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THE CHEMICAL MECHANISM OF CARCINOGENESIS- A HYPOTHESIS

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8 November 1974

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A hypothesis is presented that describes a single chemical synergistic mechanism for the production of all cancers. This mechanism depends upon the esterification within a cell of (1) an aryl hydrocarbon hydroxylase carboxylate metabolite of a polycyclic aromatic hydrocarbon and (2) a hydroxyl compound. Both of these compounds are always present within a cell; sometimes at relatively high concentrations. The specific

20. compounds and their concentrations will determine the probability of the formation of the ultimate carcinogen that can be physically and chemically incorporated into the DNA molecule during its formation. This report presents the evidence to show that only predictable specific polycyclic aromatic hydrocarbons react with all the other suspected carcinogens to form an ultimate carcinogen. These suspected carcinogens are any hydroxyl-like compounds that can form esterification type reactions or substances that can catalyse these carboxylate-hydroxyl reactions such as metal ions, asbestos, etc. Both polycyclic aromatic hydrocarbons (from pollution, cigarettes, etc.) and hydroxyl compounds (occurring naturally in the cell or from exposure to alcohol, most industrial pollutants or ionizing radiation, etc.) react synergistically. To prevent these reactions and the resulting carcinogenesis from occurring, it could be possible to purposefully cause noncarcinogenic reactions to take place by addition of other specific reactants with the polycyclic aromatic hydrocarbon-enzyme carboxylate.

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THE CHEMICAL MECHANISMS OF CARGINOGENESIS - A HYPOTHESIS

This report is the result of efforts to expand work and seek additional funds for an inhouse Independent Research program involving the determination of chemical species at tracer concentrations in biological materials. The National Cancer Institute is interested in knowing the mechanism of the initiation of cancer by trace metal ions in cells. In preparing a proposal in this area, an insight to the chemical mechanisms of carcinogenesis became evident. This report is the result of this study which was not supported by any project funds (except for typing and publication costs) of the Independent Research program.

The Independent Research program is "The Development of Delayed-Coincidence Mossbauer Spectrometry for Identification of Tracer Concentrations of Chemical Species in Materials" (Task Number MAT-03L-000/ZR000-01-01).

ROBERT WILLIAMSON II
Captain, USN
Commander

John B Wilcox

J. B. WILCOX
By direction

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INTRODUCTION

The cause of the initiation of a cancer has been ascribed to a large number of chemical substances, viruses, radiation, etc. This has come about because of the many observations of the one to one cause and effect (all other variables held constant) with (1) animal experiments when a specific carcinogenic initiator is applied or (2) statistical epidemiological analyses of humans who smoke, work in uranium or coal mines, etc.

A large effort has been made to find the mechanisms of various initiation pathways to cancer - but without success. That is, without success in being able to describe scientifically the cancer initiation mechanism and using this to predict the effect of any given carcinogenic initiator.

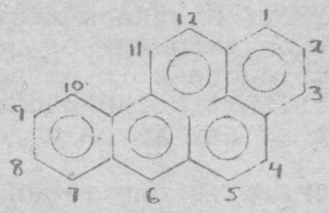
However, there is one recent exception. Seliger [1] has been able to predict which specific polycyclic aromatic hydrocarbons (PAHs) will initiate carcinogenesis and can describe the first steps in the chemical mechanism. This work appears to be of great significance because it is the only time a hypothesis has been proposed that has predicted carcinogenesis with testable scientific knowledge at the molecular level.

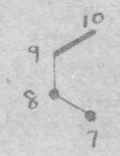
Since Seliger has been the only successful one of all those who have tried to demonstrate a predictive molecular mechanisms of the initiation of carcinogenesis, a possible conclusion could be that there is only one important mechanism for the initiation of all cancers and that all other apparent causes go through the same molecular mechanism. This would explain failures of discoveries of mechanisms from other "causes" (radiation, viral, metals, asbestos, alcohol, etc.) because there is only one important mechanism to produce cancer instead of many equally probable but different mechanisms. It is on the basis of (1) Seliger's unique discovery, (2) the above arguments, and (3) the evidence reported in this paper that forms the basis for the following hypothesis for the chemical mechanism of carcinogenesis.

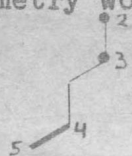
MECHANISM FOR CARCINOGENESIS WITH AROMATIC HYDROCARBONS

Seliger [1] has proposed that the cells' hydroxylase enzymes convert only the carcinogenic polycyclic aromatic hydrocarbons to an enzyme-bound $n\pi^*$ excited state product after an oxidative ring cleavage. PAHs have two active electron-dense

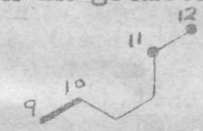
sites. The highest electron-dense site will be the position at which the PAH will attach to the enzyme. The separate but sterically related secondary electron-dense site on the PAH will be where the hydroxylation will take place. If the PAH leads to a carcinogenic compound, two separate hydroxylation reactions take place resulting in oxidative ring cleavage and an $n\pi^*$ excited state. The necessary step in the expression of the carcinogenicity of a PAH is that the internal oxidation of a monohydroxylated PAH requires a second adjacent carbon for oxygenation which occurs only for the specific electron-dense K-2 and K-3 geometries.

In 3,4 benzopyrene, 

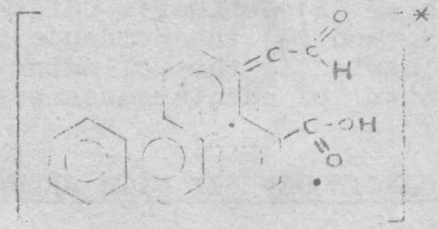
, for example, a K-1 geometry would be 

, a K-2 geometry would be 

, a K-2R geometry would be 

, and a K-3 geometry would be 

where the thick solid line indicates the electron-dense binding site and the solid dots indicate the sterically available sites at which oxygenation may occur.

The resulting enzyme metabolite carcinogen for 3,4 benzopyrene will be 

which probably remains associated for some time with the enzyme through the carboxyl group. If only the K-1 or K-2R geometries are involved, phenols, dihydrodiols or quinones are produced which are rapidly eliminated as body wastes.

In summary, Seliger has been able to predict the carcinogenic activity of specific PAHs and the chemical form of the enzyme metabolite of (1) the soluble noncarcinogenic PAH and (2) the ring-cleaved oxygenated carcinogenic PAH which passes through an identifiable excited radiative step when formed by the enzyme.

Two different mechanisms could account for the sometimes observed increased production of the carcinogenic form of the PAHs. One is the stimulation of the aryl hydrocarbon hydroxylases (AHH) (a) by attachment of the oxygenated PAH to the enzyme to make it inactive and thus stimulate more enzyme production (1) or (b) by other chemicals (from any source). The other is the chemical reaction of the carcinogenic PAH-enzyme metabolite with chemical compounds in the cell that can react to form a product that can be incorporated into DNA.

A logical test of these two alternatives is to add various chemicals in vivo in the cell and observe their carcinogenity. Some chemicals are known to stimulate enzyme activity, and for these it would not be possible by this test to tell the differences between the two proposed mechanisms. However, it will be possible to make a distinction by using those chemicals that are not expected to stimulate enzyme activity but that do enhance carcinogenity.

For the second proposed method, obvious groups of compounds that would be expected to react with the carcinogenic PAH-enzyme products are those that react (in the cell environment) with carboxyl and aldehyde functional groups. A common reaction is that of an alcohol with an acid to form an ester.

OBSERVED EFFECTS OF HYDROXYL COMPOUNDS ON CARCINOGENESIS

It has been observed by Pound (2) that skin tumor production in mice is increased if acetic acid or croton oil (contains 12-O-tetradecanoylphorbol-13-acetate and phorbol-12-13-didecanoate) is administered to the skin at the same time or prior to the addition of each of three carcinogenic PAH compounds (7,12-dimethylbenz(a)anthracene, benzo(a)pyrene, and dibenz(a,h)anthracene). Earlier work of Pound showed urethane and croton oil also increased skin tumor production. The explanation given for this by Pound was that the croton oil proliferated cell production (and thus DNA production) making the cell more susceptible to the tumor-initiating action of urethane. Also, the number of tumors produced related to the cellular proliferation.

The amounts of acetic acid or croton oil applied were the highest amounts possible without producing ulceration.

On repeated application of Croton oil alone, Roe (3) and Boutwell, et. al. (4) observed a minor carcinogenic effect. However, acetic acid did not by itself lead to the production of tumors (5).

Another observation in the work of Pound was that there was no effect on tumor yield by an increased capacity of the skin to retain hydrocarbons. The expected increase in tumor yield on the 9th day after preliminary application of croton oil was not observed over the 3rd day application.

Pound's conclusion is that, as a general phenomenon, cells that proliferate rapidly become more susceptible to the action of a carcinogen.

Another interpretation of Pound's work involving the carcinogenic effects of preliminary application of acetic acid or croton oil is that the concentration of these substances within the cell increases after application and are available for chemical reaction with the metabolic product produced by the reaction of the PAH with AHH. It is significant to observe that the addition of only croton oil produced tumors. It is suggested that increased carcinogenesis came about through the relatively small concentration of PAHs (perhaps mostly from pollution sources) always present in the cell. The AHH-PAH metabolite reacted with the croton oil to put it in a form available for substitution into DNA.

Bock (6) has studied the nature of tumor-promoting agents in tobacco and tobacco products. He has separated a methanol soluble and methanol insoluble fraction from an aqueous extract of unburned tobacco leaves and various brands of commercial cigarettes. Both fractions promote tumors. Similar experiments done with cigarette smoke condensate have shown that, in particular, a phenolic fraction can act as a tumor promoter(7,8) It was shown that the bulk of the tumor promoting activity of smoke condensate is accounted for by the phenolic fraction and various subfractions of the neutral fraction. Ether-soluble bases are of marginal activity. A logical conclusion of their work is that tumors are promoted by the more polar volatile fractions of tobacco leaves, unburned cigarettes and cigarette condensates and that these fractions would be expected to contain the compounds with hydroxyl functional groups. These hydroxyl compounds would migrate to the same cells as the PAHs where they could then react with the AHH-PAH metabolite to form the ultimate carcinogen.

Wynder and Hoffmann[9] have noted that some volatile phenols possess tumor-promoting activity. They also observed that the tumor initiating activity of a tobacco tar was about 20 times greater than could be explained by the measured, benzo(a)pyrene content. They assumed that other tumor initiators are present. Another explanation could be ascribed to the presence of phenolic promoters reacting in the cell with the AHH-PAH metabolite.

Davies, et al.[10] made a study of the dose response of mouse skin to cigarette smoke condensate. A given amount of cigarette smoke condensate was dissolved in each of two solvents and applied at various doses. The two solvents were 9:1 acetone/water and 4:1 isopropyl alcohol/acetone. Graphs of percent tumor bearing animals and percent infiltrating carcinoma bearing animals plotted against cigarette condensate dose (from 65 to 300 mg per week - log scale) always showed sigmoid curves (including condensates from two different cigarettes). In all cases the carcinogenic effect was about twice as large for the isopropyl alcohol/acetone solvent system than for the acetone/water solvent system. The authors explain this difference in effect by "either more rapid penetration of carcinogen or larger amounts of carcinogen reaching the target cells". Another reasonable assumption to explain this large solvent effect would be the presence of large concentrations of an alcohol in the cell available for esterification-like reactions with the AHH-PAH metabolite. This alcohol concentration would be above a natural background of particular compounds that could combine with the AHH-PAH metabolite.

In Davies' work three different control groups were run concurrently: (1) untreated with solvent or condensate giving zero percent tumors on 120 mice after 110 weeks, (2) acetone/water applications giving one percent tumors on 180 mice, and (3) isopropyl alcohol/acetone applications giving two percent tumors on 180 mice. These percentages are about an order of magnitude less than those for the mice treated with the low dosages of cigarette condensate in equivalent amounts of solvents.

The hypothesis proposed in this work would explain Davies' results as follows: The solvents react with the environmentally originating PAHs that are metabolized in the cell by AHH. The isopropyl alcohol solvent reacts more readily than the acetone solvent. As additional PAH is added from the cigarette condensate the same results are observed except to a greater extent. If this explanation is correct, it would imply that the observed greater number of tumors with increasing PAH dose is due to a freeing of enzymes (from the solvent reaction with the PAH-AHH complex) to react with additional PAHs instead of stimulation of the enzyme system so that there can be more enzymes to

react with the additional PAHs. As was observed above, there is a measureable increase in tumors from just the solvent alone (compared with the untreated controls). The solvent alone is unlikely to stimulate the aryl hydrocarbon hydroxylase system.

EPIDEMIOLOGICAL EFFECTS OF HYDROXYL COMPOUNDS

There is an abundance of evidence that alcohol is an important factor in causing cancer in man (11). In 1964, the World Health Organization(12) concluded that the association between excessive drinking of alcoholic beverages and cancer of the mouth, larynx and esophagus has been demonstrated in several epidemiological studies. They commented that alcoholism is associated with other factors that may be important in producing cancer, such as dietary deficiencies. Flamant(12), observing that heavy drinkers are usually heavy smokers, reported that hypopharyngeal and laryngeal cancer had a very strong relationship to both alcohol and tobacco use, while cancer of the esophagus and tongue had a very strong relationship to alcohol intake but only a strong relationship to smoking.

In addition to the mechanism proposed here for the carcinogenic effect of alcohol on man, others have been proposed that (1) alcohol is a carcinogen (with tobacco) as a trigger mechanism for a hypothetical viral cause, (2) cancer is the consequence of alcoholism which affects malnutrition, anemia, and poor hygiene, and (3) cancer is due to the possible presence of carcinogenic substances introduced in the production of some alcoholic beverages.

There have been many studies of cancer in the upper aerodigestive trace in man(11). One of these (13) shows that patients who had primary cancers of the floor of the mouth, the hypopharynx and the esophagus, had a higher drinking-to-smoking ratio than patients with primary cancers of the roof of the mouth, the larynx and nasopharynx who smoked more then they drank. A causative effect is suggested here since alcohol comes in closer contact with the ingestion tract and tobacco comes in closer contact with the inhalation tract.

Rothman and Keller(14) have made an epidemiological study of the relative risk of oral cancer according to level of exposure to alcohol and smoking. Their results are shown in Table I and support the idea that the risk of buccal and upper

respiratory tract cancer among those who use both alcohol and tobacco was greater than the sum of either risk alone. It is a result of the proposed hypothesis of this paper that the more than double risk of those who drink heavily but do not smoke is due to PAHs in the cells from mainly air pollution.

Further support of the effect of air pollution on causing cancer of the upper respiratory tract is given by epidemiological studies of Lilienfield, et al.[15]. Table II is compiled from their work. It ranks the ratio of the risk of cancer in metropolitan counties with a central city to the risk of cancer in nonmetropolitan counties in the United States for black men and women. It is a ratio of urban (mostly ghetto) to rural communities for each primary site of cancer. Air pollution would be expected to be high in the central city areas. The ratios are not as large as they probably actually are because the metropolitan counties with a central city do have some rural areas and the nonmetropolitan counties do have small cities where pollution could be high. The ranking appears to be about that expected for physical access of PAHs to the cells of the primary sites listed. It is suggested that this observed causative effect is due to two factors: (1) concentration of PAHs in the cell and (2) concentration of organic hydroxyl compounds in the cell. The effect would be synergistic and not additive.

The concentration of alcohol exposed to the cells in question is probably a significant factor in carcinogenesis. Wynder[16] has found that the relative risk of cancer of the mouth, extrinsic larynx and esophagus was much more for whiskey drinkers than for beer and wine drinkers. Another important factor might be the time sequence of smoking and drinking. The cancer risk might be greater if the smoking and drinking are done at the same time. This is the case with cocktail drinkers smoking cigarettes. Such a synergistic effect could explain the higher cancer rate among cigarette smokers compared to cigar and pipe smokers. Drinking is usually not done while smoking a cigar or pipe. The body fluids then have time to wash away and dilute the concentration of alcohol in the membrane cells.

The validity of the carcinogenesis of PAHs and alcohol hypothesis could also be made for occurence of cancer in the aerodigestive tract of coal miners (who have developed black lung disease). The author is not aware of any epidemiological study in this area.

There is abundant evidence of the effect of alcohol, tobacco, urbanization and occupation on incidence of cancer of the esophagus[11]. For example, it has been found that bartenders are twice as susceptible to esophageal cancer as the general population. Deaths from esophageal cancer occurred from those who drank primarily distilled spirits which suggests that the alcohol strength may be more important than the quantity of alcohol consumed - even when it was sufficient to cause cirrhosis (esophageal cancer correlated more with alcoholism than with cirrhosis). The high rate of esophageal cancer among Singapore Chinese correlated with drinking samsu, a strong local liquor. The custom has been to drink all drinks at "burning hot" temperatures. Here, diffusion rates of alcohol into cells would be expected to be high.

There have been many studies[11] on the correlation of alcohol consumption with cancer of the liver, lung, pancreas, large bowel, prostrate gland and stomach. It is proposed in this report that since there are PAHs and AHHs in all cells and since it is necessary for the PAH-enzyme carboxylate metabolite to react with an alcohol, the only thing that is being observed in a cancer rate increase in drinkers is an increase in the kinetics of esterification due to an increase of alcohol concentration in the cells. What is needed for elucidation of this hypothesis is (1) the measurement of hydroxyl concentrations in cells of organs, (2) carbon-14 studies on the distribution in the organs of animals of the various trace concentrations of PAHs, tobacco condensates, coal dust, etc. and (3) the correlation of these two measurements with cancer mortality.

It has been believed that alcohol is a totally foreign substance in man. However, recent work has shown that alcohol is normally present in all mammals and is continuously produced in the intestinal tract by the action of microorganisms on soluble sugars[17]. It can be calculated that the total abstainer would produce enough alcohol to equal that in about a quart of 3.2 percent beer per day. This alcohol does not reach other organs such as man's brain since ADH in the liver metabolizes almost 99 percent of it before it might move out into general circulation. It is only at the high concentrations of alcohol introduced into the circulating blood by drinking alcohol when the ability of ADH to metabolize it is overwhelmed. The alcohol passes into systemic circulation and is distributed throughout the organs of the body, including the brain, producing its well-known effects and perhaps also cancer.

ULTIMATE CARCINOGENS

What are the requirements for an ultimate carcinogen to be part of the DNA structure For one thing, it must have a structural part that is similar chemically and physically to purine or pyrimidene. This part must be attached in glycosidic linkage to deoxyribose moieties which in turn are connected by 3',5'-phosphodiester linkages. It is suggested in this report that such structures are formed by the aryl hydrocarbon hydroxylase acting of specific PAHs to form a carboxylic acid functional group after a ring cleavage. The carboxylic acid group is esterified by an alcoholic (or similar) compound that is in the cell (by natural or artifical means) to form an ultimate substance that is available for incorporation during the formation of a DNA molecule.

This ultimate carcinogenic substance could be formed in another way. Miller[18] has summarized work he and others have done on the formation of ultimate carcinogens by an esterification between hydroxyl functional group compounds formed by N-hydroxylation of aromatic amines and amides with acidic functional groups. The resulting ultimate carcinogen is the same as the enzyme metabolite-alcohol except that it was formed in the opposite way: the purine or pyrimidene portion contained the hydroxyl functional group and the cell contained a concentration of a specific acid. It would seem that of all the possible esterification reactions that could take place in the cell, only those that produce an ester that can sterically fit into the DNA structure have even a chance to cause the DNA molecule to exhibit its carcinogenic characteristics. There are probably many instances of similarly produced ester compounds that are incorporated into DNA that do not result in carcinogenesis.

Nitrosamines can form phenols in acid solutions. Aromatic nitrosamines injected into the acidic stomach can migrate into cells of the stomach wall and be available for coupling reactions or replacement reactions by -OH. For example,

$$ArN_2^+ + H_2O \xrightarrow{H+} ArOH + N_2 .$$

The phenol is then available for esterification with the PAH-enzyme metabolite (the same as the mechanism proposed in this paper for other hydroxyl compounds).

Investigators at the Roche Research Center in Nutley, N.J. have recently found that Vitamin C prevents the formation of cancers produced by the reaction of sodium nitrite with substances in the stomach. Sodium nitrite is used in meat processing to suppress the outgrowth of botulinum bacteria and to impart characteristic flavor and color to cured meat products. The sodium nitrite from this source, or from pollution or from decomposition of other nitrosamines can react in the stomach or in the cells of the stomach wall with primary aliphatic amines or secondary aliphatic amines (perhaps in peptide or protein molecules) to form a mixture of alcohols:

$$RHN_2 + NaNO_2 + HX \longrightarrow [RN_2^+ x^-] \xrightarrow{H_2O} N_2 + \text{mixture of alcohols and alkenes.}$$

For example, the reaction of n-butylamine with sodium nitrite and hydrochloric acid yields 25% n-butyl alcohol and 13% sec-butyl alcohol.

If the mechanism of carcinogenesis for nitroso compounds is through production of alcohols in the stomach, then reactions of these alcohols with such substances as Vitamin C would compete with the PAH-enzyme carboxylate metabolite.

Dinman(10) has summarized the biological evidence for occupational cancers. The chemical compounds or elements which definitely have been attributed as carcinogenic to man from studies of occupational exposure are beta-napthylamine, 4-aminodiphenyl, chromium, nickel, benzidine, arsenic, mustard gas, 4-nitrobiphenyl, alpha-naphthyamine, beryllium, and benzene. In addition animal studies implicate carcinogenesis when working with nitrosamines, N-nitrosodimethylamine, alkylating agents (nitrogen mustards, imines, epoxides, lactones), dichlorobenzidine, orthotolidine and dianisidine. Unidentified components in contaminated mixtures are mineral-derived oils, coal tars, pitches, asbestos, haematite, magenta and auramine. It is believed that all of these compounds from occupational exposure as well as all other compounds from other exposures (PAHs, urethane, radiations, etc.) either react with AHH to form a carboxyl metabolite or provide a hydroxyl compound to react or catalyze a reaction with the AHH carboxyl metabolite. It should be noted that hydroxyl radicals are one of the main products from the reaction of ionizing radiation on protoplasm.

CONCLUSION

A hypothesis has been described to explain the initiation of carcinogenesis: A molecule chemically and structurally similar to a nucleotide unit of DNA is formed in a cell by the esterification reaction of an acid and an alcohol. The acid portion of the ester is usually formed from the reaction of particular PAHs with the AHH enzyme. The alcohol portion can come from contact of the cell with an alcohol or a naturally occurring alcohol in the cell. Other suspected carcinogens simply catalyze the esterification reaction (e.g., metal ions, asbestos) or provide a source of the acid or the alcohol (e.g., radiation, nitrosamines).

Once the complex chemically inert ester is formed and separated from the enzyme, it will not be removed from the cell because of its water insolubility and thus will spend some of its time (over possibly very long time periods) in close proximity with DNA molecules. It will constantly be in equilibrium with its acid and alcohol fragments which will always be in very low concentration and dependent on cell composition and PH. The acid fragment and/or the alcohol fragment could then be available for attachment in the build up of a DNA polyester chain to the sugar (the alcohol portion) or the phosphoric acid (the acid portion). This could end the construction of this polyester chain and eventually cause the deregulation of the cell.

Support for the proposed esterification mechanism hypothesis of carcinogenesis is given by:

(1) It is possible to describe the mechanism by which PAHs are converted by enzymes to carcinogenic carboxyl metabolites and thus to be able to predict which PAHs are carcinogenic.

(2) The initial mechanism of carcinogenesis at the molecular level is known only for PAHs and no other "carcinogen" (even though extensive efforts have been made).

(3) Solvents containing hydroxyl groups can initiate a cancer even if a PAH is not also added. Some PAHs are already in cells from pollution or natural sources. The added PAH will simply mean that the carcinogenic reaction rate will be increased - which is observed.