CHAPTER 11

# PROBLEMS IN CANCER

NEW INSIGHTS INTO many pathological problems—and those of cancer in particular—are offered by the concepts we have been discussing. Let us take, for instance, the problem of just what cancer is. Classically, one is entitled to speak of a condition as cancerous when cells with cancerous character are present in the body. Whether, on the one hand, only cancer-in-situ cells are identified or, on the other hand, the patient is dying and has almost no organ or function left untouched—the condition is considered cancerous. Yet, so long as the concept of cancer is associated implicitly with the concept of malignancy, to consider clinically healthy individuals to be cancerous only because of the presence of cells with cancerous nuclear characters, when most of them will never show further development of the disease, is entirely confusing. It is essential to separate the two concepts, the presence of cancerous entities and actual malignancy.

The fact that the hierarchic levels of the organism participate in the various manifestations of cancer puts the problem in its true light. A cancerous condition does not implicitly mean malignancy when it involves only the presence of an entity with cancerous character. Other attributes must be considered. In the hierarchic progression of cancer, malignancy begins to be manifested when the cellular level participates and induces invasive cancer. With malignancy an attribute of only some of its phases, cancer can be seen to embrace many changes, beginning with those of the lowest hierarchic entities and terminating with the systemic lethal condition.

The plurality of phases of cancer, with the broad variations in time and other factors which determine the passage from one phase to the other, logically raises several immediate questions.

In view of the multiple phases, one cannot speak of pathogenesis of cancer in general, but rather of pathogenesis of the different phases. Con-



sequently, even postulating the existence of some specific original cancerous change, such a change would not, alone, induce the entire disease and determine the passage through successive phases. Different pathogenic factors must be considered to intervene in order to have cancer pass from one phase into another. The evolution of the cancerous condition has to be related to these different factors, some of them possibly more important than the original specific change. The passage of a cancer from the noninvasive to invasive phase, or from tissue to systemic, is surely more important than the appearance of a low level cancerous entity. An original change at a lower level appears, in fact, to be of very little importance, not only because of its ubiquity but also because it is not implicitly related to malignancy. From this point of view, then, cancer no longer can be defined as some single specific change in a cell, nucleus, chromosome, gene or other biological entity.

Carcinogenesis has to be conceived of in an entirely new way, in terms of plural factors and their relative values. Accepting the phases above invasive cancer as the only ones which correspond to clinical malignancy, they have to be regarded as the end result of a series of cancerous changes developed at progressive levels, with the intervention of many factors, not just one.

*Diagnostic Tests*

This view puts the problem of diagnosis of cancer in a new light. The recognition of a cancerous condition by itself, although important, has little clinical meaning. The presence of "cells with cancerous characteristics" in the prostate of almost all men 40 years of age and older, and in the thyroid, lung and stomach in a high proportion of the population, has failed to produce a general feeling of despair only because such findings are commonplace. While they still mean cancer, they do not implicitly indicate malignant disease. It appears very clear that a diagnosis of cancer is incomplete without immediate qualification as to its phase. We can no longer speak of cancer with any degree of practical meaning unless we add a descriptive adjective—noninvasive, invasive, tissular, organic or systemic.

And the search for a test to detect cancer will have no meaning as long as we have not defined in advance the information we want. A test, biochemical or immunological, which indicates the existence even of a specific anomaly in the noninvasive phase or before, while interesting, will have little significance since this anomaly exists in so many subjects and for most does not go beyond the noninvasive phase. The test will not indicate malig-

nant cancer in the clinically frightening sense. On the other hand, the processes which are added to a noninvasive phase form of cancer and turn it into the invasive, tissue, organic or systemic phase, have no character of specificity. Similar growth changes, or the appearance of lipidic predominance, which represent added factors are seen in many other conditions. By using them for diagnostic purposes we will not recognize the cancerous condition but only nonspecific intervening factors. While these factors are responsible for the changes, malignancy develops only when, and because, these factors operate on already abnormal entities, *i.e.*, cancerous entities. This explains the nonspecificity of many proposed tests and the misleading positive results obtained in conditions such as pregnancy where one of these added factors, (active growth processes) is always present.

A test for cancer, to have clinical value, would have to indicate two things: one, the specific early change which is widely distributed but represents the essential condition for the potential development of malignancy; and, two, the concomitant presence and concomitant operation of the nonspecific factors which can cause the actual development of malignancy. This kind of diagnostic test undoubtedly will come from further systematized study of biochemical changes induced by the simultaneous action of the two groups of factors.

Immunological studies represent an approach of value for diagnosis. The different phases of cancer can be interpreted, in the final analysis, to correspond largely to the intervention of the defense mechanism at different stages at the different levels. As mentioned above, a change in a phase results also from a change in the defense stage at the respective level. We have seen that the immunological aspect of cancer cannot be understood without accepting a relative independence of the levels in their different stages of defense. This view explains some seemingly paradoxical occurrences.

Cancerous cells are frequently found circulating in the blood yet this does not indicate generalized cancer. While the organism defends itself successfully at the systemic level against cancerous cells, the cancer can still progress at the lower level of the tissues. The loss at this low level of an effective defense, principally primary or allergic, which is still persistent at the systemic level, explains why the cancerous cells invade the tissues. A test indicating the presence or absence of any immunological reaction would consequently have value only when related to hierarchic levels. It must furnish indications only of what is happening at a specific level. The nature of the immunological reaction in cancer is also different from the reaction in other conditions. Defense capacity—natural defense capacity—

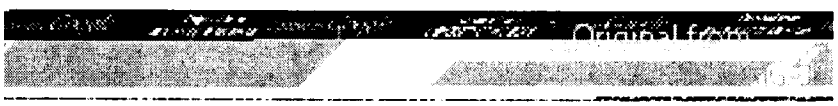
at different levels is lost as the respective level participates in the disease. This is in distinction to the immunological processes in other conditions in which the normal individual lacks specific immune bodies. An immunological test for cancer would have to reveal the loss of a previously existing defense mechanism rather than the appearance of an immunological response. This loss can be revealed in different ways. In one, the response to a cancerous antigen is investigated, and its lack would indicate the existence of a cancerous condition at this level.

In a study now in progress, we utilize pooled human tumoral tissues as antigen, and try to see if an allergic reaction can be induced with it, in two administrations, sensitizing and trigger. Two intradermic injections at the same site are made 12 days apart. They induce an allergic reaction in normal individuals, but are without effect in patients with active malignancy. If the effect is negative, a third injection is given 15 days later. A similar test is made for the conjunctiva, with sensitizing and trigger instillations of a similar antigen. No allergic reaction indicates a positive result, while a reaction is considered to be normal.

Another test which we are studying is based on the same lack of efficient defense mechanism at the tissue level. Such a lack of defense would permit a cancerous antigen to be present without the body offering a sufficiently effective defense against it. The presence of such an antigen in the tissues is revealed by inducing an allergic reaction, through the administration of specific coagulant antibodies. Sera of guinea pigs injected with pooled human tumors and having a high precipitin content are used in intradermic injections or in eye instillation. An immediate reaction indicates a positive result. As control, we use normal guinea pig sera. The studies are now in progress and the diagnostic value of these tests will be reported in a later publication.

*Circulating Cancer Cells and Surgery*

These immunological considerations have appeared important in considering a problem related to the use of surgery in cancer. The existence of a veritable flow of cancerous cells in the general circulation, largely induced by the manipulations inherent in operative procedures, has produced grave doubts as to the value of the measures taken by surgeons to prevent local spread of cancerous cells through the surgical act itself. Paradoxically, however, these precautions have been followed by good clinical results. Analysis from the point of view of the defense mechanism involved can clearly explain this situation. In the invasive phase, the systemic defense mechanism is still adequate to insure destruction of cancer-



ous cells which get into the blood. This is not true at the level of the interstitial formations, that is, at the tissular level, where such defense means are failing. The real danger during surgery consequently is not so much the presence of cancer cells in the blood, since the blood can still take care of them, but the spread of these cells at the tissular level where the defense capacity has been lost, and where a cancerous cell consequently has every chance not only to remain alive but also to grow.

The independence of the defense mechanism at different levels also must be taken into account in explaining the differences in the events which follow the appearance of a spontaneous tumor and those which occur after experimental tumor transplantation.

## EXPERIMENTAL CARCINOGENESIS

The problem of carcinogenesis appears in a new light when cancer is considered under the concepts presented above. Classically, the experimental induction of cancer is judged successful only if the result is a tumor in the invasive phase, that is, with abnormal cells invading normal surrounding tissues. This is considered to correspond to a fundamental specific change which transforms the normal cells into cancerous ones. The entire disease is held to stem from the relationship between these abnormal cells and the organism. (290, 291, 303) To these simple views of the abnormality, we have proposed another one.

In our view cancer represents a hierarchically organized condition. Its invasive form is only one phase in a long series of changes which transforms successive hierarchic entities into cancerous entities. Carcinogenesis, thus, is not simply a change of a normal cell into a cancerous one but a step by step progressive hierarchic development. A cell is cancerous only if it has a cancerous nucleus just as a nucleus is cancerous only if it is formed by cancerous chromosomes which, in turn, are cancerous if they have cancerous genes. With the same reasoning, it is possible to go far down in the organization, below genes even to nucleo-proteins or still lower to histones or even alkaline amino acids, to find that the first changes, which can be considered to be specific for cancer, take place at the bottom of the organization of the biological realm. In other words, a cell becomes cancerous after specific cancerous changes have occurred in all the hierarchically inferior entities that compose it. Thus, a successful experimentally induced cancer, *i.e.*, one that is already in the invasive phase, means that changes would have affected the entire series of hierarchic entities, including the cells, whose participation results in the invasive character. Seen under this

aspect, carcinogenesis no longer can be accepted as a simple process occurring in the cells, but must be regarded as a succession of organized processes.

This becomes still more interesting when it is realized that changes in the constituents at the lowest levels of the organization can occur on a statistical basis, that is, independently of the direct intervention of external agents. As these changes have to be developed for many successive hierarchic entities, it takes a certain time for them to be realized. This would explain why most cancers appear after a certain age. Cells with cancerous nuclei, *i.e.*, in the noninvasive phase, frequently are present, in older people, in many organs without producing clinical manifestations. Conceptually, in order for an agent to be considered a successful carcinogen, it must act upon these noninvasive entities to such an extent as to change them into invasive ones. It can thus act upon entities which have already progressed, by themselves, far enough in the hierarchic development of a cancerous process and have arrived at the noninvasive phase without any manifestation. The excessive length of time necessary, even for the most active agents, to induce invasive cancer would suggest, however, that more than a simple passage from an already existing noninvasive cancer into an invasive one is involved. A plurality of changes must be induced, some or all at levels below the cell.

We are inclined to favor this last hypothesis which obliges us to consider that a carcinogen induces changes at different levels of the organization. It is supported by a series of facts. In addition to having the capacity to induce invasive tumors, carcinogenic agents also induce precancerous lesions which correspond to cancerous entities below the invasive phase. Cells with abnormal nuclei or with only abnormal chromosomes are almost constantly seen in induced carcinogenesis. Even agents which produce a high proportion of invasive cancer consistently induce such changes at lower levels as well. For carcinogens which induce a low proportion of invasive cancers, the effects often appear to stop at lower levels. Such activity at subnuclear levels of the organization is seen in the capacity of most of the carcinogens to induce mutations and monstrosities.

In the concept of hierarchic organization, mutations are considered to result from changes taking place at the gene level, with lower levels left unaffected. Monstrosities result from changes at the chromosome level. Comparison of carcinogenesis with mutations and monstrosities has led us to consider that cancerous changes begin at levels much below those involved in mutations and monstrosities, possibly at the nucleo-protein level or, even below. The complex cancerous condition to which the invasive

form corresponds can thus be seen to be the result of a series of anomalies which have taken place at different levels below the tissular. Carcinogenesis at the invasive phase is conditioned by the existence of changes at all the lower hierarchic levels. While they can appear as the result of the development of the organization, conceivably these changes can be hastened or even induced by the carcinogens.

The concept of multiple changes in carcinogenesis has caused us to search for multiple factors in carcinogens themselves. The possibility that such factors might be found was suggested by the existence of so-called co-carcinogenic agents. These are substances without carcinogenic activity of their own but capable of inducing such activity in cases in which some carcinogens are administered in doses too small to induce invasive cancers by themselves. This peculiar intervention of co-carcinogens can be explained through the multiple factors in carcinogenesis.

It can be conceived that the factors present in a carcinogen do not have equal activity. The differences appear evident when the carcinogen is administered in very small amounts. While some factors still have sufficient potency in these small amounts to accomplish their part in the complex process of carcinogenesis, others are quantitatively insufficient and do not induce changes. The total effect is an incomplete set of changes. Under these circumstances the addition of a co-carcinogen can replace the action of the quantitatively inadequate factors, and consequently complete the plural action necessary to produce an invasive cancer. Because any one co-carcinogen can replace only certain factors, co-carcinogen activity has a certain specificity.

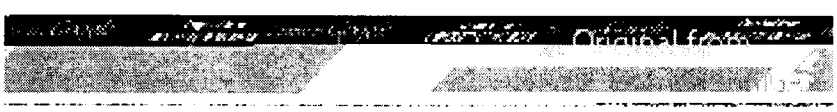
With the hypothesis of multiple actions in the same carcinogen, the next step was to try to recognize them. A study, identifying different active energetic centers in the structures of carcinogens, has substantiated the hypothesis.

We attempted, as a first step to systematize the analysis of such energetic centers in carcinogens. A short resume of this study is presented here.

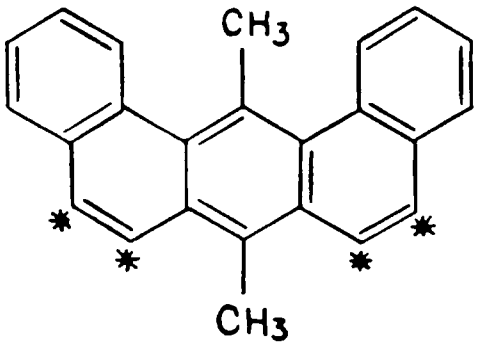
*Energetic Factors*

A well known and generally accepted concept tries to correlate carcinogenic activity with the presence of one energetic factor, identified as a "condensation of electrons," at certain regions of a molecule and revealed by the physicomathematical approach offered by Pulman and Dawdel.

Studies of the role of electron distribution in carcinogenesis were started by Otto Schmidt (43), which showed that an electron density exceeding $0.44e/a^2$ in the meso region of the molecule appears necessary to confer



carcinogenic properties. This concept was partially modified and amplified by Pulman, Dawdel and their co-workers (44) who have shown, by quantum analysis of various carcinogens, that the density of the $\pi$ electrons is increased in certain preferred regions of the molecules, the *K* regions. They showed that, when electron densities exceed 1.292e at these regions, the substances have carcinogenic properties. Figure 95 shows such a *K* region.




**9:10 Dimethyl**
**1:2:7:8 Dibenzanthracene**

FIG. 95. The regions K in carcinogenic molecules.

From our point of view, a tentatively interesting aspect of this condensation of $\pi$ electrons lies in two facts: the presence in some carcinogen molecules of more than one such K region, and the presence of different values for these K regions in different molecules. It would be the presence of more than one K region in the same molecule which would result in intervention in more than one process and thus contribute to plural activity.

Further analyses, however, suggested that the condensation of $\pi$ electrons in K regions would represent only one of the factors that would induce activity in these agents. We have identified another energetic factor in the presence of two atoms having the same electrical sign and being bound together within the molecule.

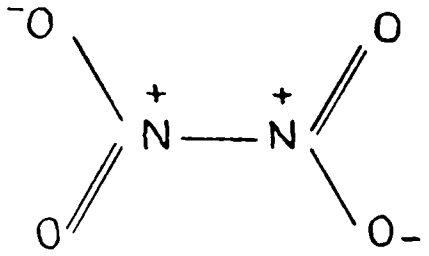
*Twin Formation*

We have considered the existence and importance of these "twin formations" as indications of energetic activity in the course of studies on electronic molecular arrangements. In a molecule, an alternation of successive atoms results in part from the alternating polarity of these atoms within a molecule and in part from the opposite characters conferred upon the

two carbon atoms when they form acetic acid, an important precursor in biological syntheses. It is through alternate polarity that an induction effect of an energetic center in the molecule propagates itself along the chain. The presence of any energetic center in the molecule represented by polar groups or a lateral chain, for instance, will enhance this alternate polarity. When one or more such inductive effects are propagated through the chain, two adjacent atoms may be found to possess the same electrical sign for their charge or ionoid character. The twin formation which results represents a center of increased molecular reactivity. This reactivity can be so intense as to lead to breaking down of the molecule, something which occurs often in inorganic substances. This has led Pauling to believe that this condition, called "adjacent charge rule," cannot exist.

"Pauling has pointed out that the mutual potential energy of two electrical charges of the same sign is so high that a canonical structure having net residual charges of the same sign on any adjacent atoms would have too high an energy level to contribute appreciably to the real molecular structure." So notes William A. Waters in "Physical Aspects of Organic Chemistry." (45)

The form suggested for nitrogen peroxide ($N_2O_4$), *(Fig. 96)* would appear to be impossible because of the high energy developed at the two positive nitrogens.

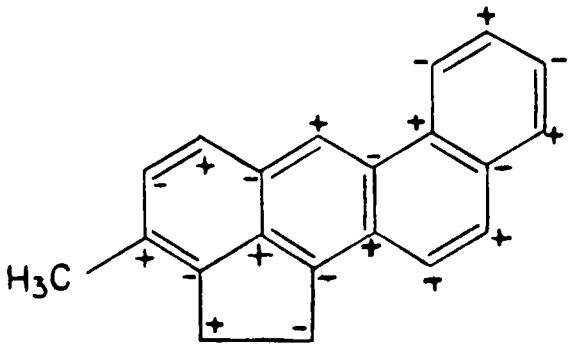



**Nitrogen Peroxide**

FIG. 96. The existence of nitrogen peroxide molecule is prevented by the high energy developed at the two adjacent positive nitrogens.

However, the forces that exist in most of the organic molecules are much weaker, so that the resulting "twin formations," although energetically potent, are not strong enough to induce the breaking down of the molecule. Consequently, they would exist and represent important energetic centers.

We have studied a number of carcinogenic agents, seeking twin formations. Analysis of the ionoid character of the carbons of the methylcholanthrene molecule reveals the presence of twin formations which could be localized at various points of the molecule. Figure 97 shows the energetic aspect of methylcholanthrene and the ionoid character of its carbons. It is the presence of the cyclopentane group in the molecule that induces the same sign in two adjacent carbons. The presence of the methyl group would determine the electrical character of $C_{20}$ and consequently the succession of alternate signs. On the other hand, the double bonds will determine the probable localization of these twin formations in the molecule at the K formation itself, that is, at $C_5$ and $C_6$.



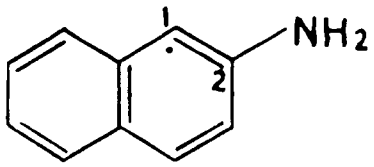

20-Methylcholanthrene

FIG. 97. The energetic aspect of methylcholanthrene, with twin formations.

Twin formations can be found in many carcinogens. It must be emphasized, however, that unequal energetic values can be recognized easily for different twin formations and would explain differences in their activity, a fact which would confer possible plural properties upon this group of qualitatively similar energetic formations.

Another aspect of the relationship between these formations and carcinogenesis appears to be even more interesting. While no twin formations can be found in several agents, the formations are present in the substances resulting from metabolism of these agents in the body. The relationship of twin formation to carcinogenic activity can be suspected when such changes appear simultaneously with carcinogenicity.

For example, no twin formation occurs in 2-naphthylamine, *(Fig. 98)* whose direct carcinogenicity is questioned, but such a formation appears in heterocyclic 3:4:5:6 dibenzcarbazole, one of its intermediates (46), which is known for its carcinogenic properties. *(Fig. 98bis)* This is also true for

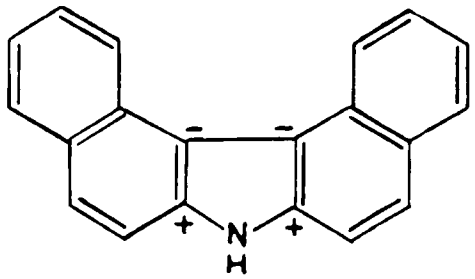



(a)

2-Naphtylamine

FIG. 98. No twin formations exist in 2-naphthylamine.

aminofluorene, which is also related to 2-naphthylamine. *(Fig. 99)* The existence of a twin positive carbon group or a twin negative in the same molecule can further explain the diversity of the tumors produced by this carcinogen and its acetyl derivative, which has the same energetic picture. (47, 48, 49, 50, 51, 52)

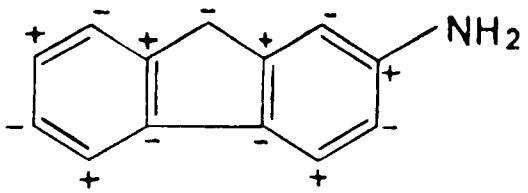



(b)

3:4:5:6 Dibenzcarbazole

FIG. 98bis. A twin formation appears in the intermediate 3:4:5:6 dibenzcarbazole.

Twin carbons can be correlated with the degree of carcinogenicity of the sulfur isosters (53) in each of which a thiophene nucleus replaces the benzene ring of 9:10 dimethyl 1:2 benzanthracene. This also applies to the azo compounds with twin formation at the level of the azo bond. Figure 100 shows the presence of a twin nitrogen at the level of the azo bond, due to the influence exerted by the symmetric rings.

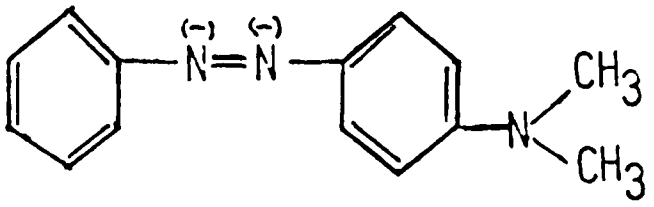



2-Aminofluorene

FIG. 99. A twin carbon group is present in aminofluorene.

Furthermore, it is the relationship of twin formation to carcinogenicity which indicates the need for considering the metabolism of various carcinogens in the organism.

Dimethylamino-azobenzene, butter yellow, which has a twin formation and is an active carcinogen, can become still more active through the metabolic changes occurring in the body which lead to products with twin carbons. The same 2:2′-azonaphthalene, with a twin formation, becomes more active because of its transformation into amines passing through hydrase compounds. 2:2′-diamino-1:1′-dinaphthyl, with twin car-



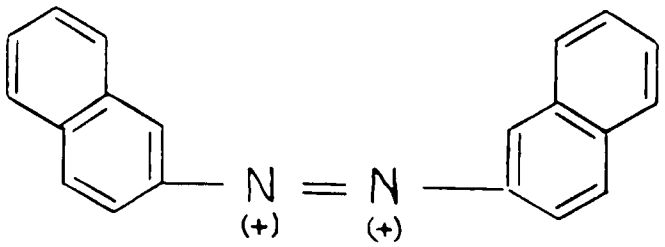

4 - Dimethylamino azobenzene

FIG. 100. A twin formation is present in 4-dimethylamino-azobenzene at the level of the azo bond.

bon formation, is more active than the precursor, 2:2 Azonaphthalene. (54), *(Figs. 101 and 102)*

It is possible that benzidine rearrangements of the hydrazo derivative determine twin formation and thus explain its carcinogeneity.

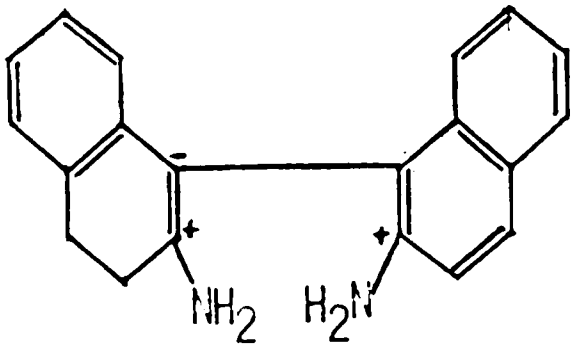
The similarity in kinds of tumors produced by the derivatives of 4-aminostilbene *(Fig. 103)*, and the aminofluorene derivatives (55), makes us think that twin formations can appear in this case through changes occurring in the organism.




2:2' Azonaphthalene

FIG. 101. 2:2' Azonaphthalene has only a slight activity.

Some artificial estrogens of high potency (56) diethylstilbestrol and triphenylethylenic acid, (57), *(Figs. 104 and 105)* are known to have carcinogenic activity. While a twin carbon is present in both, such a formation is assumed to appear more active in the latter, as the result of metabolic changes in the body.



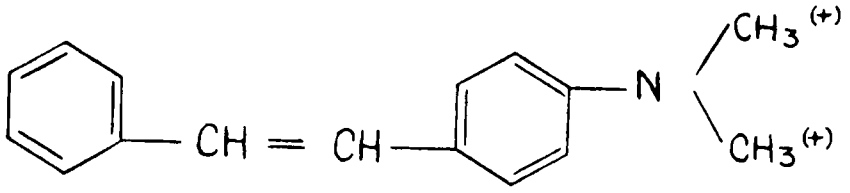

2:2' - Diamino
1:1' - dinaphthyl

FIG. 102. The passage of 2:2' azonaphthalene into the active 2:2'-diamino 1:1'-dinaphthyl results in the appearance of an active twin carbon formation due to the influence exerted by the amino-group.

An interesting aspect is furnished by urethane and other esters of carbamic acid. Figure 106 shows that no twin formations can be seen directly or through a change in the molecule. This accords with these substances' lack of capacity, noted by many authors, to induce cancerous lesions or even tumors. (58, 59, 60) Orr (61) relates lesions produced by carbamic

acid esters to chronic inflammations, noting their regression when treatment is discontinued. *(Note 1)*

From analyses of the substances able to induce invasive cancers, it can be observed that many present a twin carbon or nitrogen formation, usually activated by the induction exerted by a polar group or by double bonds. Some of these substances originally without twin formation become carcinogens only when changes occur in the body leading to the appearance of a twin formation.

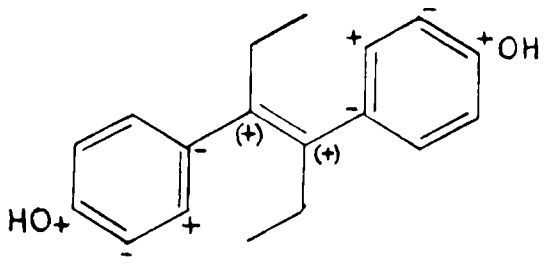



4-Dimethylaminostilbene

FIG. 103. 4-Aminostilbene derivative.

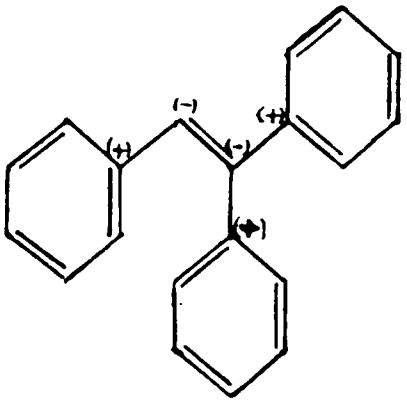
It must be emphasized, however, that according to the concept of plural factors in carcinogenesis, twin formation does not appear to be an obligatory condition for carcinogenic activity; other factors can produce such activity.

It is interesting to note that in most carcinogens, especially in the hydrocarbons, the twin formation is electrophobic due to its richness in electrons. For the present, we wish to stress only that in substances considered to be actively carcinogenic, *i.e.*, capable of inducing invasive cancer, twin




Diethylstilbestrol

FIG. 104. A twin formation exists in diethylstilbestrol.




Triphenyl-ethylene

FIG. 105. The position of the twin formation in triphenyl-ethylene.

formation appears to be an added factor which insures complex activity. Intervention of groups of two energetic centers with the same character, in carcinogens, places in a special light a group of agents which, under particular circumstances, induce tumors. One group with alkylating activity, is formed by the nitrogen mustards, diepoxides, polyethylene amines and dimethanesulfonoxyalkanes. One of the physicochemical characteristics of this group is the presence of two electrophilic centers near enough to each other to permit joint action. Still more important seems to be the fact that, through changes in all these substances, new formations may appear which energetically could be ultimately considered similar to twin formations. Through this character, their activity could also be parallel to that encountered in the carcinogens mentioned above.

$NH_2 - CO - OC_2H_5$

Urethan

FIG. 106. Urethan has no twin formation and apparently—according to many authors —no *direct* carcinogenic activity.

*Nitrogen Mustard Derivatives*

The most representative and better studied substances of this group are the nitrogen mustard derivatives, characterized by the 2-haloethyl amine group *(Fig. 107)* attached to a radical which can be aliphatic or aromatic. It seems that it is through hydrolysis that the compound becomes biolog-

ically active, and Haddow has shown that activity is present only if hydrolysis is sufficiently high. (62) The inequality of hydrolysis in different members can be related to the influence exerted by the radical bound to the nitrogen. It seems that the presence of a stronger energetic center, as it appears in positively or negatively charged atoms bound to the cyclic radical, reduces the dissociation of the chloroethyl group. Generally, nucleophilic groups would retard the dissociation. Sufficient evidence exists to show that biological activity follows the elimination of the chloride ion and the appearance of a carbonium ion as a reactive intermediate. A further passage into the ethyleneimonium ion, considered more stable and

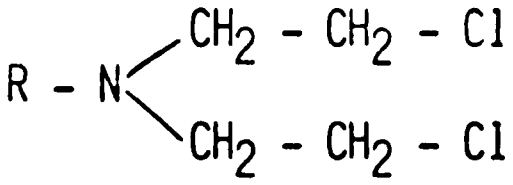



FIG. 107. The nitrogen mustard derivatives.

therefore less reactive, seems to complete the transformation. Figure 108 shows these changes.

The haloalkyl side chains in the molecule appear indispensable for biological activity. (63, 64, 65, 66) They lead to the immediate appearance of two positive electrostatic energetic centers. This does not represent a minimal condition, according to Landing and co-workers. (67, 68) These investigators have shown that in nitrogen mustards, cytotoxicity increases with the number of haloalkyl side chains. It is to be noted that a double electrophilic center is found not only in the two original haloalkyl side chains, but also in the later product, the ethyleneimonium ion. In view of the more frequent appearance of this ion also for other agents, the analysis of the relationship of this group to twin formation appears interesting.

In the ethyleneimonium group, while a negative charge can be seen at the nitrogen, a positive charge appears to be present between the two $CH_3$, providing a certain polarity. With two carbons positively charged and in a relatively fixed position, this group is similar energetically to a positive twin carbon group. A two-step change, with the imonium group in the first, and a carbonium in the second, can explain, as we shall see below, the strange biological activity of the nitrogen mustards which have a carcinogenic activity only through changes which take place in the organism.

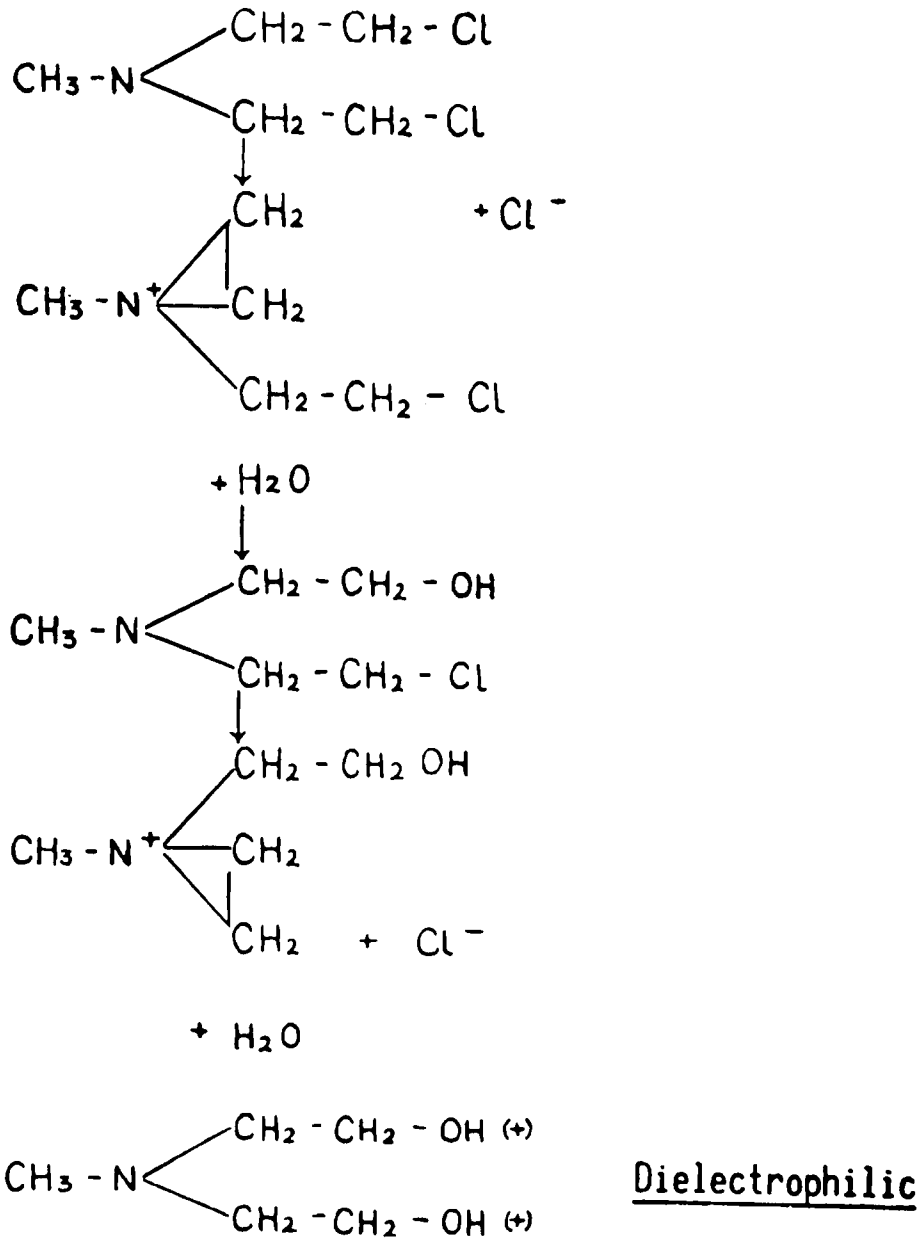
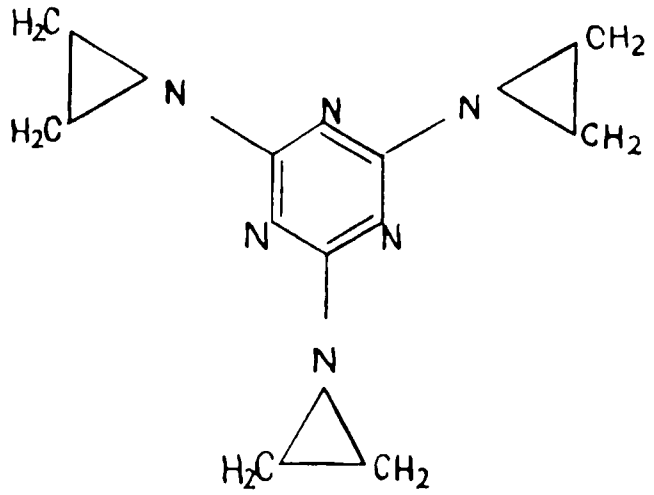



FIG. 108. The changes occurring in the nitrogen mustard leads to ethyleneimmonium in which an energetic aspect similar to that of a twin formation is present.

This agrees strongly with the nature of the more recently studied relatively active carcinogens, the ethyleneimines, where similar centers are seen. *(Fig. 109)* The biological effect of the ethyleneimine group has been considered to be related to a reactive intermediate.

Generally, if sufficient influence is exerted by another center in the molecule, the imine group becomes active. This center can be a nitro group as in 2:4 dinitrophenyl-ethyleneimine, or other ethyleneimine groups as

in methyleneimine 1:3:5 triazine. *(Fig. 110)* Through the influence exerted by these centers, the ethyleneimine group can have its carbons charged sufficiently to become a dielectrophilic formation. The possibility of a reactive intermediate and a more stable electrophilic form thus appears common to the two groups, mustards and ethyleneimines.




Triethylenimine

2-4-6- Triazine

FIG. 109. Ethyleneimines are active carcinogens, probably related to their energetic aspect with a formation energetically similar to the twin formation.

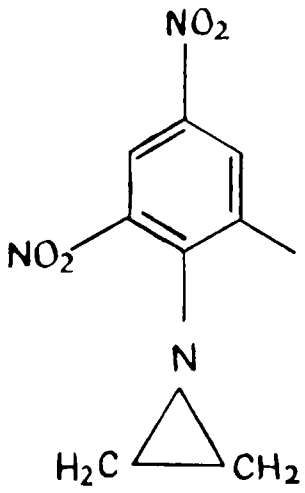
*Epoxide Carcinogens*

A similar condition is also found in the epoxide carcinogens. Carcinogenic activity has been recognized for substances having two epoxide centers in close proximity in the molecule. The epoxide center by itself can lead to a formation similar to that of carbonium ion, as seen in Figure 108 and thus to the same formation found in mustards and ethyleneimines. The analogy goes still further. The energetic center appears insufficient to accomplish biological changes without an inductive activation. In the case of epoxides, this is usually brought about by another similar epoxide group in the same molecule.

As no carcinogenic activity has been found in substances with only one epoxide center or with two epoxide centers far apart, the inductive centers seem to be of primary importance. The two energetic centers forming the epoxide group, similar to those of the ethyleneimines, do not alone appear

sufficiently reactive to induce important changes. Only when enhanced by reciprocal induction is their reactivity adequate to induce either the appearance of a reactive intermediate or a sufficient charge in the ethylene carbons to produce biological activity. These changes can be measured by the reaction with thiosulfate ion and consequently can be related to the reciprocal positions of the two epoxide centers.

The biological activity of dimethanesulfonoxyalkanes can also be related to a similar energetic formation. Such a formation appears when the




2:4 Dinitrophenyl Ethylenimine

FIG. 110. Through the influence exerted by the nitro group upon the ethyleneimine, the imine group of 2:4 dinitro-phenyl-ethyleneimine, becomes dialectrophylic.

molecule is metabolized, with the difference that the two $CH_2$ in this instance seem to come originally from other chains. *(Fig. 111)* For the methylolamides, it is possible that a similar process occurs during the changes that take place in the organism.

Some corroboration can be found in the fact that two forms can be observed in these last groups of carcinogens. One is electrostatically active; that is, it has a certain ionic character. The second has a dual electrophilic activity which can be related to a twin formation with molecular reactivity.

Thus, twin formation, with its special reactivity, appears common in many carcinogenic agents. To be biologically active, the twin formation has to be sufficiently strong and this is insured by an induction effect exerted

by other formations in the molecule, such as double bonds in parallel position or polar groups. A twin formation as energetic center in the molecule would exert a molecular field effect. It would thus represent a center of molecular reactivity which has to be considered as such in the analysis of plural activity.

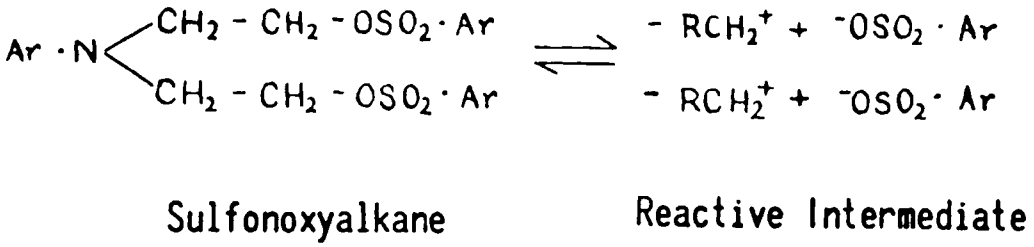



FIG. 111. Changes occurring in sulfonoxyalkanes leading to two active $CH_2$ centers.

*Synjugated Formations*

The study of various carcinogens has permitted us to recognize and relate to complex carcinogenic activity another energetic influence exerted by two or more double bonds when present in a parallel reciprocal position in cyclic molecules. This led us to the concept of "synjugated formations" with 2, 3, 4 or more such parallel double bonds.

In studying methylcholanthrene, one of the most potent of the known carcinogenic agents, the curve of its absorption in ultraviolet light was considered. This curve is shown in Figure 112. The place and form of the peaks could be interpreted in a peculiar way when conjugated double bond formations were considered. In the curve of methylcholanthrene, we could recognize portions that correspond to an inverse of the curves obtained from various conjugated polyenes. Furthermore, the curve obtained through the spectral analysis of methylcholanthrene can be considered to have high similarities to the inverse of the curve of a mixture of conjugated polyenes. Figure 113 shows the spectral analysis of conjugated cod liver oil fatty acids, while Fig. 114 shows the inverse curve of mixture of conjugated fatty acids of cod liver oil in which conjugated di-, tri-, tetra-, penta- and hexaenes are identified. Figure 115 shows the comparison between the curve of methylcholanthrene and the inverse of the peaks of the mixture.

We were thus led to consider the conceptual interpretation of these curves in terms of the special relationship that exists between double bonds in the same molecule. In the classical concept, two double bonds are considered conjugated if two of their carbons are joined by a single bond. In the zig-zag representation of aliphatic molecules, the conjugated double

bonds fulfill this condition. *(Fig. 116a)* Applying this relationship to cyclic molecules, what was considered to correspond to conjugation, according to this criterion, did not show properties similar to conjugated aliphatic members. *(Fig. 116b)* This made us consider, as the condition for the properties present in conjugated formations, another character: the reciprocal parallelism between double bonds present as they appear in the aliphatic mole-

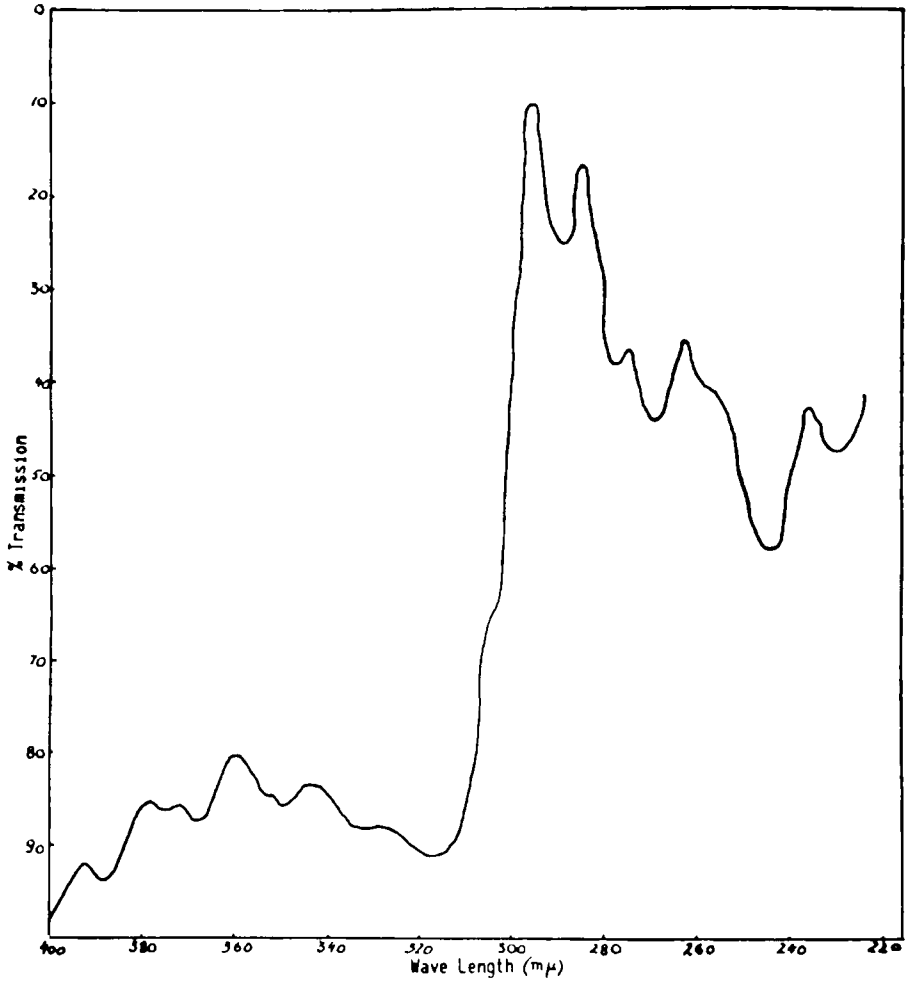



FIG. 112. *An interpretation of the spectral analyses of methylcholanthrene.* Curve (a) shows the spectral analysis in ultra-violet of methylcholanthrene.

cule. Two or more double bonds in a cyclic molecule would thus realize a similar kind of energetic formation when parallel, and would do so independently of the number of the single bonds present in-between. *(Fig. 116c)* For didactic purposes, we have applied the term "synjugated" to energetic formations resulting from parallel double bonds separated by more than one single bond.

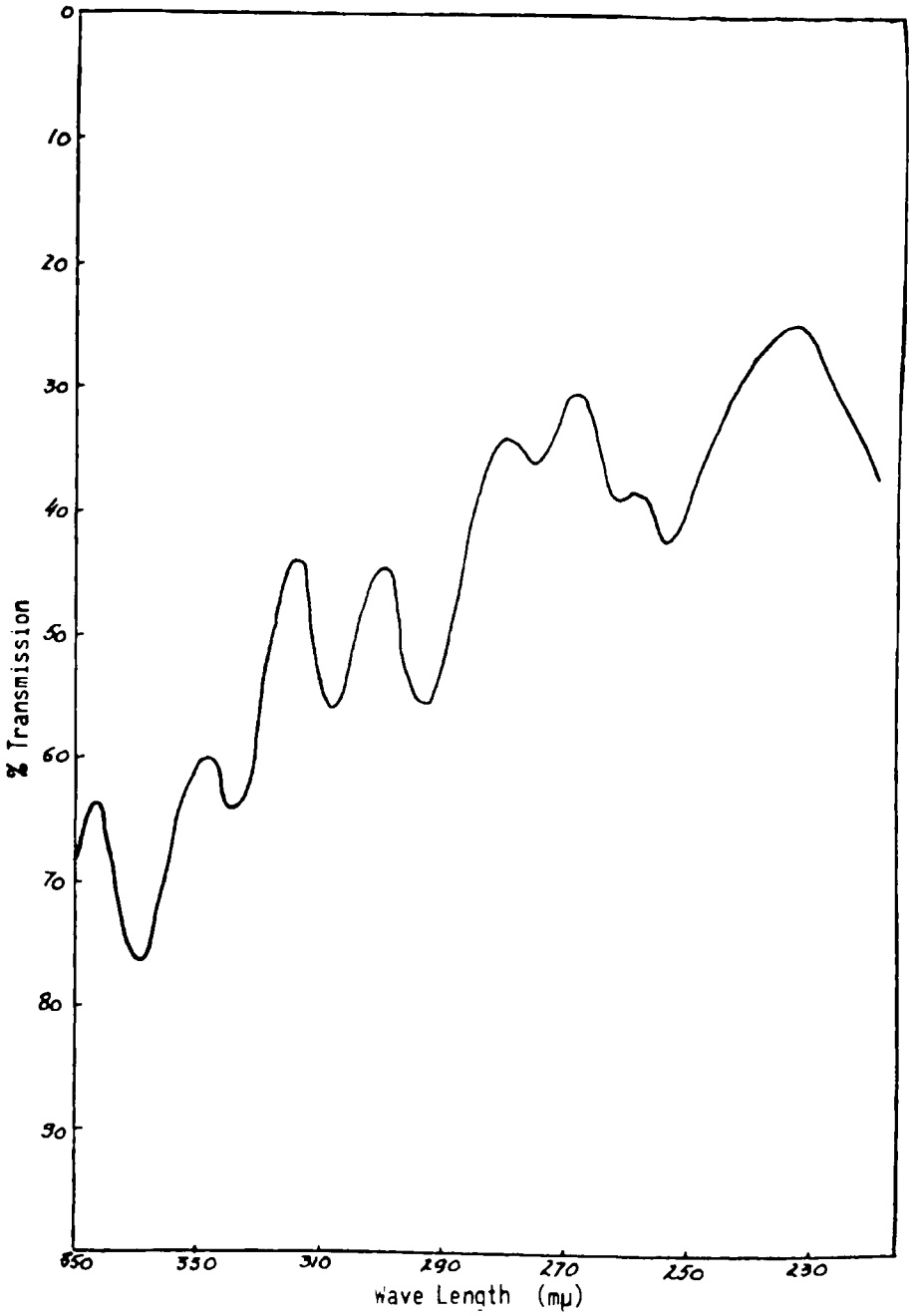



FIG. 113. The curve shows the spectral analysis of the mixture of conjugated fatty acids with members having from 2 to 6 double bonds, as obtained by treating cod liver oil fatty acids with KOH.

Thus, in the methylcholanthrene molecule, there exist formations composed of two, three and four parallel double bonds *(Fig. 117)*, which we call di-, tri-, and tetraenic synjugated formations. It is logical to assume that they are important in determining the energetic aspect of this molecule

when the relationship of its spectral analysis to the curve corresponding to the inverse of conjugated di-, tri- and tetraenes can be recognized. From the point of view of its relationship to the plurality of factors determining the carcinogenicity of a substance, the presence of parallel double bonds,

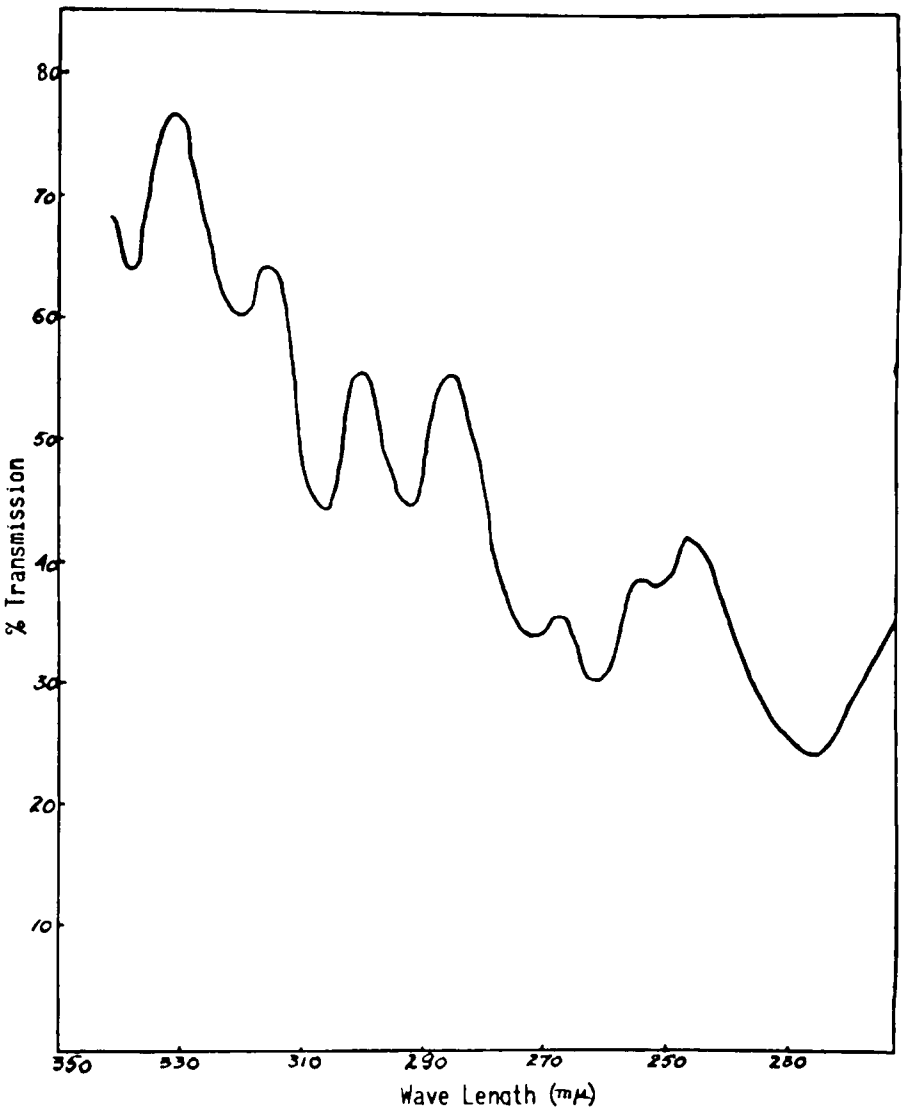



FIG. 114. This curve is the inverse of the curve of Fig. 113.

and the synjugated formations which they constitute, is interesting. Theoretically, each one of these synjugated formations would by itself represent a reactive possibility. Although qualitatively similar, they would show manifest quantitative differences. It must be noted that, while they are not present in all carcinogens, they are in most active, realizing di-, tri-, tetra- and

even penta-synjugated formations. According to the concept of plural activity in carcinogenesis, synjugation, while not indispensable for carcinogenetic activity, would represent one of the factors that can make it possible.

Together with the condensation of the $\pi$ electrons in the K regions and the presence of polar groups, the twin and synjugated formations would

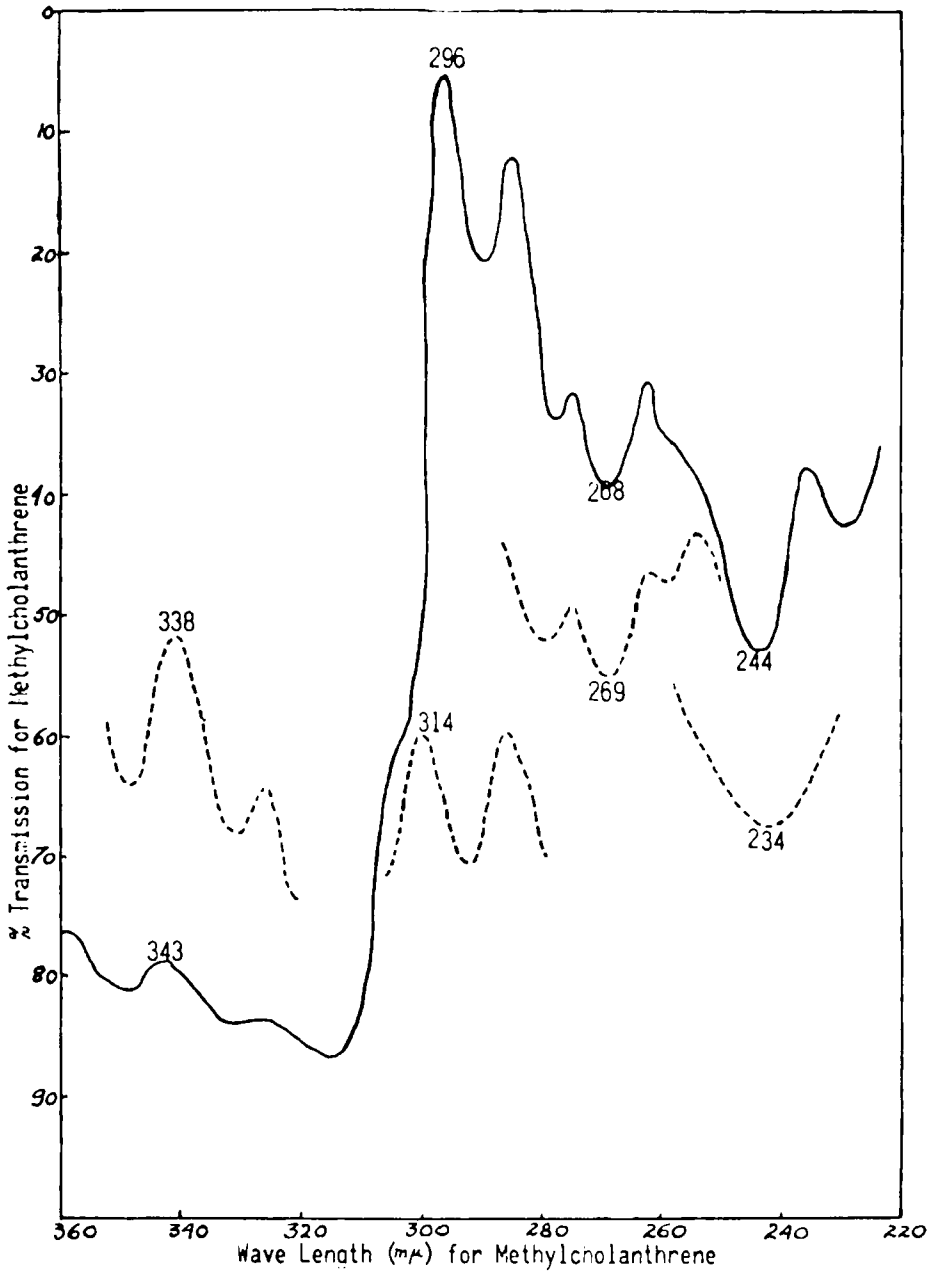



FIG. 115. Direct comparison between the curve of the spectral analysis of methylcholanthrene and the inverse of the peaks characteristic for the different conjugated fatty acids as seen in Figs. 113 and 114.

confer high plural activity upon the molecules of active carcinogens. An energetic spectrum of a carcinogen can be established in which these factors can be presented systematically.

Figure 118 shows a spectrum for 9:10 Dimethyl 1:2:7:8 Dibenzanthracene.

In the light of this analysis, it appears logical to conceive that the carcinogenicity of a chemical compound is a result of many factors, and that the great differences in carcinogenic properties of various agents is the re-

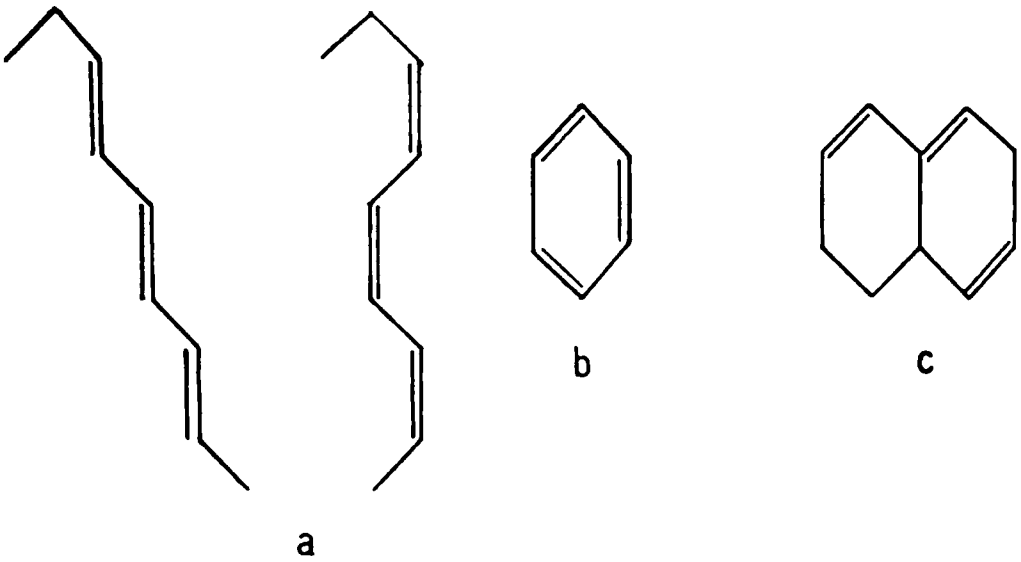
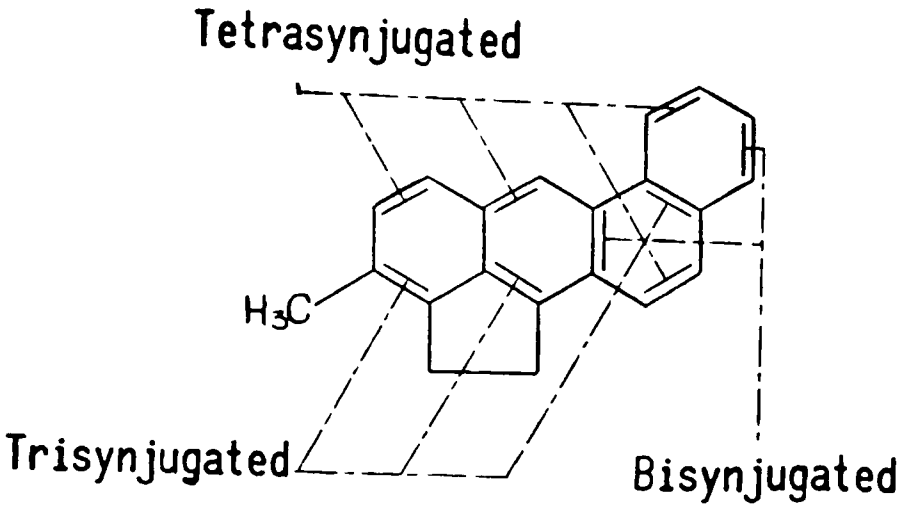



FIG. 116. *Conjugation and synjugation.* In the aliphatic chain (a) the presence of single bonds between the double bonds induce the parallel position of double bonds. It is this parallelism, which through the reciprocal influence exerted, induces the energetic characteristics of the conjugated formations. In the benzene molecule (b) where the double bonds, although separated by single bonds, are not parallel, the lack of this parallelism explains the lack of the properties characteristic to the conjugated formation. The parallelism when present in cyclic molecules (c) realizes the "synjugated" formations.

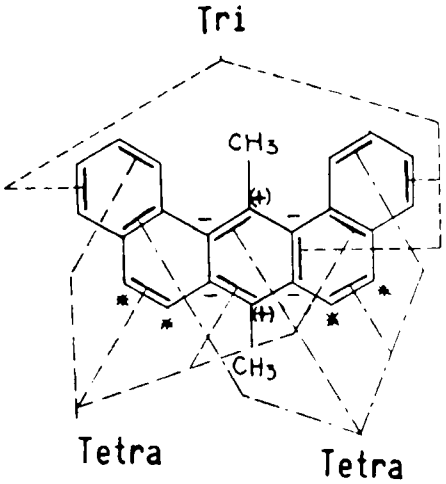
sult of differences in their energetic spectra. The differences are consequently qualitative as well as quantitative. From this viewpoint, it is possible that the great carcinogenic activity recognized for some substances would correspond to the presence in them at once of a great number of energetic factors.

The study of the correlation between the presence of various energetic centers and carcinogenesis has been facilitated by relating carcinogenic changes to levels of organization. Taking place at different levels, the induced processes can be seen to correspond to an entire series of manifestations which, while present also in invasive cancer, often can be recognized




## Synjugation in 20-Methylcholanthrene

FIG. 117. The parallel position of the existing double bonds in methylcholanthrene corresponds to a bi-, tri-, and tetrasynjugation.




Two K Regions * *
Two Twin Formations
One Trisynjugated Bond
Two Tetrasynjugated Bonds

## 9:10 Dimethyl 1:2:7:8 - Dibenzanthracene

FIG. 118. The energetic picture of 9:10 dimethyl, 1:2:7:8 dibenzanthracene, shows the presence of two K regions, two twin negative formations, one trisynjugated bond and two tetrasynjugated bonds.

in cases in which an invasive cancer is not induced. Following this view, it can be expected that carcinogenesis is the summation of a whole series of actions induced in the organism, some exogenous and others endogenous.

Consideration of the plurality of the factors which intervene in chemical carcinogenesis and make it a complex process leads us to consider viruses in the etiology and pathogenesis of cancer in a similar light.

## VIRUSES AND CANCER

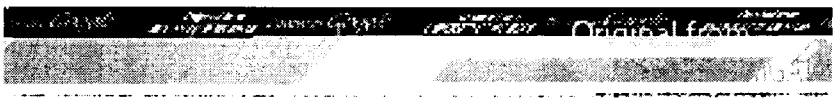
More than fifty years ago, Borrel presented his hypothesis of viral origin of cancer based primarily on analogies. Since then, although an enormous amount of material on this subject has accumulated, much of it has been contradictory and it has appeared to be impossible to arrive at any clear-cut concept of the role of viruses in the pathogenesis of tumors. However, it seemed that an attempt to correlate most of the data with information furnished by the study of chemical carcinogenesis in the light of the concept of cancer as a complex hierarchic disease might be of some value for an initial simplification of the problem. (293, 312) *(Note 2)*

Some theoretical considerations have helped in systematizing the data and in indicating the probable limits of viral intervention in carcinogenesis. Just as with chemical carcinogens, it could be assumed that virus intervention may bring to bear multiple factors. An analysis of the processes which occur under viral influence indicates that this hypothesis is plausible.

Even more than chemical carcinogens, viruses are able to act only at certain levels of organization. Their intracytoplasmatic and often intranuclear development conditions the intervention of these agents at these levels. Fundamental differences in carcinogenic effect could be expected if viruses are able to influence the subnuclear levels, or the nucleus, or, on the other hand if their activity is limited to the cytoplasm. This view has permitted us to understand the striking difference in influence exerted by various viruses which, although recognized by any worker in the field, has not been the subject of any special consideration.

### *Two Types of Carcinogenic Effects*

The difference lies in the time needed for a virus to produce carcinogenic effects. Inoculation of fowls with purified Rous sarcoma virus, for instance, has been seen to produce a clear-cut, immediate effect. Changes have been recognized within 48 hours at the site of inoculation. They take place in the nuclei of fibrocytes and consist of swelling, appearance of a more distinct nuclear membrane, cleared nucleoplasm, margination of chro-

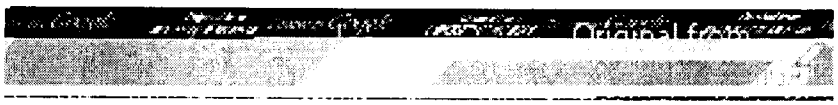


matin and one or more enlarged nucleoli. In as few as one or two days, cytoplasmic changes are also evident. There is manifest basophilia with swelling of the cell which becomes greatly enlarged. Concomitant with these changes, the abnormal cells invade the fibrillar tissue. The tumor which develops has the character of the classical spindle cell Rous sarcoma.

Thus a cancerous tumor in the invasive stage, with typical nuclear and cytoplasmic cell characters, is induced in only a few days at the site of inoculation. This is characteristic of one type of viral carcinogenesis, the extremely active one. Integrated in the concept of complex carcinogenesis presented above, it would mean that the entire series of changes—from those at the lowest level which determine the cancerous character to the cytoplasmic changes which produce the proliferative, invasive cancer—has been achieved by the virus in this short time. In fact, this tumor grows rapidly, is palpable even at the fifth day and fatal in two to three weeks.

Almost diametrically opposed to this type of carcinogenesis are tumors which represent another type of virus intervention, such as certain mammary cancers in mice. Viruses that produce such tumors can be obtained from various organs, even from those of animals without apparent tumors. They induce the appearance of tumors but only under very characteristic circumstances. Preferably introduced in the first days of life—subcutaneously, intraperitoneally or even orally—they will produce their effect only after many months or even after one or two years, as tumors of a specific organ, such as the mammary gland, for example. However, such tumors appear almost only in females who have had one or more pregnancies. In this case, the virus acts only upon highly differentiated cells and acts independently of the site of inoculation. The extremely long period without manifestation, the fact that the virus can be found to some extent in various organs which show no change, and the specific localization in a highly differentiated organ such as the mammary gland, would indicate that the carcinogenic intervention of the virus is highly related to a specific character of these cells, their particular differentiation. This would place virus intervention at the cytoplasmic level where differentiation occurs.

Under this interpretation, the length of time necessary for tumor appearance would be related to the time needed for a natural evolution of the mammary cells to the point where they are sufficiently differentiated. It appears probable that this length of time corresponds to that needed by abnormal hierarchic entities of the mammary cells to have arrived, independently of the virus, at a state corresponding to that of precancer or noninvasive cancer. Intervention of the virus at the cytoplasmic level would then transform the relatively advanced but still noninvasive cancer cells



into invasive cancer cells. Viruses would act, in this case, as cytoplasmic carcinogens.

These two types represent the extremes of carcinogenesis in which viruses play a role. They help to interpret many of the other data furnished by experiments. For didactic purposes, we shall regard as "broad-scale" viruses those which act from very low to high levels of the organism, and as "cytoplasmatic" those which act at the higher cellular level only.

Some of the rapidly acting broad-scale viruses will induce evolving tumors in a much shorter time than any known chemical or physical carcinogen, a fact that can be interpreted as meaning that these viruses are more capable of inducing not only the cytoplasmatic carcinogenic changes but also the entire scale of preparatory changes leading to invasive cancer. Viruses differ from the usual active chemical carcinogens in their special capacity to induce changes easily at the cytoplasmic level where they are particularly capable of multiplying and acting. This would contrast with most chemical agents which generally have low carcinogenic activity at the cellular level. Chemical and virus carcinogenic activity would complement each other. This is in accord with experiments of Russian scientists, which have shown that cultures of cells treated with methylcholanthrene in vitro become highly carcinogenic when inoculated in animals if a cancerous virus is also added.

This view of the activity of cytoplasmatic viruses at the higher cellular level, as contrasted with many chemical carcinogens usually more active at lower levels, appears also to be in agreement with the experiments of Rous and Kidd (69, 70) which demonstrated the capacity of coal tar extracts to localize the Shope papilloma virus. The high cytotropic character of this virus is well known. It would easily act upon cells already transformed from normal into noninvasive form by chemical agents which are active at the lower levels. These chemical agents thus "localize" the viral activity. This is in accord with the ability of chemical agents in the Rous and Kidd experiments to increase the percentage of invading carcinomas as compared to the papillomas present. According to the view presented above, the papilloma as a benign tumor would represent changes similar to those seen in cancer but limited exclusively to the higher levels, without cancerous entities at the lower levels. The addition of an agent with a broad-scale of carcinogenic activity, that is, acting also at the lower levels, such as the chemical agent, would give the resulting lesion the entire cancerous scale, that is, the character of malignancy.

The integration of viral carcinogenesis in the concept of cancer as a complex condition and recognition of the two extreme types of viral car-

cinogenic activity permits us to understand the reserve of most authors over the viral etiology of cancer. For instance, many have refused to accept as a carcinogenic factor the virus shown by Bittner (71) to be present in maternal milk and to influence the appearance of mammary carcinoma in mice. The refusal is based upon comparison of this virus with that of the first type seen in fowl tumors. With the systematization presented above and the concept of broad-scale and cytoplasmatic carcinogenic viruses, this reason is not valid. Furthermore, a carcinogenic virus should not be considered to be the indispensable factor able to induce proliferative cancer in animals which usually have a virus cancer. This would explain why, in certain breast carcinomas in mice, a viral agent could never be found. (72)

The specific capacity of a virus to act upon a differentiated cytoplasm explains the fact that a virus may be widely distributed among organs but does not induce tumors except in special cells. Previous preparatory changes seem necessary for the cytoplasmatic virus to intervene. This is in accord with a low incidence of tumors in certain strains of mice despite an abundant presence of the "milk factor" virus. (73)

These facts shed a new light on the entire problem of the relationship between viruses and tumors. Viruses can multiply in organisms without inducing cancer. The virus of mammary carcinoma in mice can be transmitted to females through spermatozoa and can be found in large amounts throughout the organism. The virus is present a long time before any cancerous lesions are seen and is present in organs that will never have tumors and even in animals that never develop cancer. The development of this virus, like all viruses, takes place in the cell cytoplasm which does not necessarily mean the induction of carcinogenesis as long as other factors are not present. No tumors appear as long as the cell has not undergone the prior changes required if the cytoplasmatic carcinogenic effect is to take place. Without the previous changes, the virus will not influence the cell any more than many other noncarcinogenic viruses. It is only in the presence of an advanced cellular change that the virus will produce an invasive cancer.

### *Plural Activity*

The capacity of a broad-scale virus to induce an invasive tumor in a short time through plural activity at different levels has been related to its richness in lipids. Its analysis makes us suspect the presence of several parts in the virus, each one able to act at a different level, as in the case of active chemical carcinogens. A similar plural influence can be seen

exerted by viruses other than those with carcinogenic activity. The study of bacterial viruses has shown the existence of such plural parts. *(Note 1)*

Luria (74) has shown, after irradiating a bacteriophage with ultraviolet light, that if lytic activity can no longer be obtained by the intervention of a single one of these particles, it can be induced with two or more of them. They act as though several parts, which usually are present in the virus but which were unequally inactivated by the irradiation, would be necessary in order to induce the process of lysis. This agrees with the experiments of Debruck and Hershey (75), which have shown that new types of viruses with new properties can be obtained when units of the same phage strain or related strains are mixed together. The new properties are combinations of those of the mixed units. (298)

Similar changes in the plural constitution of the viruses would explain other peculiarities observed in bacterial phages. Bacteria can carry phages for generations before any lytic activity occurs. The lysogenic strains (76) of bacteria are examples. It is possible that a virus may undergo temporary changes under certain circumstances; this would explain the frequent impossibility of finding a virus immediately after it infects a bacterium, and the "disappearance" of some viruses in animals immediately after infection. Since, in both cases, the virus is found later, a change which makes it unable to act and thereby be detected is plausible. The "masked" virus would be one with only some of its plural properties present. The possibility of recovering a lost property was demonstrated in the Berry-Dietrich phenomenon, when a heat-inactivated myxoma virus recovered its lethal capacity if inoculated along with a fibroma virus.

This concept of plural activity finds further application in the explanation of many phenomena observed in viruses in general and in variations in carcinogenesis. The "self-sterilization of the neuro-infections" described by Levaditi has to be regarded rather as partial inactivation of the viruses especially if the viruses can be reactivated. This occurrence must be separated from cases where a total destruction of the virus can be supposed to have taken place.

The lethal infection induced in mice injected intraperitoneally with salivary gland viruses of certain strains is an example of the latter. The presence of the inclusion bodies in liver and other organs, and the total inability to produce the disease in other mice (77) can be interpreted as a sign of a destruction of the viruses in the organism. The inclusion bodies can be interpreted as resulting from an agglutination of the viruses themselves as shown by Nicolau in herpes. (78) In other cases, such as protracted herpes infection in rabbits (79), or vaccinal infection in rabbits

(80), only partial inactivation can be considered to occur since electrophoresis, repeated passage, or even dilution restores pathogenicity. The restorative factor can be of varied nature. Cases in which pathogenicity is restored by a nonviral agent—activation of the virus of swine influenza in the presence of Hemophilus influenza (81), for example—are most revealing.

*Virus and the Host*

This concept of plural activity explains the relation between tumorgenesis and destruction induced by viruses. Often "neoplastic" infection and "destructive" infection are induced by the same virus. (82)

The herpes virus thus induces necrotic lesions in the chick embryo when introduced in early stages, but if the embryo is more developed, the same virus produces proliferative changes. (83) The myxoma virus induces more proliferative lesions if attentuated than does the unchanged virus. (84) Under special circumstances, such as in older animals, sheep pox virus induces papilloma instead of pustular infection (85). It must be remarked that these different results are not limited to viruses; they occur with radiation or even with other infectious agents. (86) Bartonella bacilliformis, which induces often-lethal Oroya fever, seems to be the cause of "verruga peruviana," a fibroangiomatous tumor often seen in subjects recovering from the acute disease.

The differences in activity of the same virus appear to be related to the age of the host. Generally, youth of the host increases the virus' capacity for acting at more levels. The virus can produce lethal destructive disease in young animals but only a neoplastic response in adults, as seen for the fibroma virus in rabbits. Furthermore, the neoplastic response also occurs in young animals but only if a small amount of virus is inoculated, or if an attenuated virus, such as a long-stored one, is used. (87) This is clear in the case of the Rous sarcoma and other chicken tumors.

When injected into very young animals, Rous sarcoma and other chicken tumor viruses produce a hemorrhagic lesion (88) but they will induce tumors in adult animals. The destructive effect can be repeated with repeated passages of the virus in very young animals but in adults each passage produces the neoplastic response. This is also true for some strains of lymphomatosis virus (89) which induce tumor formation in adults and necrotizing processes in young animals or embryos. It is also true for the virus of neurolymphomatosis (90), and of gliomas. (91) These viruses, although selective for the nervous system, induce inflammatory or neoplastic lesions according to the age of the infected animal.

In a general way, it has been postulated that for viruses, as for bacteria, the young animal represents a favorable terrain, while a certain resistance is encountered in the adult. Waters and Bywaters (92) have shown that the filtrable agent isolated by Prickett and Belding (93) is not transmitted spontaneously if the animal is older than 40 days. It is transmitted through the eggs, although months elapse before there are manifestations. (94)

The problem cannot be limited to the host, since, by passing the virus through young or old animals some of its properties can be changed. Gross (95) has shown that a cell-free extract obtained from leukemic mice of the AK strain, would induce the condition in $C_3H$ mice, provided the inoculation is given within a few hours after birth. The results published by Gross and the inability of other authors to reproduce them (96) could be explained by differences in the virus strains used. (97)

This was shown in the experiment of F. Duran-Reynals (98), in which the Rous sarcoma virus undergoes changes during passage in the adult chicken, which make it adaptable to another species, namely ducks. The virus growing in young chicks seemed unable to induce the disease when inoculated in ducklings or in older animals. Tumors obtained even through cell suspensions, some of them very large tumors, could not be transmitted for more than one or two generations. However, filtrates of tumors from older chickens, when injected into ducklings no more than a few days old, induced tumors which easily could be passed to young as well as adult ducks. This change in the virus was strictly conditioned by the age of the chicken; it occurred only if the animal was between three and ten months old. If the animal was more than 19-20 months old, injection of the filtrates was always unsuccessful, and injection of the cells only rarely induced tumors.

These changes in the virus are explained by mutation. Among various resonance forms which appear on a purely statistical basis, one different from those previously predominant finds favorable conditions for its development—conditions that are not favorable for the predominant forms. These experiments have permitted us to further correlate the intervention of viruses with the influence exerted by several chemical factors upon the complex tumor pathogenesis.

*Virus and Lipoids*

In studying the influence exerted by lipoids upon viral activity, we could show that the presence of free fatty acids, especially polyunsaturated, induced changes opposite to those induced by the anti-fatty acids.

In rabbits, administration of various preparations of fatty acids, espe-

cially polyunsaturated, induced an unusual degree of resistance. Animals previously given subcutaneous injections of fatty acid preparations showed a reduced general response to chicken pox inoculation as compared with controls, and practically no response in the skin at the site of the fatty acids injection. On the other hand, administration of insaponifiable lipid fractions obtained from tissues of receptive species was followed by manifest responses localized in the zone of injection, even in species otherwise refractive to viral infection. It is under this special influence of lipoids that we have further investigated the intervention of the viruses in carcinogenesis.

Cells vary in their content of lipids. We could see that richness in sterols of a group of cells increases their receptivity to, and favors the development of, viruses in general, while richness in fatty acids, especially polyunsaturated, has an opposite effect. The local increase in a tissue's richness in sterols makes it more susceptible to the localization and development of a virus, as is shown in the following experiment.

In rabbits, intracutaneous or subcutaneous injections of a colloidal suspension of cholesterol were made on epilated skin at several sites. Twenty-four hours later, the animal was injected intravenously with suspension of smallpox vaccine. Characteristic lesions were observed to develop at the sites of the cholesterol injections.

The general effect of sterols upon receptivity to viruses, noted in many experiments in animals, was also recognized in humans. The following observation appears interesting. Mrs. D. R. had always appeared refractory to smallpox vaccines. Until the age of 40, repeated inoculations produced constantly negative responses. She was treated at that time with a cholesterol preparation for precordial pain, receiving daily 2-3 cc. of a 2.5% solution of cholesterol intramuscularly. After three weeks of this treatment, she was obliged to go abroad and it was necessary for her to have the routine smallpox vaccination. For the first time in her life, a characteristic positive result was obtained.

The relationship between sterols and viruses, which would explain the affinity of most viruses for the nervous system and skin, since both are of exodermic origin and particularly rich in free sterols, would also explain why young cells similarly richer in sterols are more susceptible to viruses, and the facility with which almost all viruses develop in embryos, such as in chicken embryos.

Changes in richness in lipids were observed under natural circumstances other than those related to age. Thus seasonal changes could be noted, the cold season leading to an increase of fatty acids while the summer season

brought an increase of sterols. This would help to explain the seasonal changes usually observed in naturally occurring viral infections. (99)

The epidemiology of poliomyelitis may be related to the organism's richness in sterols in the summer, particularly on hotter days. Seasonal changes were noted in naturally occurring tumors in which a viral etiology is seen. A certain resistance appears in the fall and increases in the winter in the case of leukoses and possibly in other natural viral tumors. (100) This would explain the manifest seasonal changes observed by us in the transplanted Walker tumor in rats or in grafted tumors in mice in general, and in the induction of tumors through carcinogens. Similarly, the induction of teratomas in testes through local administration of zinc chloride was noted to be influenced by the seasons. (101)

The influence exerted by sterols would explain the fact that viruses able to act only at a higher level, as in the cytoplasm, tend to develop in animal cells abnormally rich in sterols. It is highly probably that once it has penetrated, a virus will develop within a cell only under favorable conditions, and these are insured by the presence of sterols. The virus will persist, interfering little with the fate of the cell until other changes occur at lower levels. These other changes take many months or even years to be completed, and only then would the influence of the virus be apparent through its activation of the noninvasive abnormal cell. Activation can occur regardless of seasonal changes in sterol richness. It seems superfluous to note that this relationship holds more for cytoplasmatic viruses than for those with broad-scale activity. The latter are also more active in young animals.

Changes in age of the host and other circumstances can modify the character of viral carcinogenesis, leading to rapid or very slow development, or even to complete lack of response. This was often noted for Rous sarcoma. In young animals, small amounts induced rapidly growing tumors with multiple hemorrhagic metastases rich in filtrable virus. In adult animals, despite the large amount of virus necessary, tumors took months to appear, seldom metastasized, and could be transmitted with difficulty, or not at all, by filtrates or even by cells. (102)

The relationship between viral carcinogenesis and lipids has been the basis for a group of experiments in which we tried to influence the carcinogenic activity of a virus by administration of sterols. Experiments still in progress, using sterols obtained from chicken embryos, seem to indicate that lipids can strongly change viral carcinogenic activity. In general, they induce an increased response to viruses.

Many other peculiarities of the relationship between viruses and carcinogenesis have been analyzed in terms of intervention of lipids as an

intermediary factor. The capacity of a virus to induce tumors in different organs—as seen for leukemic tissue cell-free extracts in mice, which induce peculiar salivary gland tumors (103), or tumors in the adrenal gland or in the subcutaneous tissues (104)—can be explained by certain peculiar affinities of the viruses for differentiated tissues, possibly related to certain specific lipids found in these tissues. A similar affinity for the salivary gland is seen for rabies virus. There are also the affinities shown by the neuro- and dermatotropic viruses, and by the neoplastic viruses for mammary gland, lymphatic tissue, neuroglia, etc. While affinity for the adrenal gland could be related to its richness in sterols, affinity to others sites could be related to other lipids.

The plurality of localizations of viruses also can be related to affinities of mutated agents for different cells. The experiments of Gross (105), which show the possibility of separating out of the same filtrate the agents responsible for salivary gland tumors and for leukemic changes, would indicate that a change in the virus must be also considered. However, the change can be interpreted as a mutation and can be related to the influence exerted by lipids upon the virus. Treatment of virus with lipids has shown the possibility of inducing changes in its behavior. Data showing the influence upon tissular receptivity of such changes will appear in future publications.

With this concept of the role of viruses in the pathogenesis of cancer, it seems possible to explain other peculiarities that have led to confusion in this field.

It has been noted that viruses act as factors determining the change to a cancerous entity which, once induced, can continue to develop without need of further intervention of the virus. This poses the problem of the relationship between viral carcinogenesis and development of the virus in the tumor itself. Even in a tumor, the multiplication of the virus has to be separated from that of the growth of the tumor. Although often interrelated, they must be considered as two different processes. The growth and even direct transmissibility of the tumor can continue, independent of the presence of the agent that originally induced it. When tumors have been induced by a chemical carcinogen, they can be transmitted in continuous generations over many years, producing large tumors each time, a fact which would preclude any possible direct intervention of the agent in these later tumors. Similarly, tumors once induced by virus can be further transmitted by cells, the virus no longer being apparent in the tumors. A tumor induced by a virus often serves as a medium for the multiplication of the virus. However, even while the tumor can continue to grow, it can become an adverse medium for the further multiplication of the virus.

This explains the peculiar fact that tumors induced by a virus can be rich in or can lack an appreciable amount of virus, as often seen in a Shope papilloma (106), or even in tumors in fowl (107), which pass through periods when transmission through cell-free filtrates becomes impossible, while transmission through the transplant of tumor cells still continues. The virus multiplication capacity can vary not only with the host but with the virus itself, thus explaining the changes noted above.

The results of the interesting studies of Bryan, Galman and Maloney (108) who have investigated the relationship between richness in virus of an induced Rous sarcoma and the percentage of positive results in induction easily can be interpreted under this view. The chances of inducing tumors increase in cases in which the host is also a favorable medium for the multiplication of the virus, and vice versa. This would explain why these authors found little or no active virus in cases in which the injected material produced less than 50% positive results, but cases originated by a material that induced a large proportion of positive results were rich in active virus. The capacity to multiply after inoculation in the host itself thus increases the ability of the virus to act as a carcinogenic agent, which seems logical. The relative independence of the two processes—the multiplication of the virus and the induction of tumors—appeared clearer in the cases mentioned above, where the virus develops in the entire body of mice, even in successive generations, without inducing tumors.

The presence of viruses in the organism, even without inducing tumors, helps to explain the rather puzzling experiment in which a transmission through filtrates, considered characteristic for viruses, was seen to occur for tumors induced by chemical carcinogens. Carrel has claimed to have transmitted through filtrate passages tumors induced by arsenic, tar preparations and even indoles. These tumors were of the Rous type obtained in fowl. More recently, similar tumors transmitted through filtrates were observed by McIntosh and Selbie (109), Maisin, Haddon and Haagen (110), and Oberling and Guerin (111) after injection of methylcholanthrene, especially in fowl. The considerations presented above furnish a logical explanation for these observations.

A first factor to consider is the presence, in animals regarded as normal, of a virus able to intervene to produce a neoplastic effect under special circumstances. We have noted that such a virus can be present without inducing tumors. Fowl appear to be especially susceptible to viruses (112), statistics showing that viral lymphomatoses are responsible for 50% of the malignancies in chickens. Even while some species display an inborn resistance to viral infection, others are highly receptive, as seen for the viruses

of sarcomas (113) and lymphomatosis. (114) Viruses have been found in as many as 10% and even 20% of chickens, according to some reports. (294)

The number of animals with viruses and no tumors must be considered still higher when presence of viruses is revealed by antibodies. Duran-Reynals, in collaboration with the East Lansing Agricultural Experiment Station (115), has shown that, while not one of 23 chickens kept isolated and free of lymphomatosis showed antibodies in the blood, hundreds of chickens taken at random did have the antibodies. This fact makes it highly probable that the presence of the viruses in the chickens used for carcinogenic studies was independent of the administration of the chemical carcinogen. Furthermore, the role of chemical agents acting alone as carcinogens can be discounted because their effects differ widely. In the series of McIntosh, the tumors appeared far from the site of injection which is very unusual for methylcholanthrene. Furthermore, the agents used by Carrel, except tar extracts, generally have little or no carcinogenic activity.

The two hypotheses—one, that chemical carcinogens alone can induce filtrable tumors; the second, that this is only coincidence and the tumor is entirely of viral origin—can be reconciled under the concept of plural intervention. Thus the chemical carcinogen would induce only part of the process; the remainder, at higher levels, would result from viral intervention. The change that occurs in the cells through the influence of the chemical carcinogen could also favor the change in the virus, making it not only more active but also of neoplastic character. The concept of plural changes needed to induce active carcinogenesis permits us not only to integrate the intervention of viruses in the concept of carcinogenesis in general, as presented above, but also to consider this intervention in relation to other factors.

Hormones can play a part; they are needed to induce the degree of differentiation that is a condition for viral co-carcinogenic intervention. Inoculated intraperitoneally, the Bittner milk factor, although active, will rarely induce mammary tumors in virgin females although the virus can be proved to be in the body. The hormonal changes related to pregnancy and lactation influence the mammary gland and cause a differentiation. Since this differentiation represents a condition for viral neoplastic activity in these cases, hormonal intervention can be integrated as an added factor important for the viral carcinogenic activity. The hormonal factor would appear to be an indirect co-carcinogen, and it is under this aspect that its role in carcinogenesis has to be studied.

The concept of plural co-carcinogenic intervention permits us not only

to relate the different pathogenic factors involved in carcinogenesis; in addition, by relating these factors to various levels of organization, it allows us to obtain new insight into the genetic factor.

## GENETICS AND CARCINOGENESIS

The genetic factor in carcinogenesis can be understood in terms of phylogenetic hierarchic development. Such development results from a series of progressive changes which lead to successive hierarchic levels of organization. As we have seen, for each level of the organization a series of different solutions are available when a new hierarchic entity is to be realized. This results first from the fact that various numbers of entities take part in the constitution of the principal parts. Since different constituents can form the secondary parts of these new hierarchic entities, the number of solutions is increased. The resulting solutions can be considered on a statistical basis. Many of these new entities will die immediately; others will subsist as such; still others will progress. Their fate results largely from their interrelationship and the conditions present in the environment in which they find themselves.

The striking similarity to the resonance process studied in the lowest levels of organization, such as atoms or molecules, has led us to consider that changes at higher levels are of the same fundamental nature. Of all the resonance forms that occur at each of the levels, there are some that, on a statistical basis, persist and develop. These persisting resonance forms make up the normal organism. The favored resonance forms are determined by heredity and also by environmental conditions. While the resonance forms appear on a statistical basis, the environmental condition can vary and new resonance forms will mark the intervention of external factors. As a normal entity is composed of the persistent resonant forms, abnormality occurs when such an entity persists. The characteristics of any individual are provided by the resonance forms which have developed phylogenetically and also ontogenetically. These predominant forms are "isotropic." For didactic purposes, we called the others "allotropic."

### *Allotropic Resonance Forms*

It must be accepted that, originally, it was the intervention of allotropic resonance forms which permitted the appearance of new forms able to respond well to the environmental changes. The phylogenetic development of different phylae, species, strains and even individuals, can be seen as resulting from such different solutions for the same problems. When, how-

ever, an allotropic resonance form appears, during ontogenetic development, it results in an anomaly. At the level of genes or chromosomes, it produces a mutation or monstrosity. At a still lower level, such allotropic resonance forms may result, not in monstrosities or mutations, but in cancerous entities. The concept of cancer as a hierarchically organized disease accords with this view of allotropic resonance forms. A first cancerous entity would, therefore, develop when an allotropic resonance form occurs at a low level of the organization. Under favorable conditions, the allotropic entity would develop hierarchically, passing on through the different levels of the organization, and realizing allotropic chromosomes, nuclei, cells and tissues.

In order to have an invasive cancer, it would thus be necessary that an entire succession of favorable conditions be present insuring the development of a continuous line of hierarchic cancerous entities. These favorable conditions can occur spontaneously at each level, and both cancerous and normal entities may have many allotropic forms. Carcinogenesis would correspond to the creation of these favorable conditions, and, as seen above, it can take place at the different levels of the organization. The result is a hierarchic succession of the persisting forms of the allotropic series of respective cancerous entities.

The long time usually necessary for a cancerous condition to appear accords with this mechanism. The intervention of any external factor considered capable of inducing, by itself, the development of cancerous allotropic forms, has to be regarded as favoring the conditions necessary for the persistence and development of the succession of allotropic cancerous entities. (304)

This would also explain the relationship between hereditary factors and carcinogenesis. Just as the individual has the capacity to realize the successive allotropic forms, so a strain or even a species can inherit the tendency to develop such allotropic forms. This explains the persistence of mutation forms. It applies to the development of strains with high or low incidence of spontaneous tumors. It would also explain the vast differences among different species and strains in their response to carcinogens.

The concept of cancer as corresponding to a series of allotropic resonance forms at the successive hierarchic levels is of importance in terms of the intervention of external factors. Such environmental influences can establish conditions favorable for the development of allotropic forms at successive levels, permitting the progression of the allotropic line. The inequality of their action at different levels accounts for the big differences

seen between the various carcinogens. It is under this special aspect that we saw above the important problem of induced carcinogenesis.

The relationship between carcinogenesis and the defense mechanism can be understood in terms of the differences in defense capacities of allotropic and normal entities. From the study of cancerous and normal subjects, it appears that the latter have the capacity for defense responses. It appears highly possible that the characteristic of the "normal" resonance forms resides in their ability to resolve noxious interventions. The allotropic forms lack this ability. The loss of the defense mechanism at various levels, which is characteristic of cancer, can be regarded, up to a certain point, as being the result of the intervention of allotropic resonance forms, which would appear to be fundamentally inadequate to oppose the hierarchic progression of cancer. Incapable of responding with the full defense mechanism when confronted by continuous noxious interventions, cancerous entities use the simpler, primitive defense forms, and especially the lipidic prolonged form. This lipidic predominance represents the principal factor in producing the actual manifestations of cancer, with their dualistic nature.

The intervention of noxious factors in carcinogenesis is well known. Trauma and microbial viral infections in particular are such factors. Co-carcinogens such as croton oil, and some solvents such as benzene and toluene, can be considered noxious factors. (295)

This view of a plurality of factors intervening together to realize the hierarchically complex condition of cancer has another value. It helps to reconcile various explanations of the pathogenesis of cancer, each attributed to a different etiological factor, and each based on incontestable evidence. According to our view, with the possible exception of the broad-scale virus, which leads to the appearance of cancer cells in a couple of days, in all other cases a number of factors of different nature intervene. To resonance changes, would be added chemical, viral, metabolic, hormonal or defense influences, at the same or at different levels, in order to provide the necessary circumstances for cancer development. The fact that, regardless of the nature of agents used to induce them, cancers, once induced, differ very little or not at all makes plausible the hypothesis of plural exogenous factors acting to bring about the necessary favorable conditions.

The above presentation—a resume of our research—must still be considered to be a working hypothesis.

Other aspects of the cancer problem have been analyzed anew in the light of the concept of pathogenesis discussed above.

*Lipids and Carcinogenesis*

As we have seen above, the recognition of the fact that a series of successive changes take place in carcinogenesis has invalidated the concept that in order to consider a substance active in this field, it has to induce the entire series of changes by itself, including the passage into the phase of invasive cancer. An agent can be considered active if it induces only a part of the successive series of changes. Didactically, we can thus consider changes concerning the subnuclear, nuclear, cellular and metazoic levels. We have investigated the intervention of lipids in carcinogenesis from this specific point of view.

The coordination of data from various observations and experiments has indicated that some of the lipids would act especially at the cellular and tissue levels.

Statistical data have indicated a greater proportion of cancer of the cervix in non-Jewish women as compared to Jewish women. This was tentatively related to the circumcision of the respective males and this correlation was confirmed by statistical data concerning other groups of population practicing circumcision, such as the Moslems. The probable role of smegma was seen in experiments in animals made by different workers. It was reported that in mice, smegma, sterilized or non-sterilized, introduced in the vagina of mice followed by the suture of the vagina, would induce papilomatous and cancerous lesions of the cervix.

Statistical studies (324) showed a similar correlation between cancer of the prostate and circumcision, with a lower proportion of cancer in circumcised individuals than in those not circumcised. Entirely different results were obtained by other workers. Studies made by the group of Memorial Hospital in Cleveland, concerning cancer-in-situ, showed no differences between Jewish and non-Jewish women. Similarly, several workers have reported that the prostates in individuals over 40 years of age, who died of conditions other than cancer, have present in high proportion cancer-in-situ cells. No correlation with circumcision could be found in these cases, the same proportion being found in all the ethnic groups examined. (325)

We tried to interpret these totally discordant conclusions of the two groups of statistics and found the explanation in the concept of plural intervention of carcinogens. In the first group of statistics in the cases of both females and males, the cancerous processes considered were those in the invasive cellular stage, while in the second group of statistics, the non-invasive cancer phase was considered. It appears thus quite clear that the intervention of the circumcision and respectively of the smegma exists but

has to be placed at a precise point in the progressive changes in the evolution of the cancerous condition, at the passage from noninvasive into invasive cancer. Without any influence upon the appearance of the cancer-in-situ, the smegma would act manifestly by changing the proportion of active cancer present. Its influence appears thus to be exerted at the cellular level, where the occurring changes result in the passage of the noninvasive into the invasive cancer, and at the tissue level where the loss of the capacity to defend itself against the cancer cells permits their invasion. This led us to the hypothesis that the cancerous process in the cervix and prostate, evolves independently of circumcision until the cancer-in-situ step, but will make the next step toward invasive cancer predominantly under the influence of the smegma of noncircumcised males. Without such intervention at this stage, such a change may occur but in an impressively lower proportion. The invasive cancer of the cervix is thus almost entirely non-existent in virgin nuns. This consideration and the richness of smegma in positive lipids has permitted us to go farther and consider the problem of the role played by these lipids in carcinogenesis.

We have tried for a long time, but with litle success, to induce cancer in animals through the administration of unsaponifiable fractions alone. The several positive cases obtained in mice with repeated injections of the unsaponifiable fractions of placenta, chicken embryos, eggs or butter, have not appeared sufficiently consistent to warrant any conclusions.

In our attempt to investigate an intervention of the positive lipids for the specific change, corresponding to the passage of the noninvasive to the invasive cancer, we carried out the following experiment. We selected female mice with at least two previous pregnancies. After a third pregnancy, when in lactation, the mammary gland was injected with small amounts of the above mentioned unsaponifiable fractions. The results seem to indicate a higher proportion of mammary carcinoma in these mice than in controls.

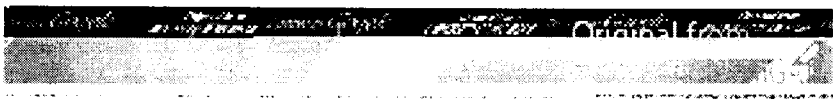
Similarly, we have tried to influence cells of the cervix in mice, through the introduction of the same different preparations of unsaponifiable fractions in the vagina of ex-breeder mice, followed by suture of the vagina. The first results have shown a high proportion of malignant tumors. The concept of the intervention of abnormal lipids in carcinogenesis has led us to utilize in similar experiments, instead of the above preparations, unsaponifiable fractions considered to be heterogenized by being heated above 320°C. At the moment, these experiments which are in progress seem to indicate the existence of such an influence.

It is of even greater interest to note the influence exerted by the unsaponifiable fractions upon animals that had received urethane, according

to the experiments of Berenblum. By combining three factors, urethane for the first changes in the amino-acids, several pregnancies for the changes until the noninvasive phase and unsaponifiable fractions especially heterogenized for the passage into the invasive phase, a high proportion of invasive mammary and cervical cancers seems to be induced in the first experiments. Of importance appears the factor that a certain lapse of time is necessary between each two factors which are applied in the above mentioned succession. This concords with the concept of plural successive changes in carcinogenesis discussed above.

*Carcinogenic Activity of Urethane*

The interesting research of Berenblum has brought an important contribution not only for the largely debated role of urethane as carcinogen, but also for the problem of carcinogenesis in general. The fact that croton oil, applied to the skin, induces the appearance of malignant tumors in animals previously fed with urethane, concords largely with the concept of plural changes taking place in carcinogenesis. The analysis of the influence exerted by carbamic acid upon amino-acids would place the intervention of this agent at the first members of the biological realm. It can thus be seen that the bond between the amino-acid group and the carboxyl and amine groups of carbamic acid occur in a way similar to that which occurs between two amino-acids with the big difference that in the first case it would result in the appearance of the CNCN formation. *(Fig. 201)* As mentioned above, this CNCN formation represents the group which characterizes the first biological entity. The place of this CNCN group, not at the end of the molecule opposed to the carboxyl as in the alkaline amino-acids, but as corresponding to the bond which results in polymers, represents the anomaly, which according to the work hypothesis we advance, would correspond to the first cancerous entity. The fact that the specific activity of urethane takes place at the lowest levels of organization, explains the necessity that a certain time separates its intervention from that of croton oil, which would act only at the higher levels, probably inducing the passage from noninvasive to invasive phase. This time is necessary for the first cancerous changes to build up the series of cancerous hierarchic entities since the cocarcinogen, croton oil, would act only in those more evolved cancerous entities. In experiments in progress, the passage of the urethane-induced noninvasive cancerous entities into invasive cancer, is successfully obtained by treatment with preparations of unsaponifiable fractions of placenta or eggs.



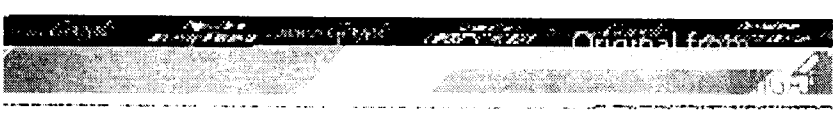
## INTERVENTION OF PSYCHOLOGICAL FACTORS IN CANCER PATHOGENESIS

The role of psychological factors in the pathogenesis of cancer, although still obscure, has been of increasing interest in recent years. Various theoretical considerations (*i.e.* the relationship between the known effect of emotions upon hormonal and biochemical balances and the possible effect, in turn, of the latter upon neoplasms), as well as a number of clinical reports and experimental and statistical studies, point toward psychological influence in cancer pathogenesis, but little is actually known in this area.

In order to explore this matter further, a research program has been carried on in our Institute since 1952 by Dr. L. LeShan. This study has included the evaluation of projective personality tests given to over 300 cancer patients; interviews of 2 to 8 hours each with over 150 patients; and extensive exploratory psychotherapy (of from 60 to 400 hours) for 25 patients. Control groups were also included in each category. For patients undergoing psychotherapy, regular comparisons were made between the personality picture and various biochemical activities reflected in blood and urine analyses.

A "back-and-forth" method between the three techniques of personality evaluation has been employed. Hypotheses formulated from data obtained with one technique have been evaluated, refined and clarified by data from the others. When a hypothesis was consistently supported by all three approaches, an attempt was made to formulate it in terms permitting it to be subjected to critical test by experimental or statistical technique.

As an example, an hypothesis was developed that the cancer patient, more often than chance would allow, had lost a major emotional relationship, and had been unable to find a satisfactory substitute, some time before the first apparent symptom of cancer. This hypothesis appeared to be validated by data from all three techniques. It was then formulated in terms by which it could be tested. If the hypothesis were true, then certain social groups which, a priori, had known higher rates of such losses should also have a higher cancer mortality rate. Thus, for example, if marital status were taken as the only variable, then, after age was cancelled out, we should expect the highest cancer mortality rate in the "widowed," the next in the "divorced," the next in the "married" and the next in the "single." Published data, such as census material, could be used to explore the accuracy of this prediction. Various predictions of this type—all based on the hypothesis—were made. When tested against published statistical data, all were demonstrated to be valid. (116, 117)



At this point of the research, one general, emotional pattern has been found in over 50% of the 300 studied cancer patients and in approximately 10% of the equated controls: An early life history with much self-doubting and some anxiety over relating to others; the establishment of one personal relationship that afforded a high degree of satisfaction, meaning and validity to the individual and provided him with a "raison d'être"; and the loss of this relationship, followed by inability to find a substitute, and a period of intense (if often concealed) depression. This has been elaborated upon, and case histories presented in various publications. (118, 119, 120, 121, 122)

In summing up this research in a recent paper (123), the following conclusions could be reached by LeShan:

1. There seems to be a correlation between the existence of neoplastic disease and the persistence of certain types of psychological situations.
2. The most consistently reported, relevant psychological state has been the loss of a major emotional relationship. Often the psychic state resulting from this loss could be traced to a period shortly before the first noted symptoms of cancer.
3. There appears to be some relationship between personality organization and the evolution of the cancerous condition.
4. There may be some relationship between personality organization and the type or location of a cancer.

It would seem as if future research in this area, to be as useful as possible, must focus upon the chemicophysiological changes which result from variations in psychic states in general. It is highly probable that these changes are mediated through the endocrine system. Through the linking of psychic states with hormonal changes, we may be able not only to integrate psychological factors with the many other factors influencing development of cancer, but also to relate them to certain levels of organization. It may be possible to establish the relationship of psychological factors to other influences which favor or even induce passage of cancer from one phase to another. This may permit a more complete understanding of cancer and help in finding new points at which some therapeutic value might be expected from psychological intervention.

The relationship between the adrenals and psychic states on the one hand, and between the adrenals and the lymphatic system on the other hand, could explain why the influence of a psychologically unresolved problem is most evident, among all cancerous conditions, in lymphomas. LeShan has been able, by analyzing a sizable number of these lymphoma cases, to

recognize more clearly and more consistently than in other conditions the existence of a pattern of psychological changes occurring prior to clinical illness. Recurrences of symptoms, or periods of exacerbation, also could be connected to events which had deep repercussions upon the psychological state.

Research along these lines, seeking information on psychic factors in the pathogenic mechanism of cancer, is being pursued actively by LeShan and his co-workers at our Institute.