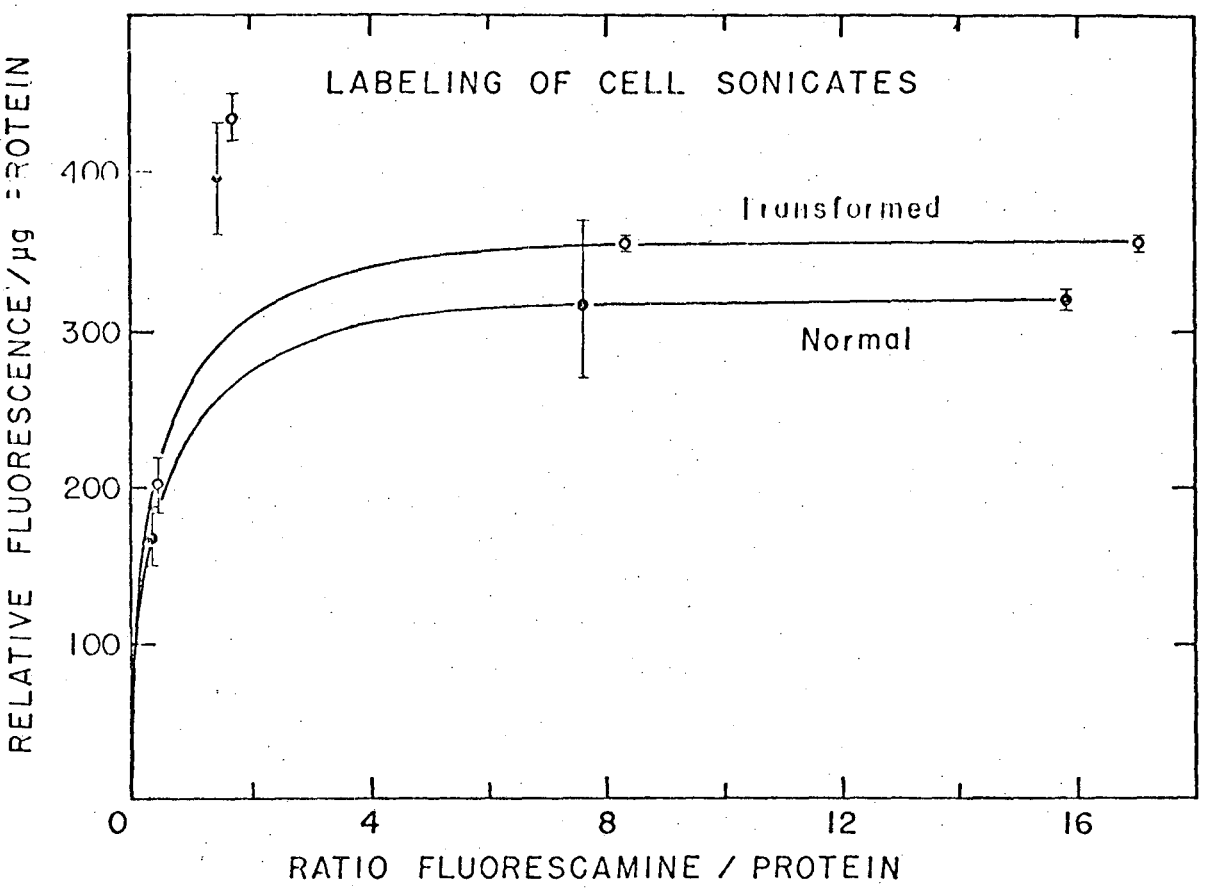
Fluorescamine + H_2O —B→ Non-fluorescent hydrolysis products

A $t_{1/2}$ 100–500 msec

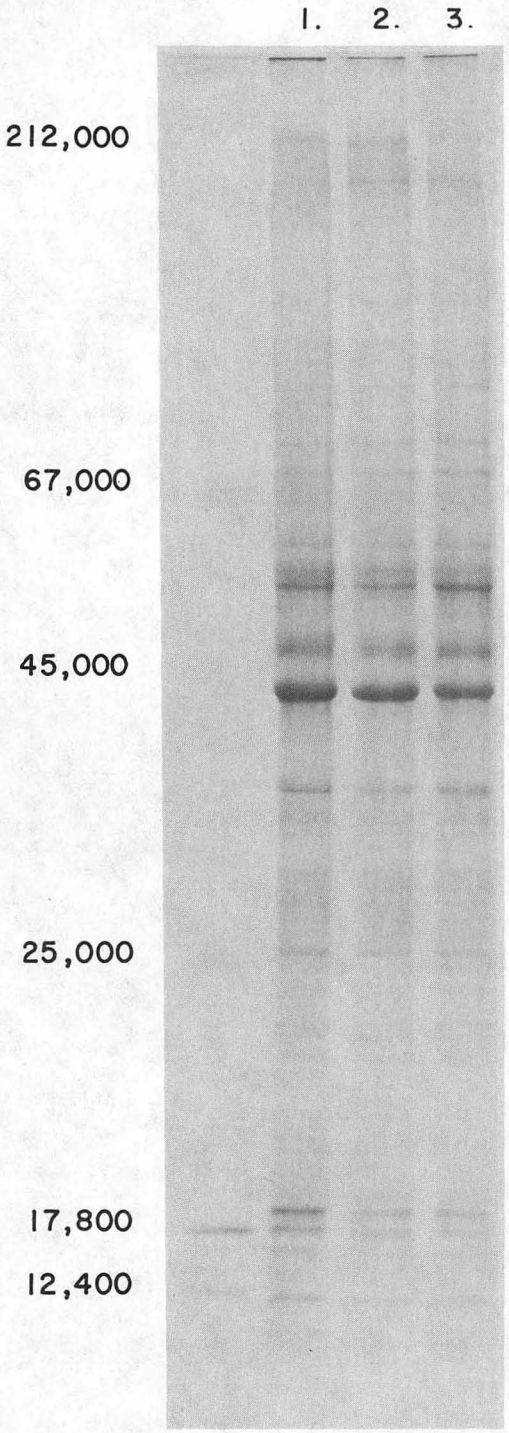
B $t_{1/2}$ 5–10 sec

XBL7510-8732


FLUORESCAMINE LABELING OF INTACT CELLS
% RELATIVE FLUORESCENCE
100
80
60
40
20
0
100
200
300
400
500
μg FLUORESCAMINE / ml BUFFER
Normal
Transformed
XBL7510-8725



XBL7510-8735



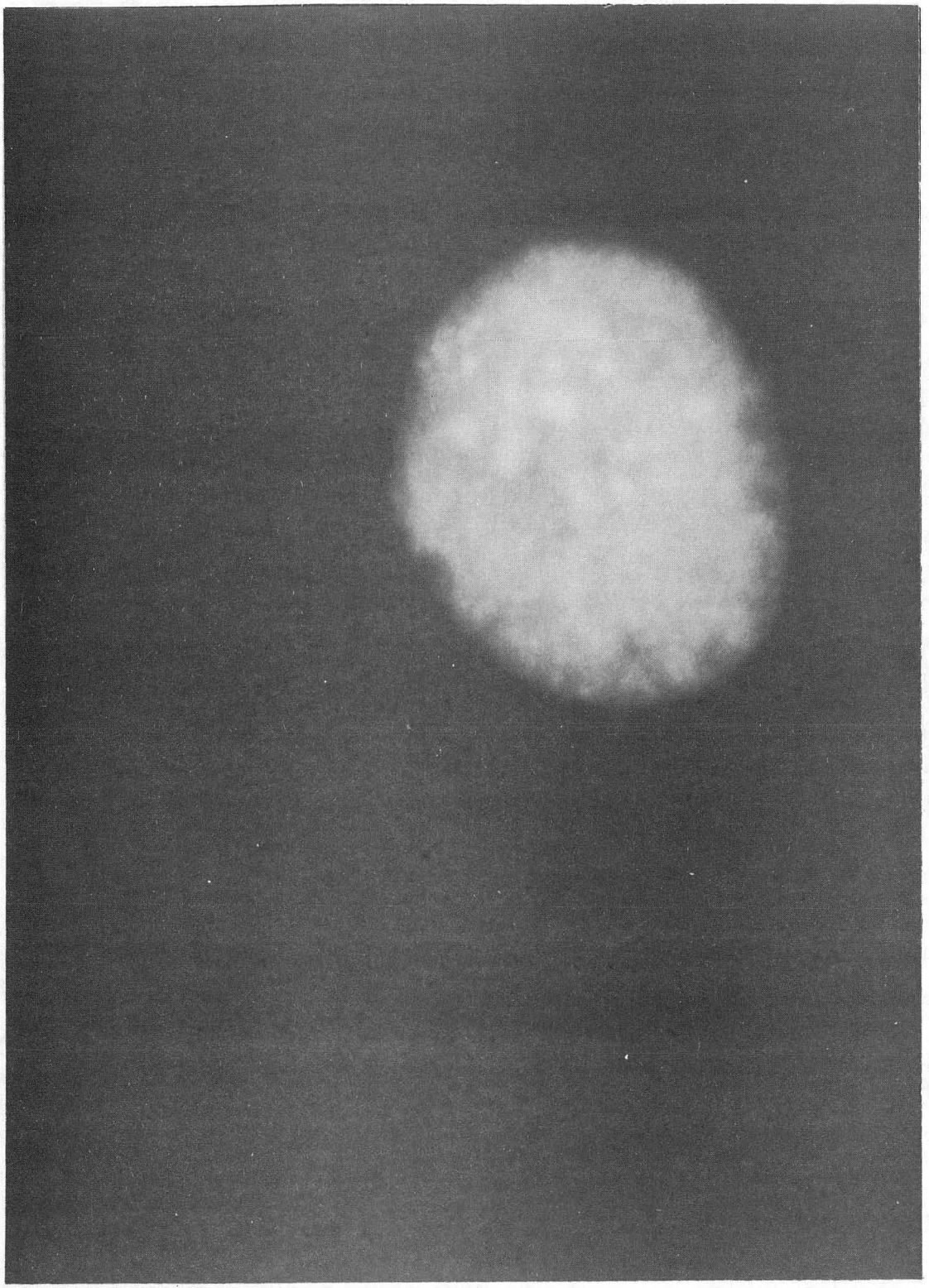
1. Normal chick embryo fibroblasts (CEF)

2. Fast growing CEF

3. Transformed CEF

10-20% Acrylamide gel

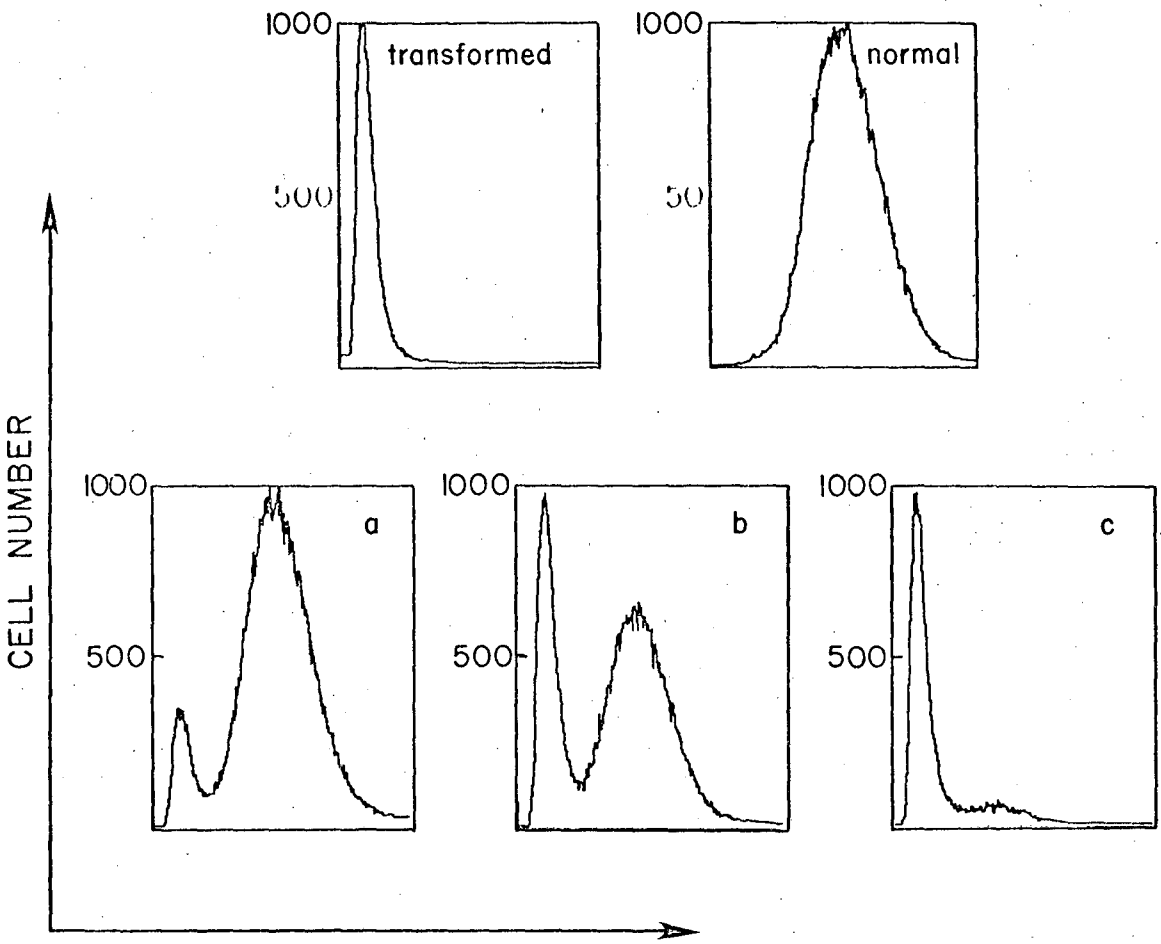
XBB 7510-7804



CBB 768-7699

Fig. 17

DETECTION OF TRANSFORMED CELLS USING FLUORESCENT PROBES ON MSV INFECTED BALB 3T3



XBL 768-9533

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