of theories do not exist, there is an ever expanding pool of knowledge derived from experimental investigations that leads us closer to the answers.

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A BRIEF REVIEW OF CHEMICAL CARCINOGENESIS

Advancement of chemical technology is the primary mission of the Texas Institute for Advancement of Chemical Technology. The purpose of this report is to advance technology through an informed public.

Charles D. Holland, President
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and
Professor Emeritus of Chemical Engineering
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Special Report 1, 1989

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ACKNOWLEDGMENTS

This report is based on a vast volume of literature describing an untold number of years of experimentation. The following reviewers were of inestimable value to me in the evaluation and interpretation of the literature, and I am most grateful for their help.

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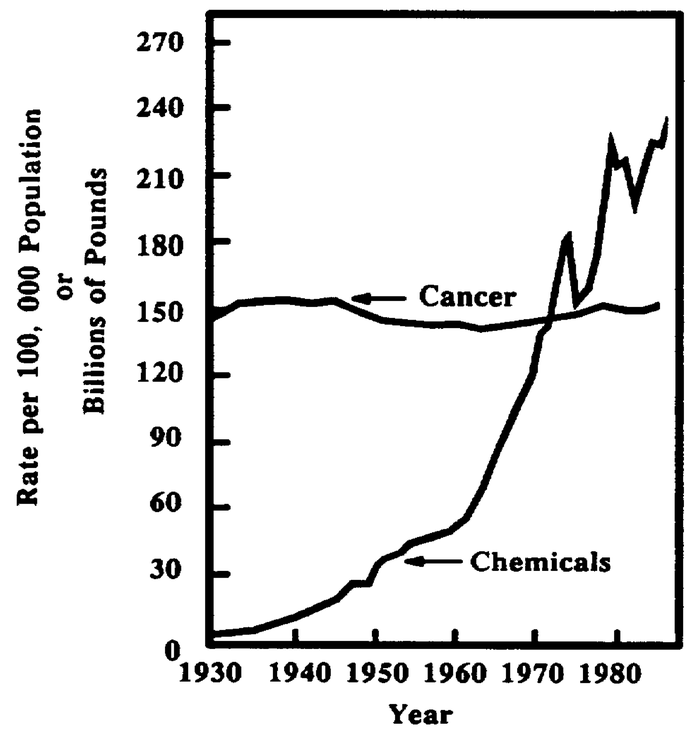

Figure 1. The overall cancer rate has remained essentially constant while the production rate of organic chemicals has increased over two hundred fold during the past 50 years. [Age-adjusted to the 1970 U. S. Census; United States Trade Commission,[6] and U. S. National Center for Health Statistics and U. S. Bureau of the Census.[7]]

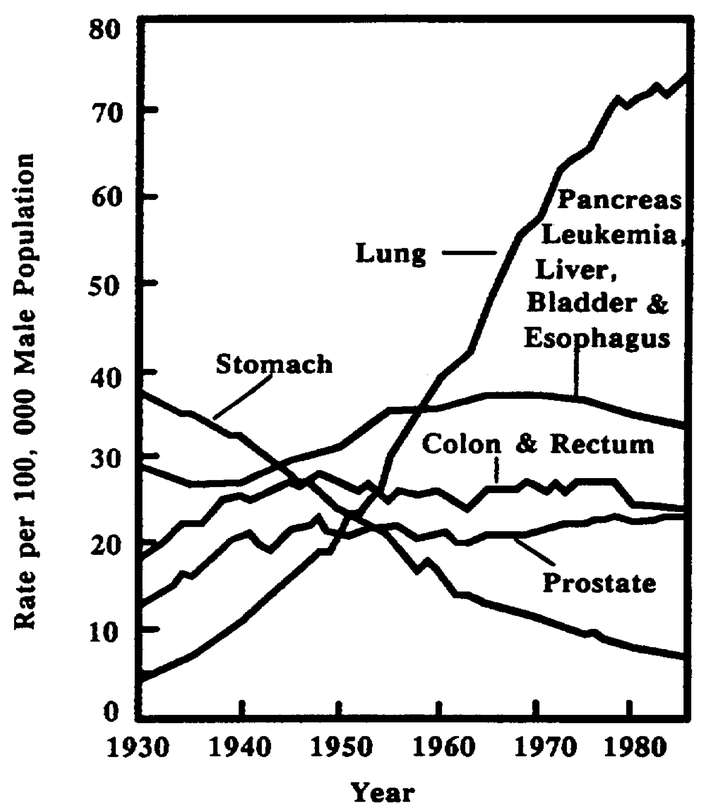

Figure 2. Age-adjusted cancer death rates for selected sites, males, United States. [Age-adjusted to the U. S. 1970 Census. Source of data: U. S. National Center for Health Statistics[6] and U. S. Bureau of the Census.[7]]

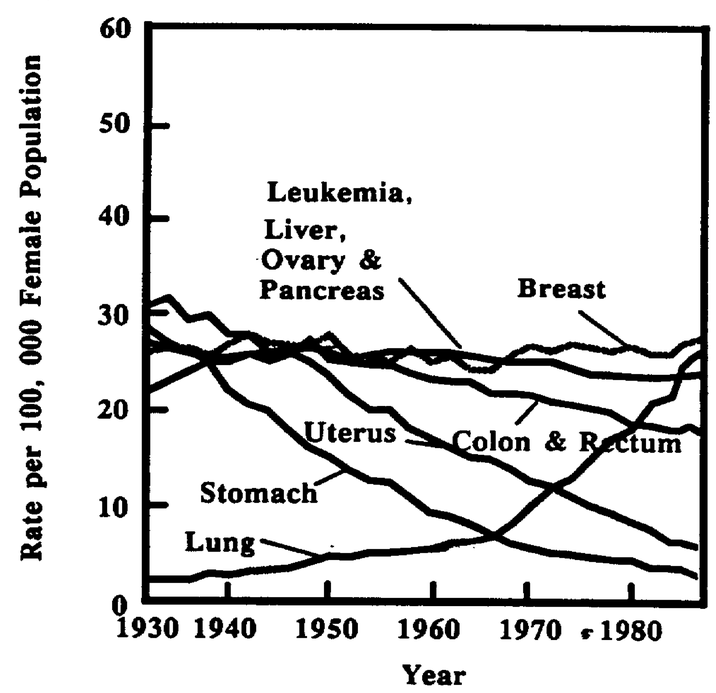

Figure 3. Age-adjusted cancer death rates for selected sites, females, United States. [Age-adjusted to the U. S. 1970 Census. Source of data: U. S. National Center for Health Statistics[6] and U. S Bureau of the Census.[7]]

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A BRIEF REVIEW OF CHEMICAL CARCINOGENESIS

The purpose of this review is three-fold: (1) to provide managers and engineers with an in-depth overview of the developments in the field of chemical carcinogenesis,(2) to provide useful facts and graphs that refute exaggerated claims and false beliefs held by the public, and (3) to provide the background material for the preparation of the next issue of INSIGHTS which is to be entitled "Chemical Carcinogens and Cancer in Perspective."

Our inability to find cures for all of the dreaded set of diseases of cancer makes cancer an emotional issue. Out of confusion, the general public has become an easy prey for those who claim to know all of the answers. Exaggerated claims in the 1970's by a number of people, including some with scientific training, that almost all cancer was caused by industrial pollution have been discredited by the careful studies of Doll and Peto[1]†, Higginson[2], Higginson and Muir[3], Cole[4], and Wynder and Gori[5] in which the major causes of cancer have been identified. The work of Doll and Peto[1], published in 1981, is the most detailed analysis of the causes of cancer that has been presented up to this time, and parts of it are presented in subsequent sections.

Many have heard only the exaggerated claims and have formed opinions on that basis. There are, however, real risks which must not be confused with the exaggerated claims. The purpose of this report is to "set the record straight," present facts provided by scientists, and give an assessment of just where we are.

Comparison of Cancer Mortality with the Production of Chemicals

A cancer epidemic has been alleged and attributed to "man-made chemicals." As shown in Figure 1, the facts do not support this allegation, as pointed out by Burack.[8] A disease may be highly prevalent, but this fact alone does not make it an epidemic. If a disease is growing rapidly throughout a population at a rate clearly in excess of that normally expected, it is classified as an epidemic. Cancer is a prevalent disease but it is not an epidemic because the total mortality rate of cancer has remained essentially constant from 1930 to 1985 [To correct for yearly changes in population, it is customary to age-adjust all rates relative

† The study of Doll and Peto[1] is the most thorough and massive of those made to date, and it is drawn upon heavily throughout the present analysis. At the time this study was published, Sir. Richard Doll was Honorary Director, Imperial Cancer Research Fund, Cancer Epidemiology and Clinical Trials Unit and Warden of Green College, Oxford, United Kingdom, and Mr. Richard Peto was Imperial Cancer Research Fund Reader in Cancer Studies, Nuffield Department of Clinical Medicine, University of Oxford, Radcliffe Infirmary, Oxford, United Kingdom.

This study by Doll and Peto is a report commissioned by the Office of Technology Assessment, U.S. Congress, to provide background material for their assessment of "Technologies for Determining Cancer Risks From the Environment" (OTA, 1981).

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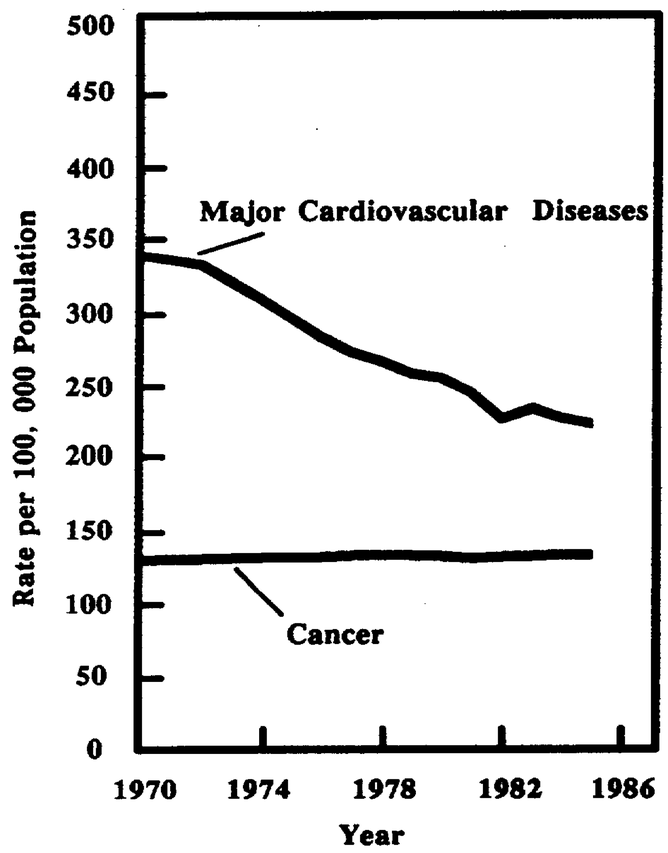

Figure 4. Age-adjusted death rates for major cardiovascular diseases and malignant neoplasms. [Source: Vital Statistics of U. S. Department of Health and Human Services: age-adjusted to the 1940 U. S. Census.[7]]

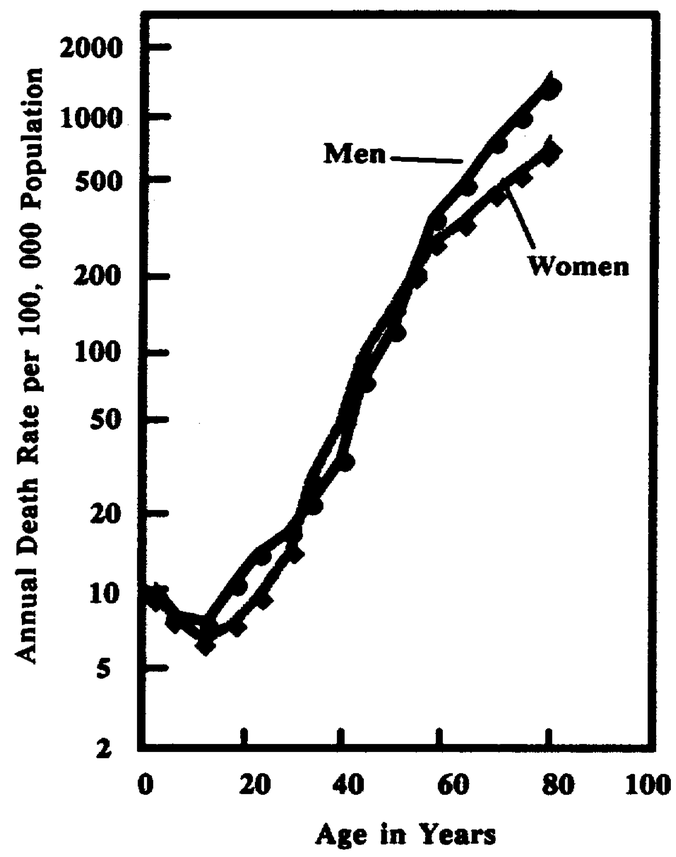

Figure 5. Cancer death rates by age and sex. Source: Vital Statistics of the United States, 1977. [From Schottenfeld, D. *Cancer*, 47(5), 1095 (1981

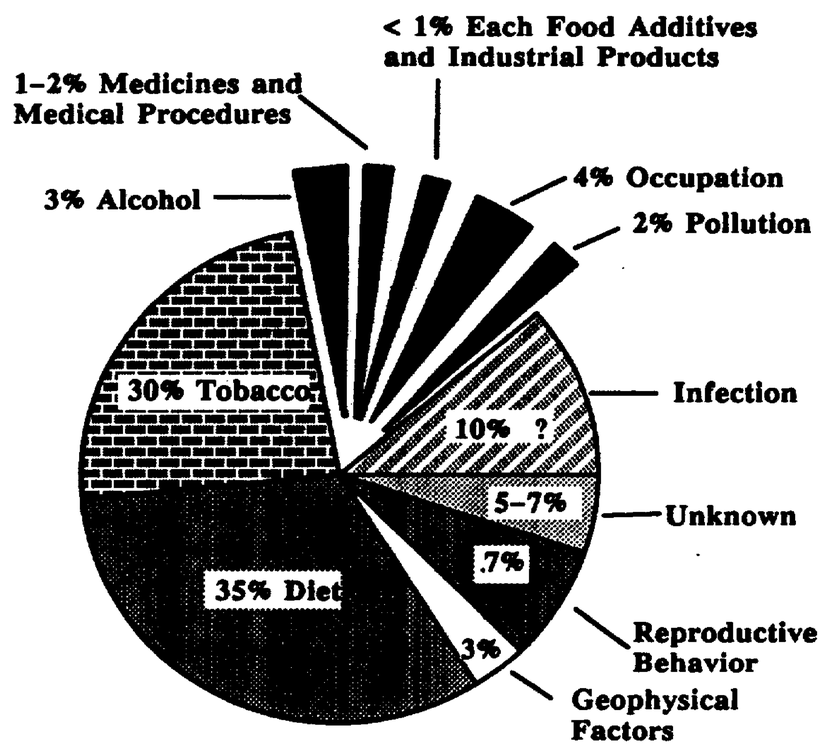

Figure 6. Distribution of cancer proposed by Doll and Peto.[1] [Age-adjusted to the 1970 U. S. Census.]

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to the population for a particular year.] Over this period of time, the production rate increased over two hundred fold. Likewise emissions from automobiles and diesel-powered vehicles (which contain some carcinogenic material) have increased enormously but total cancer mortality rate has not. Even accounting for a latency period of 15 to 25 years for cancer to develop after exposure, the data do not demonstrate any detectable cancers from the above exposure.

The history of cancer deaths from 1930–1985 is displayed in Figure 2 for men and in Figure 3 for women. Observe that the decrease in stomach, colon, uterus, and ovarian cancer rates is approximately equal to the increase in the lung cancer rates. If it were not for lung cancer, the total cancer mortality rate would have declined. These figures demonstrate that there is no epidemic of cancer in the United States except possibly for lung cancer which is known to be caused primarily from the use of tobacco. The perception that there is a significantly higher cancer rate today than there was in the past results in part from the following reasons. First, because of the discovery of preventions and cures for other once fatal infectious diseases, cancer has become a more prevalent cause of deaths. Also, as illustrated by Figure 4 great strides have been made in the treatment of the major cardiovascular diseases. Since everyone must die of something, the prolonging of life allows time for tumors and cancers to develop with the result that cancer will become even more prevalent than it is today as a "disease of old age". Thus, our success in the treatment of other diseases has contributed to cancer's becoming more and more a disease of the elderly; 60% of cancer deaths occur in persons 65 or older, 34% in persons 45 to 64, and 6% in persons under 45 years of age, as shown by Schottenfeld[9] in Figure 5. Second, many cancers were not diagnosed in the past, particularly in the elderly, whose deaths were frequently attributed to "natural causes" and "old age". Diagnostic methods have improved and are continuing to do so, leading to the identification and treatment of cancers which would have been missed in the past. Third, people are more open today about the diagnosis of cancer and the subsequent treatments than they were in the past. There was a tendency to keep the diagnosis a secret and in many instances it was even withheld from the patient.

Leading Causes of Cancer

On the basis of an analysis of data compiled on various types of cancer mortality up until 1980, Doll and Peto[1] deduced the distribution of the causes of cancer mortality shown in Figure 6. According to their analysis, diet is the leading cause of cancer with 35% . Doll and Peto point out that although the figure of 35 % is a plausible total, the parts that contribute to it are uncertain in the extreme. The range of acceptable estimates is taken to be 10 to 70%, as shown in Table 1. Use of tobacco is second in the cause of cancer, and accounts for 30% of the mortality with an acceptable range of estimates from 25 to 40%. A figure of less than 1% was attributed to food additives with a range of accepted estimates of -5 to 2%. The -5% allows for a possible protective effect of antioxidants and other preservatives which may behave as anticarcinogens.

The figure of 4% allocated for occupational hazards by Doll and Peto was thought to be accurate by a factor of about two, and thus the acceptable range of estimates was given as 2

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Table 1. Range of Best Estimate for the Cause of Cancer Shown in Figure 6. [Taken from Doll and Peto.[1]]

Cause of Cancer	Percent of All Cancer Deaths: Best Estimate	Range of Acceptable Estimates
Tobacco	30	26 to 40
Alcohol	3	2 to 4
Diet	35	10 to 70
Food Additives	< 1	< -5 to 2
Reproductive and Sexual Behavior	7	1 to 13
Occupation	4	2 to 8
Pollution	2	< 1 to 5
Industrial Products	< 1	< 1 to 2
Medicines and Medical Procedures	1	0.5 to 3
Geophysical Factors	3	2 to 4
Infection	10 ?	1 to ?
Unknown	5 to 7 *	?

* The "?" used by Doll and Peto to denote their uncertainty has been replaced by 5 to 7 so that the best estimates have a sum of approximately 100.

Table 2. Group I – Cancer not Known To Be Produced by Occupational Hazards. [Based on Doll and Peto.[1]]

Types of Cancer	Number of Deaths Recorded in 1978 (United States): Male	Female
Lip, Tongue, Pharynx, Small intestine, Gallbladder, Other uterine cancer, Ovary, Melanoma, Breast, Cervix uteri, Other female genital organs, Eye, Male genital organs, Thyroid, Myeloma (or bone marrow)	13, 386	67, 499
TOTAL DEATHS: Male plus Female	=	80, 885

Table 3. Group II – Cancer that Possibly May Be Produced by Occupational Hazards. [Based on Doll and Peto.[1]]

Types of Cancer	Number of Deaths Recorded in 1978 (United States): Male	Female
Mouth, Esophagus, Stomach, Colon and Rectum, Pancreas, Kidney, Brain, Hodgkins disease, Non-Hodgkins lymphoma	73, 084	63, 212
Postulated due to Occupational Hazards	731	316
TOTAL DEATHS: Male plus Female	=	136, 296
TOTAL DEATHS DUE TO OCCUPATIONAL HAZARDS: Male plus Female	=	1, 047

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to 8%. A token figure of 2% was assigned to pollution, due chiefly to the uncertain effect of the combustion products of fossil fuels in urban air. Because of the uncertainties involved in attempting to account for the effects of pollution, the acceptable range of estimates of less than 1 to 5% was said to be rather arbitrary.

Although there is no cause for alarm from trends in cancer incidence, many industrial products have been so recently introduced that their effects would not yet be apparent. Even though less than 1% was attributed to cancer mortality caused by industrial products with a range of acceptable estimates of less than 1 to 2%, Doll and Peto cautioned that there is too much ignorance for complacency to be justified. The figure of 1% for medicines and medical procedures has a range of acceptable estimates of 0.5 to 3 %. Geophysical factors such as radiations may account for about 3% which is quite reliably known, with a range of acceptable estimates of 2 to 4%.

The best estimate of 10% for cancer caused by infections contains a lot of uncertainty, and only the lower number of 1% was given for the range of acceptable estimates. In the case of unknown causes, Doll and Peto give no numbers, and the figure shown in Figure 6 was obtained by difference. Doll and Peto point out that the firmest estimates in Figure 6 are those for tobacco, alcohol, and geophysical factors. Tobacco and diet are seen to be by far the largest contributors to cancer mortality.

To estimate the cancer mortality attributable to occupational hazards, Doll and Peto[1] divided all cancer mortality into three groups and estimated the number in each group which could have been caused by occupational exposure. In the first group shown in Table 2, no cancer mortality were attributed to occupational hazards. In the second group, presented in Table 3, the evidence that any of the cancer mortality resulted from occupational hazards is weak to inconclusive. Thus, in the absence of definite evidence, Doll and Peto allocated a token of 1% of the cancers in males and 0.5% of those in females for a total of 1,047 mortality.

Table 4. Group III – Cancers which Can Be Caused by Occupational Hazards. [Based on Doll and Peto[1]]

Types of Cancer	Number of Deaths Recorded in 1978 (United States)	
	Male	Female
Mesentery and Peritoneum (membranes), Liver, Larynx, Lung, Pleura, Nasal sinuses, Bone, Skin (other than melanoma), Prostate, Bladder, Leukemia, and Others	131, 867	52, 907
Postulated due to Occupational Hazards	14, 046	1, 976
TOTAL DEATHS: Male plus Female	=	184, 774
TOTAL DEATHS DUE TO OCCUPATIONAL HAZARDS: Male plus female	=	16, 022

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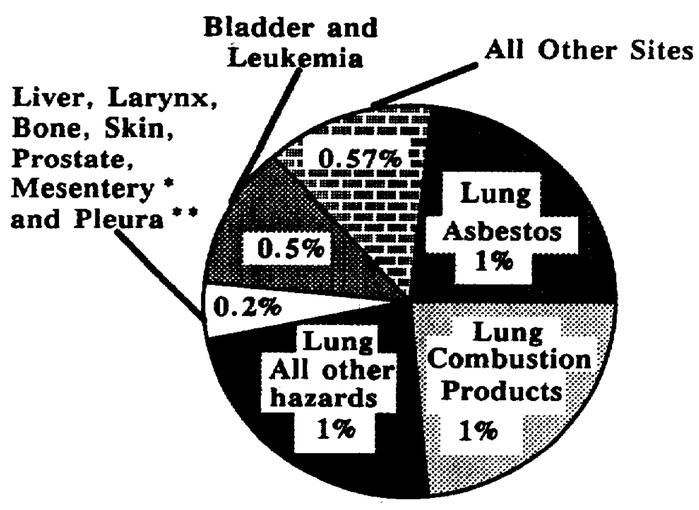

Figure 7. Distribution and types of the 4% occupational cancer mortality estimated by Doll and Peto.[1] [Total number of cancer deaths was 401, 955, age-adjusted to the 1970 U. S. Census. Of these, 17,069 were attributed to occupational hazards.]

* Membranes that attach organs to body walls.
** Membranes that facilitate movement of lungs.

Table 5. Causes of Cancer as Deduced by Higginson and Muir[3] and by Wynder and Gori[5]

Cause	Birmingham Region England Cancer Mortality in %[Higginson and Muir[3]]		United States Cancer Mortality in % [Wynder and Gori[5]]	
	Male	Female	Male	Female
Tobacco	30	7	28	8
Tobacco / alcohol	5	3	4	1
Diet			40	57
Life-style	30	63		
Occupation	6	2	4	2
Sunlight and Ionizing radiations	11	11	8	8
Iatrogenic (medical treatments)	1	1		
Exogenous (external) hormones				4
Congenital (present at birth) and unknown	17	13	16	20

Table 6. Comparison of Cancer Mortality Rates in the United States with Areas of the Lowest Rates for People Under 65 Years of Age.* [Selected entries taken from Doll and Peto.[1]]

Type of Cancer	Male rates (per million)			Female rates (per million)		
	Connecticut registry	Low incidence registry and location		Connecticut registry	Low incidence registry and location	
Mouth	31.8	0.8	Japan, Miyagi	11.8	2.4	Japan, Miyagi
Colon	137.2	13.7	Nigeria, Ibadan	140.7	11.6	Nigeria, Ibadan
Bronchus	325.8	9.0	Nigeria, Ibadan	96.9	8.7	Nigeria, Ibadan
Prostate	92.3	5.3	Japan, Miyagi	—	—	—
Bladder	113.1	17.8	Japan, Miyagi	32.8	7.3	Japan, Miyagi
Melanoma	40.8	8.0	United Kingdom Liverpool	38.6	18.4	United Kingdom Liverpool
Tongue	19.8	4.1	New Mexico, Spanish	6.7	2.7	Israel, Jews
Esophagus	34.6	6.5	Norway, rural	8.3	1.3	Norway, rural
Total all Cancers	1, 590	321		1, 775	408	

Total all cancers male and female in Connecticut = 1, 590 + 1, 775 = 3, 365

Total all cancers male and female at locations of lowest rates = 321 + 408 = 729

Avoidable Cancers = 3, 365 − 729 = 2, 636

$$\% \text{ Avoidable Cancers} = \frac{2,636 \times 100}{3,365} = 78.3\ \%$$

* Cancer rates are age-standardized to the U. S. 1970 census.

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In the third group, those types of cancer, some of which may be caused by occupational hazards are listed in Table 4. For each type of cancer, the total number of deaths are given as well as the number attributed to occupational hazards. Although some of each of the types of cancer shown in Table 4 can be attributed to occupational hazards, only one, bladder cancer has been studied. Cole et al.[4] attributed 18% of the bladder cancers for men and 6% for women; Davies et al.[10] attributed 5% for men and 2% for women, and Hoover et al.[11] in the most extensive study for the entire United States attributed 8% of the bladder cancers for both sexes combined to occupational hazards. On the basis of these investigations, Doll and Peto[1] attributed 10% for men and 5% for women of the bladder cancer to occupational hazards. Cancer resulting from occupational hazards are thought to be primarily lung cancers. In particular, asbestos was thought to have caused 5% of the lung cancer mortality, combustion of fossil fuels was responsible for another 5%, and other hazards could have caused no more than 5%. On this basis, Doll and Peto attributed 15% of the male cancer cases and 5% of the female cancers to occupational hazards.

A summary of the above results follows:

Total Cancer Mortality in Groups I, II, III = 401,955

Total Cancer Mortality in Groups I, II, III

attributed to **Occupational Hazards** = 17,069 or 4.25%

The estimates described above which were made to deduce the 4.25% figure for cancer mortality due to occupational hazards are shown in Figure 6 and Figure 7. [The numbers shown in Figure 7 were rounded. The precise numbers were: 2.94% for all lung cancer, 0.23% for liver and prostate, 0.51% for bladder and liver, and 0.5% for all other sites.]

The estimates of Doll and Peto are supported by the fact that similar studies have been carried out by others and there is general agreement, although not exact, between the various studies. Those by Higginson and Muir[3] and Wynder and Gori[5] are shown in Table 5. Higginson and Muir refer to "life-style" rather than diet, meaning factors such as lack of dietary fiber, caloric intake, excess fat, and possibly hormone carcinogenesis.

Avoidance of Cancer

Doll and Peto[1] estimated that 70% of all cancers are avoidable. This estimate was deduced by identifying those countries, or areas of countries, having the lowest cancer mortality rate for each type of cancer and comparing these rates with those for the United States. As a basis for the United States, the mortality rates from the Connecticut registry were used. Selected entries from the tabulation of Doll and Peto are shown in Table 6. The difference in the cancer rates for Connecticut and those for the respective areas of lowest incidence may represent the avoidable cancers. These studies suggest that our higher cancer rate for each type of cancer can be attributed to some aspect of our lifestyle, general environment or genetic constitution which differs from those of the country having the lowest incidence. (While some of these cancers may be individually avoidable, it should not be inferred that the full 70% are avoidable because of the many conflicting factors unaccouted for by Doll and Peto's simple comparison.) An extension of this approach offers the possibility for the determination of the cancer causing foods in our diets as well as the cancer causing aspects

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Table 7. Comparison of Cancer Mortality of the United States with Lowest and Highest Rates for 50 Countries 1982–83, Age-adjusted Rates per 100, 000 Population.

Type of Cancer	Male Rates and Rankings: United States	Male: Lowest Rate and Location	Male: Highest Rate and Location	Female Rates and Rankings: United States	Female: Lowest Rate and Location	Female: Highest Rate and Location
Oral	5.3(22)	1.1(50) El Salvador 1.3(49) Peru 2.1(48) Israel	18.6(1) France 16.0(2) Singapore 11.4(3) Hungary	1.8(13)	0.0(50) Martinique 0.6(47) Chile 0.6(47) El Salvador	6.9(1) Singapo 5.0(2) Kuwait 3.7(3) Panam
Colon and Rectum	24.6(19)	0.8(50) El Salvador 2.3(49) Kuwait 2.5(48) Martinique	36.4(1) Luxembourg 35.5(2) Czechoslovakia 33.3(3) New Zealand	17.5(20)	1.0(50) El Salvador 3.2(49) Peru 3.5(48) Mexico	29.1(1) New Ze 25.3(2) Denmar 24.5(3) Hungar
Lung	72.7(11)	2.5(50) El Salvador 8.2(49) Malta y Gozo 8.6(48) Dominican Rep	106.9(1) Scotland 104.2(2) Belgium 103.5(3) Netherlands	23.7(4)	1.7(50) El Salvador 2.7(49) Paraguay 3.5(48) Peru	31.0(1) Scotlan 28.2(2) Iceland 24.6(3) Singapo
Breast				26.7(16)	1.7(50) El Salvador 5.8(49) Peru 6.2(48) Japan	34.9(1) Malta & 34.5(2) England Wales
Uterus				7.8(38)	4.9(50) Israel 5.2(49) Greece 5.5(48) Iceland	32.4(1) Surinam 27.9(2) Paragua 24.9(3) Venezuel
Stomach	7.8(49)	6.1(50) Dominican Rep 8.1(48) El Salvador 10.4(47) Kuwait	63.2(1) Costa Rica 58.8(2) Japan 51.8(3) Chile	3.7(49)	3.3(50) Dominican Rep 5.0(48) El Salvador 6.0(47) Canada	27.8(1) Costa Ri 27.5(2) Japan 22.3(3) Chile
Prostate	23.1(21)	3.3(50) El Salvador 4.6(49) Japan 5.4(48) Singapore	52.1(1) Martinique 32.0(2) Switzerland 31.3(3) Luxembourg			
Leukemia	8.4(6)	1.6(50) Surinam 2.2(49) Peru 2.4(48) Dominican Rep	14.2(1) Luxembourg 9.4(2) Denmark 8.8(3) Malta & Gozo	50(17)	1.6(50) Surinam 1.3(49) Dominican Rep 1.6(48) Barbados	6.9(1) Martiniq 6.1(2) Costa Ri 5.7(3) Hungary

Source: *Facts and Figures*, 1988, American Cancer Society and World Health Statistics Annual 1983–1986.

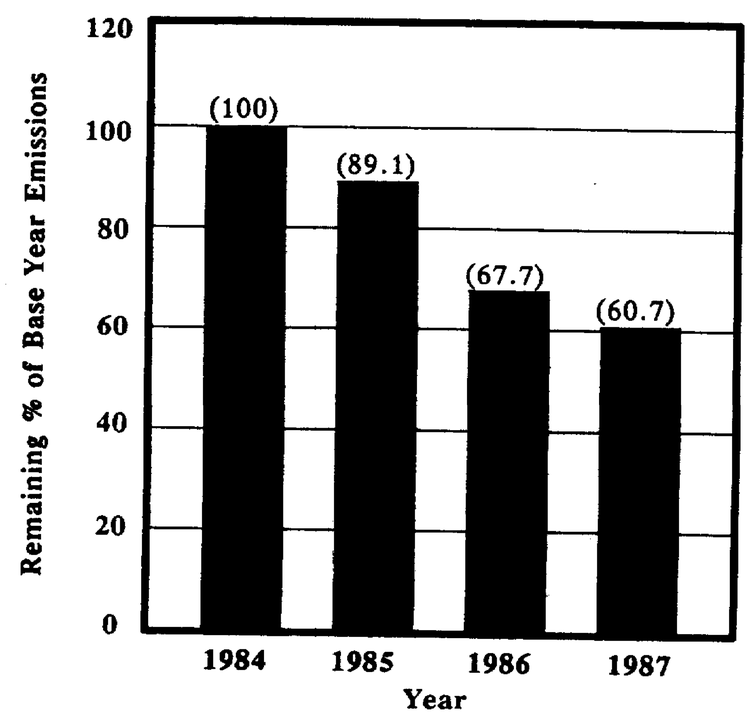

Figure 8. Air emissions by Dow Chemical U. S. A. were reduced 40% from 1984 to 1987, and from 1974 to 1984, they were reduced by 70%. [Taken from *The Point is ...* , No. 117, June 30, 1988.]

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of our lifestyle. Obviously, knowing that 35% of the cancer may be attributed to diet is not very helpful in avoiding cancer unless we can determine the foods to be avoided. Actually, the 35% is probably not fully avoidable because trace nutrients can be both nutritionally necessary and carcinogenic, and the best diets must contain some trace nutrients.

To provide an idea of how the United States cancer mortality rates compare with those of countries having the highest and lowest rates for specific types of cancer, the tabulation shown in Table 7 was prepared from a recent report of the American Cancer Society.

The Environmental Movement

During the 1960's an environmental movement was born because of pollution problems, many of which were severe. The movement was strongly fueled by scientists such as Rachel Carson[12], a biologist in the Fish and Wildlife Service. Her book, *Silent Spring*, focused mainly on pesticides of which DDT was her greatest concern. Her dramatic approach led to a needed tightening up of the regulations and use of pesticides.

Rachel Carson was followed by others who predicted a catastrophic end to our way of life by as early as the 1980's and certainly by the early 2000's. Among them were Paul Ehrlich [an entomologist and ecologist], Barry Commoner [a biologist] and an MIT group. Their remedy was "zero growth" and a redistribution of Western wealth to Third World nations. Commoner also called for a change in our constitution and system of government. Recently he gave a full exposition of his views along these same lines in the *New Yorker* magazine (June 15, 1987).

In 1972, an MIT computer study [conducted at the request of the Club of Rome] called the *Limits of Growth* appeared. In this study an "uncontrollable decline" was predicted in which industrial resources, industrial output, food supply, and the population would crash somewhere near the year 2005. Although the MIT study [published by Meadows et al.[13]] produced the conclusion that life on earth was on the verge of ending, there was little data available to support this conclusion as pointed out by Edith Efron.[14]

Obviously our way of life has not come to a catastrophic end. More people are employed today than ever before. Industry has made tremendous strides in handling of its waste problems. Typical of the reduction of emissions throughout the chemical industry is that shown in Figure 8 for air emissions by Dow Chemical Company. From 1974 to 1984, Dow's air emissions were reduced by 70%, and from 1984 to 1987 they were further reduced by 40%.

There are, however, some areas in obvious need of improvement. For example, waste disposal in many cities, particularly in the northeast, has become a serious problem. Air pollution caused by automobiles continues to be a problem in our cities, regardless of whether they are located in industrial or non-industrial areas.

Ranking of the Various Countries in Cancer Mortality

In the middle 1970's, those who had led the environmental movement in the 1970's joined by

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others and with widespread publicity in the press turned to cancer, which they attributed to "man-made chemicals", as their rallying cry. The same solutions offered for their pending environmental disaster were offered as the solution to the cancer problem.

Dan Rather opened his documentary entitled *The American Way to Death* on October 15, 1975 with this statement: "The news tonight is that the United States is number one in cancer. The National Cancer Institute estimates that if you are living in America, your chances of getting cancer are higher than anywhere else in the world."[15]

The truth is that the United States ranked eighth for Blacks and 25th for Whites out of a total of 36 countries, as determined by Mitsui Segi[16] two years prior to the broadcast. Segi's analysis was based on the death rates of the nine most common cancers, buccal, esophagus, colon and rectum, lung, stomach, breast, uterus, and leukemia.

Unfortunately, the public never learned of the actual ranking of the United States at the time of Rather's statement nor the ranking by the World Health Organization[17] which ranked the United States 19th for men and 18th for women out of 44 nations in 1970–1971. According to the most recent study, shown in Table 8, the United States ranks 22nd for men and 21st for women, out of 50 nations.

Table 8. Cancer Mortality for All Sites (oral, colon & rectum, prostate, breast, uterus, stomach, lung, leukemia) for 50 Countries 1982–83, Age-adjusted Rates per 100, 000 Population.

Country	Rates and Rankings Male	Rates and Rankings Female	Country	Rates and Rankings Male	Rates and Rankings Female
Luxembourg	310.9(1)	150.5(7)	Iceland	195.7(26)	149.4(12)
Hungary	286.1(2)	166.3(3)	Japan	194.3(27)	105.6(36)
Belgium	284.3(3)	147.6(13)	Chile	192.1(28)	144.4(15)
Czechoslovakia	281.2(4)	150.1(10)	Costa Rica	186.5(29)	137.6(20)
Netherlands	270.2(5)	141.8(17)	Yugoslavia	186.1(30)	107.8(35)
Scotland	268.0(6)	172.7(2)	Sweden	177.0(31)	131.7(25)
France	262.9(7)	121.3(31)	Martinique	172.9(32)	92.9(44)
Singapore	258.4(8)	141.4(18)	Cuba	172.4(33)	123.8(30)
Uruguay	255.7(9)	150.0(11)	Portugal	170.1(34)	105.2(37)
Denmark	248.1(10)	175.2(1)	Bulgaria	166.2(35)	102.4(38)
Germany, F. R.	245.5(11)	150.3(9)	Greece	160.7(36)	100.9(39)
England & Wales	243.7(12)	157.3(9)	Israel	157.8(37)	132.0(24)
Austria	241.8(13)	150.4(8)	Barbados	155.7(38)	118.4(33)
Switzerland	241.4(14)	135.5(22)	Romania	153.6(39)	99.7(41)
Poland	236.5(15)	131.1(27)	Kuwait	149.8(40)	109.4(34)
Finland	227.5(16)	124.2(29)	Surinam	148.4(41)	100.5(40)
New Zealand	226.4(17)	157.6(5)	Puerto Rico	140.3(42)	89.6(46)
Northern Ireland	224.9(18)	147.4(14)	Venezuela	133.5(43)	120.8(32)
Canada	221.2(19)	139.6(19)	Panama	115.1(44)	93.5(43)
Ireland	219.8(20)	158.6(4)	Mauritrius	102.4(45)	79.9(47)
Australia	218.2(21)	131.0(28)	Paraguay	91.8(46)	97.1(42)
United States	216.6(22)	136.5(21)	Mexico	91.5(47)	92.8(45)
Germany, D. R.	214.7(23)	132.1(21)	Peru	75.8(48)	76.3(48)
Malta & Gozo	208.3(24)	143.6(16)	Dominican Rep.	67.3(49)	65.0(49)
Norway	198.3(25)	131.6(26)	El Salvador	37.5(50)	48.7(50)

Source: *Facts and Figures*, 1988, American Cancer Society and World Health Statistics Annual 1983–1986.

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Exaggerated estimates of cancer mortality caused by "man-made chemicals" began to appear in the middle 1970's prior to the publication of Doll and Peto's[1] careful study in 1981 in which they attributed 4% of the cancer mortality to occupational hazards and 2% to pollution, for a total of approximately 6% or 9% if alcoholic beverages are included, recognizing that exposure to alcohol is largely a matter of lifestyle. [This estimate is based on the assumption that the net contribution of food additives and industrial products (shown in Figure 6) and medicines are zero because of the possible anticarcinogenic effect of food additives and the fact that medicines save more lives than they take.]

Professor Corbett in his book, *Cancer and Chemicals*,[18] published in 1977, said that it was man who was creating the chemical pollution that was causing 80% of the human cancer. In 1976, James Bishop Jr.[19] said on *CBS Face the Nation*, that it was generally accepted that 60% to 90% of all cancer was caused by "man-made chemicals". In 1977, Larry Agran, placed the figure at 90% in his book, *The Cancer Connection.*[20]

Edith Efron[14] called attention to the equivocation of Agran and Epstein[21] in attributing 90% of all cancer to "man-made chemicals" because they give little consideration to natural occurring carcinogens such as arsenic, asbestos, nickel, and chromium. Also, Commoner, Epstein and others managed to equate environmental factors with "man-made chemicals" and industrial pollution. As pointed out by Higginson and Muir,[3] the word "environment" included not only discrete chemical carcinogens but also life styles such as dietary, social habits, tobacco smoking and alcoholic beverages as well as occupational hazards. As early as 1969, Higginson[22] had published the estimate that 90% of all cancer (exclusive of those related to skin pigmentation) were influenced by external factors and were preventable. Higginson used the word "environment" in the broad sense of all external factors (diet, tobacco, life style, natural carcinogens, and air and water pollution); whereas, the aforementioned and others appeared to equate environmental factors with "man-made chemicals." Only a negligible increase in lung cancer over that caused by tobacco was attributed to atmospheric pollution.

The claims of 60% to 90% cancer mortality from "man-made chemicals" are easily refuted by simply comparing the cancer rates in industrial and non-industrial areas. For example, the incidence of cancer (exclusive of tobacco-related lung cancer) in non-industrial Geneva, Switzerland is higher then industrial England. Also, non-industrial San Francisco has a higher cancer incidence than nearby industrial Pittsburgh (American Industrial Health Council[23]).

In 1978, a widely circulated but unpublished and unsigned report by the Department of Health, Education, and Welfare asserted that as much as 38% of cancer was due to occupational exposure. This report[24] called the "Estimates Paper" has never been widely accepted by the scientific community and Doll and Peto[1] said that it was written "for political rather than scientific purposes."

Higginson[25] attributed the over estimates of HEW to the fact that these people found it hard to accept that general air pollution, smoking factory chimney etc. are not the major causes of cancer. They really hoped to be able to prove this. Then they would be able to say "let us regulate everything to zero exposure and then we have no more cancer."

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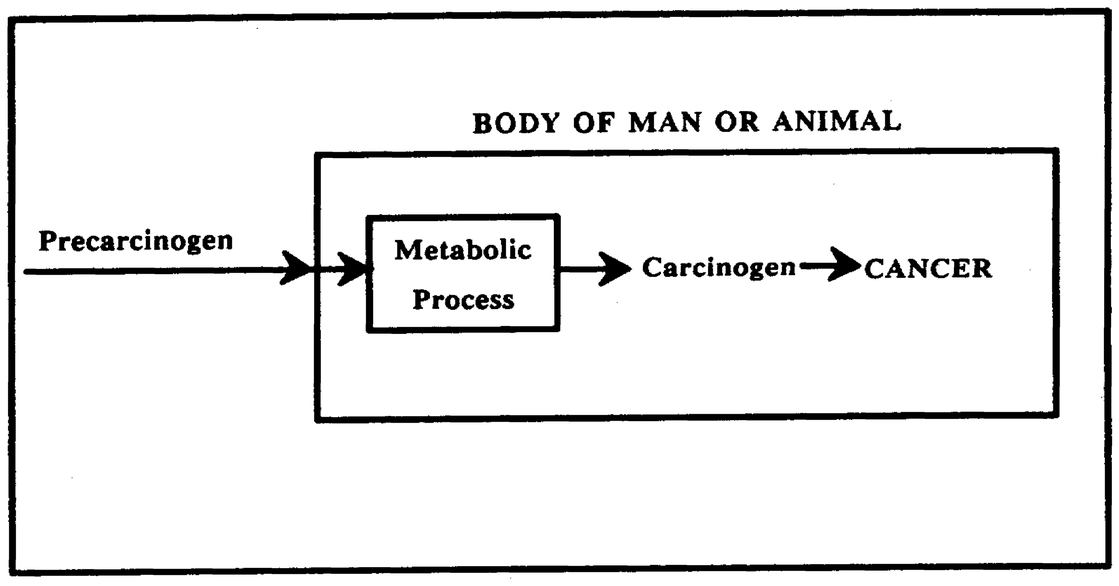

Figure 9. The metabolic processes of the body may convert a precarcinogen into a carcinogen.

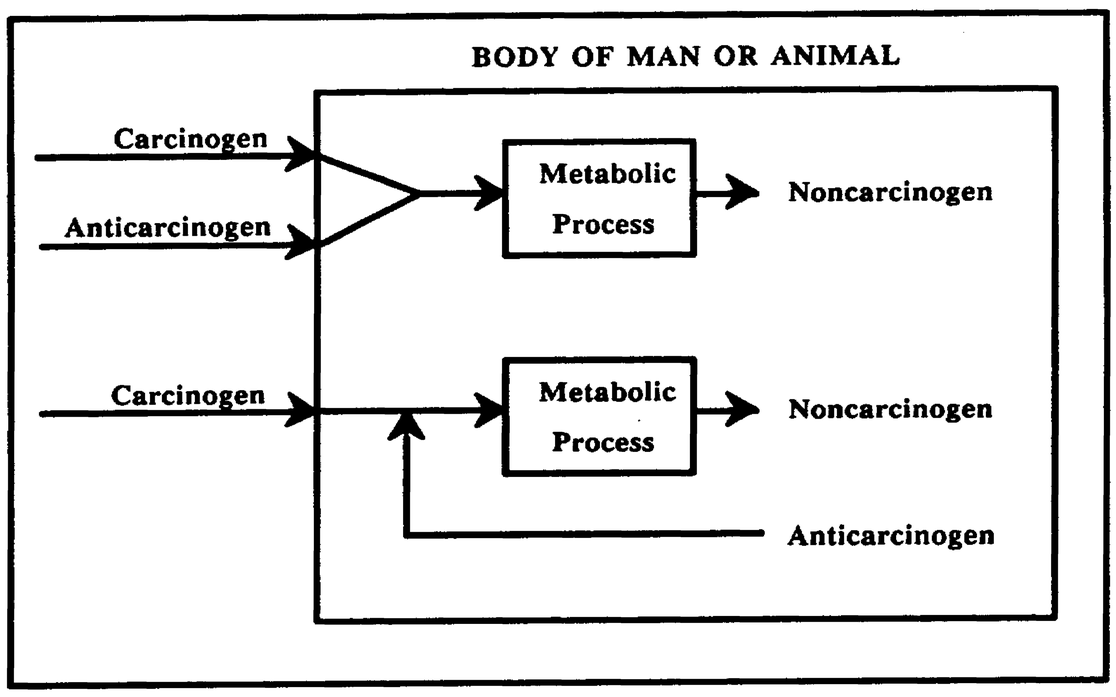

Figure 10. Carcinogens may be converted to noncarcinogens by anticarcinogens from either internal or external sources.

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Research Developments — Discovery of Metabolism of Chemicals to Carcinogens, Inhibitors, Anticarcinogens, Cocarcinogens, and Promoters

A chemical **carcinogen** may be defined as any substance which causes cancer. From a practical point of view, this definition is subject to many ambiguities as indicated in the discussions which follow in subsequent sections. There are two major classes of chemicals that induce cancer, direct acting and indirect acting carcinogens.

During the year 1970–74, the Millers[26] demonstrated that indirect- carcinogens (or precarcinogens) are metabolized to carcinogens upon entering the body of an animal as shown in Figure 9. On the other hand, direct acting carcinogens are in their carcinogenic form prior to entering the body. The Millers' discovery led to major problems because of the difference in mammalian metabolism in different species. As a consequence, the extrapolation of the results from animal tests to humans tends to break down.

Chemicals known as **inhibitors** and **anticarcinogens** were discovered in 1929 by Berenblum.[27] When applied or taken simultaneously with a known animal carcinogen, anticarcinogens can delay the appearance of tumors, diminsh the number of tumors, prevent tumors from occurring, or reverse the early phases of the carcinogenic process. In particular, Berenblum discovered that dichloroethyl sulfide inhibited the formation of cancer in a mouse which had been exposed to carcinogenic tar – a known animal carcinogen. This concept is illustrated in Figure 10. Subsequently, many other anticarcinogens were discovered and their role in the cancer process amplified. Inhibitors are generally regarded as those substances which inhibit or delay cancer formation by decreasing the rate of cell division.

Other chemicals called **cocarcinogens** were also discovered by Berenblum.[28] The names "cocarcinogens" and "promoters" have somewhat overlapping meanings. The name "cocarcinogens" is usually reserved for those chemicals which enhance the action of carcinogens, particularly in the initiation phase of the cancer process as illustrated in Figure 11. On the other hand, the term "promoter" is generally used to mean any chemical which promotes the cancer process by increasing the rate of cell division of the initiated cells. In addition to substances which are carcinogenic, it is well known that radiations such as ultraviolet, X-rays, and gamma rays can cause cancer, through the formation of free radicals from chemicals in the near neighborhood of the DNA or from the DNA itself, which can react with the DNA.

Research Developments — The Two–Stage (or Initiator–Promoter) Theory of Cancer Formation

The discovery of initiators and promoters led to the **two-stage** or **initiator-promoter** theory of cancer which is illustrated in Figure 11. It was shown by Berenblum[28] that the carcinogen benzo(a)pyrene initiates a cancer when applied to the skin of a mouse. The cancer grew much faster when croton oil was applied either simultaneously or consecutively with the benzo(a)pyrene. Since the croton oil had no effect if it was applied prior to the application of benzo(a)pyrene, it was concluded that the process is two-stage with the promotion stage always following the initiation stage.

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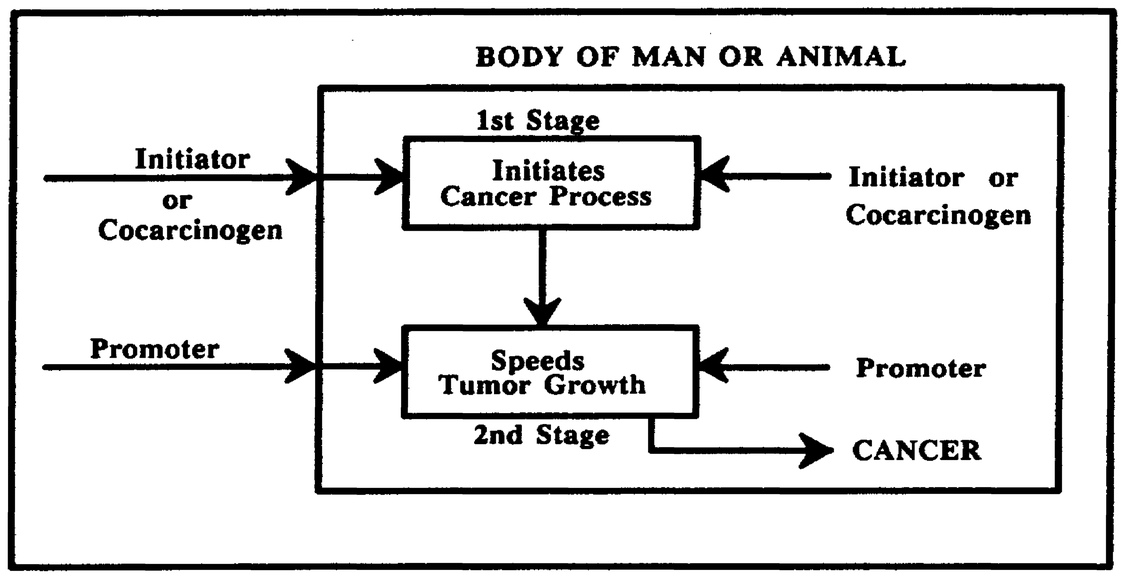

Figure 11. Graphic illustration of the "Two-Stage" or "Initiator-Promoter" theory of cancer formation.

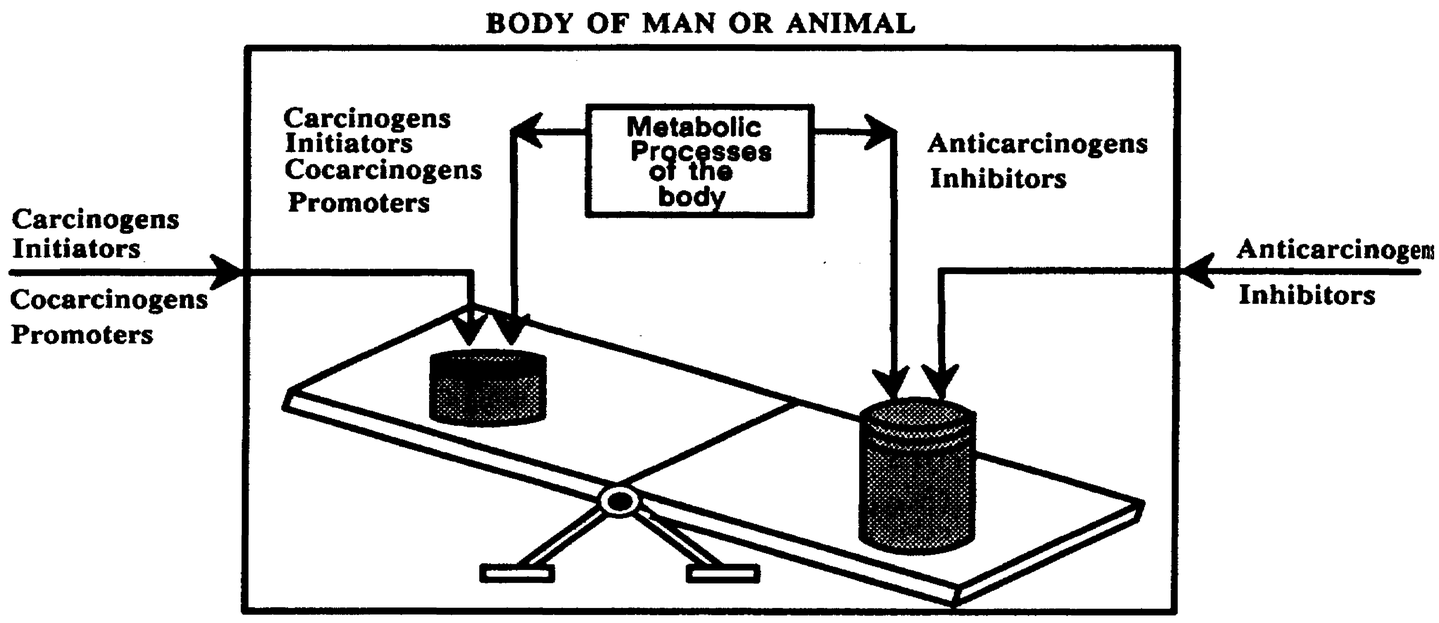

Figure 12. Nature has heavily counterbalanced the body defenses against the development of cancer.

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The initiation stage is thought to consist of a mutation of the DNA molecule by an initiator molecule (or activated metabolite) which becomes chemically bound to or reacts with the DNA or other macromolecules. Consequently an initiator is also frequently referred to as a **mutagen**.

The concept that cancer was caused by mutations was a long held theory dating back as far as the 1920's. Support for the initiator stage was also provided by Ames et al.[29] with the development of a simple biological test for the mutagenicity of chemicals, which merits some discussion. The test consists of the use of a rat or human liver homogenate (S-9Mix) for the carcinogen activation (thus supplying the mammalian metabolism) and a set of *salmonella* histidine mutants for mutation detection. The homogenate, bacteria, a TPNH enzyme generating system, and the compound to be tested are then incubated directly on a petri plate. Compared with animal testing, the Ames test is both more rapid and economical.

In an initial testing program, Ames et al.[29,30] found that 90% of the animal carcinogens were also mutagens. However, as further tests were run, it was found that for certain classes of chemical carcinogens , the test did not show them to be mutagens. Also, the converse was found; namely, not all mutagens were carcinogens, and not all noncarcinogens were nonmutagens. The final conclusion was that the Ames test could not be regarded as an absolute test for the identification of carcinogens, but that it could be useful for screening purposes.

The second stage, the promotion step involves the subsequent progression and cell proliferation through a series of pathological states heading eventually to a malignant tumor. Cell proliferation can be accelerated by chronic cell-killing viruses, such as the human carcinogenic virus, hepatitus B (a major cause of liver cancer), and human papilloma virus 16 (HPV16) [a contributor to cancer of the cervix]. The promotion stage can also be accelerated by hormones which may be the major factor in causing all hormonally influenced cancers such as cancer of the breast.[31] Chemicals may act as accelerators of the promotion step. As an example, alcohol causes cirrhosis of the liver which leads to cancer of the liver. There is increasing evidence that small amounts of promoters are not active, but when taken in large amounts cause chronic irritation, cell killing, and cell proliferation.

While the cancer mechanism has been represented by two major stages, it has been thought to consist of many stages; perhaps as many as six. The two-stage mechanism properly defined may be the most prevalent of the cancer mechanisms. Although radiations are not substances, they can be included in this mechanism by virtue of the fact that they act as mutagens by damaging the DNA directly or indirectly by damaging macromolecules such as enzymes that are involved in its formation. Molecules damaged by radiation are highly reactive and tend to react with body chemicals in their immediate vicinity. To further complicate the picture of the cancer mechanism, it is now known that initiators, cocarcinogens, promoters, inhibitors, and anticarcinogens are produced internally in the body, independent of outside influence.

However, all of the cancer causing agents either introduced to or produced within the body have been heavily counterbalanced by an abundance of repair mechanisms, anticarcinogens, and inhibitors provided by nature, as illustrated in Figure 12.

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Research Developments — The Two–Mutation Theory of Cancer Formation

This model, recently investigated by Moolvagar and Knudson[32], and Wilson[33] was also called a "two-stage" model. However, to avoid confusion with the two-stage model described above, the Knudson-Moolgavkar model is referred to herein as the "Two-Mutation" model as suggested by Wilson[33] because the two-stages shown in Figure 11 have in effect been replaced by two critical mutations.

These two mutations on the critical path for the transformation of a normal cell to a cancerous one are assumed to be irreversible. An important feature of the newer model which enables it to account for a greater variety of cancers is the recognition of the role of increased cell division throughout the cancer formation process. Since cocarcinogens are known to increase normal cell division rates, a cocarcinogen (both external and internal) may be one of the inputs to the first mutation in the same manner as shown for Stage 1 in Figure 11 for the two-stage model.

By increasing the rate of cell division, the probability that damaged DNA will undergo division before it can be repaired is increased. Thus, the mutation becomes fixed irreversibly in all future cells. Increased rates of cell division also increase the probability of the occurrence of mutations from uncontrollabe events such as those resulting from errors in DNA gene transcription, cosmic radiation, and natural mutagens. Normally all or nearly all damaged DNA macromolecules are repaired. However, any damaged DNA which is not repaired before cell division leads to a genetic change or mutation becoming fixed in future cells.

The assumption that only two mutations lie on the critical path between normal and cancerous cells has not been generally established. However, it has been shown to rigorously hold for some cancers such as cancer of the retina in children. In this case, some children are born with an inherited mutated gene (which constitutes the first event), and the second mutation usually occurs in early childhood leading to cancer.

Chemicals (and other agents) can contribute to cancer incidence in two ways. First, the agent may react directly with DNA, increasing the number of damaged DNA molecules thereby increasing the probability that a damaged DNA molecule will escape repair before cell division. Second, the agent may react with some other component in the cell which causes an increase in cell division or the agent may kill the cell. If it kills the cell, there follows a temporary increase in the rate of cell division such as is observed in the formation of scar tissue, which in turn contributes to cancer formation by increasing the probability of occurrence of mutations resulting from uncontrollable events such as those enumerated above. Thus, the concept of accelerated cell division provides an explanation for the causation of cancer by agents such as viruses which may not react directly with DNA but which do cause damage and chronic irritation.

Not only does the feature of the rate of cell division of the two-mutation model aid in the explanation of essentially all types of cancer formation, it also provides one possible answer to the avoidance of cancer: namely, the reduction of the rate of cell division. An increase in that class of anticarcinogens, called inhibitors, which have the capacity to inhibit or decrease the rate of cell division would consequently prevent or reduce the formation and proliferation of cancer. Slowing cell division of the initiated cells after the first event

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reduces the probability of occurrence of the second critical event. Slowing of cell division after the second event reduces the proliferation of the cancer and makes it dormant.

Research Developments — Inhibited Gap Junction Communications: A Nongenetic Based Model

This report deals primarily with mechanisms based on genetically caused cancers because most of the work, beginning with Armitage and Doll[34], has been done in this area. However, there are significant cancers which cannot be directly attributed to genetic changes in the sense that the carcinogens causing these cancers are not mutagens. For example, potent carcinogens such as polybrominated biphenols (PBB) do not exhibit mutagenic behavior according to all of the short-term tests.

The Gap Junction Intercellular Communication (GJIC) model proposed by Trosko[35] provides an explanation for carcinogens whether they are either mutagenic or nonmutagenic. This model was the first to recognize intercellular communication and its importance in inhibition of cell division of initiated or mutated cells. In this model it is supposed that the process is initiated by the mutation of a cell by any means (some mutagenic or uncontrollable event independent of the carcinogen in question). Once formed, these initiated cells are held in check by surrounding normal cells through intercellular communication. This process functions through the transfer of negative growth factors in the form of calcium ions, pH changes, free radicals, and small molecules which are transferred across a membrane-protein interface (called a Gap Junction) from one cell to another.

Any substance such as a carcinogen or an event (a cosmic ray or a cell death) can inhibit or block the intercellular communication which releases the initiated (mutated) cells from the restraining effects of surrounding normal cells. Once the gap junctions have been blocked, the cell division process of the mutated cells is no longer restricted, and uncontrolled cell division occurs with the progression to the formation of malignant cells.

As recognized by Trosko, the Gap Junction Intercellular Communication model possesses the same complications associated with all models of cancinogenicity such as species, tissue and cell type specificity. Also, many chemicals (PCB, DDT, TCDD, phenobarbital) under one set of biological conditions act as carcinogens and under some other set, act as anticarcinogens.

Research Developments — Identification of Human Carcinogens by Epidemiologists

From the standpoint of the public, the definition of a carcinogen given above is taken to mean any substance which causes cancer in humans. Since substances which cause cancer in humans do not necessarily cause cancer in animals and conversely, carcinogens should be classified into two subgroups : "**human carcinogens**" and "**animal carcinogens**", and further subdivided into mouse, rat, etc. However, confusion in the general public exists because only the single designation, "carcinogens" is used for both humans and animals.

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Identification of human carcinogens has been through the use of epidemiological studies rather than experimental investigations of the potential cancer causing substances. That is, epidemiology begins not with the study of all possible pollutants in a given city which might have caused cancer deaths, but with all of the deaths which have occurred in the city over a given period of time, and tries to determine the major causes of these actual deaths. By use of an analysis of death certificates, case histories and personal interviews, they attempt to establish the major cause of each death and the exposure and life style of the decedent. A graphical representation of this process is shown in Figure 13.

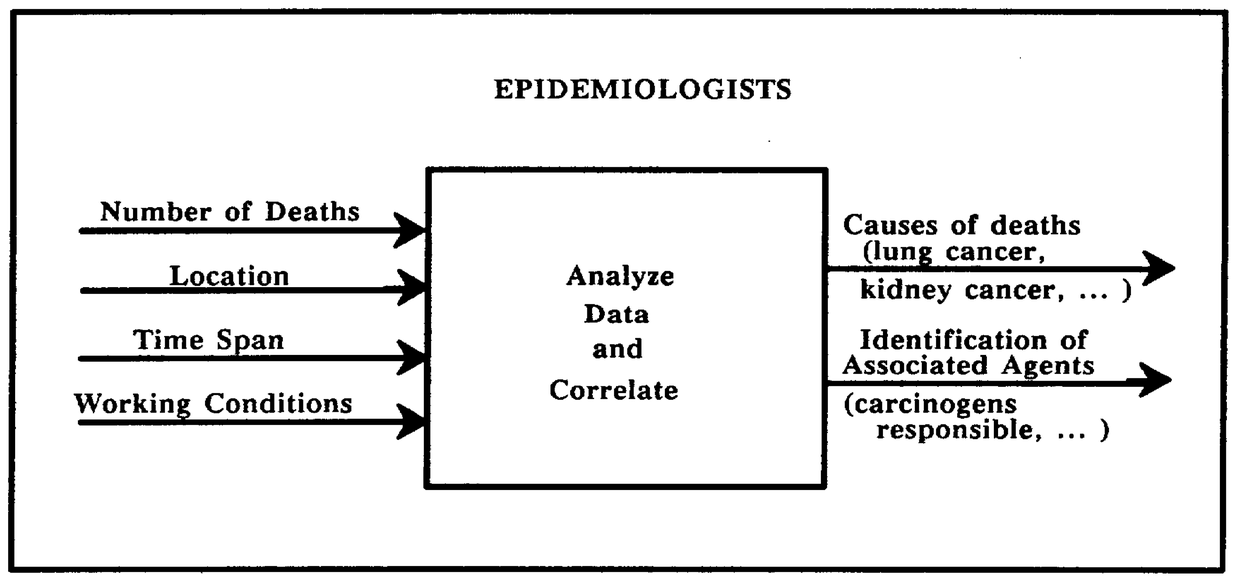

Figure 13. Most if not all human carcinogens have been identified by epidemiological studies.

This approach has led to the discovery of most if not all of the presently known human carcinogens. Of the more than 600 chemicals and chemical mixtures evaluated by the International Agency for Research on Cancer (IARC) expert working groups, only 23 chemicals and 7 processes have been associated with cancer in humans.[36] A tabulation of the commonly accepted human carcinogens and processes is presented in Table 9. According to Wilkinson no new major causes of cancer have been identified by the IARC within the past 10 years.[37]

Research Developments — Animal Tests and Associated Problems

In order to avoid the possibility of introducing chemicals into the environment which would cause cancer, an extensive effort has been under way to develop laboratory tests which would predict the carcinogenicity of a substance in humans. However, animal tests are very expensive.

Although animals are used for testing substances for carcinogenicity, this approach is

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Table 9. Human Carcinogens – Industrial Processes and Chemicals. [From *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans*, Supplement 4 (1982).[36]]

1. **Industrial Processes and Occupational Exposures**
   - Auramine Manufacture
   - Boot and Shoe Manufacture and Repair (certain occupations)
   - Furniture Manufacture
   - Isopropyl Alcohol Manufacture (strong-acid process)
   - Nickel Refining
   - Rubber Industry (certain occupations)
   - Underground Haematite Mining (with exposure to radon)
2. **Chemicals and Groups of Chemicals**
   - 4-Aminobiphenyl
   - Analgesic Mixtures Containing Phenacetin[(a)]
   - Arsenic and Arsenic Compounds[(a)]
   - Asbestos
   - Azathioprine
   - Benzene
   - Benzidine
   - N, N-Bis(2-chloroethyl)-2-naphthylamine (Chlornaphazine)
   - Bis(chloromethyl)ether and technical-grade chloromethyl methyl ether
   - 1, 4-Butanediol dimethanesulphonate (Myleran)
   - Certain Combined Chemotherapy for Lymphomas[(a)] (including MOPP[(b)])
   - Chlorambucil
   - Chromium and Certain Chromium Compounds
   - Conjugated Oestrogens[(a)]
   - Cyclophosphamide
   - Diethylstilboestrol
   - Melphalan
   - Methoxsalen with Ultra-violet A Therapy (PUVA)
   - Mustard Gas
   - 2-Naphthylamine
   - Soots, Tars and Oils[(a), (c)]
   - Treosulphan
   - Vinyl Chloride

This list does not include known human carcinogens such as tobacco smoke, betel quid, and alcoholic beverages.
(a) The compound(s) responsible for the carcinogenic effect in humans cannot be specified.
(b) Procarbazine, nitrogen mustard, vincristine and prednisone.
(c) Mineral Oils may vary in composition, particularly in relation to their content of carcinogenic polycyclic aromatic hydrocarbons.

fraught with difficulties because of the different responses exhibited by different animal species to chemicals. Responses may differ not only from one species to another, but from male to female of the same species, and also from animals to humans. For example, mice get cancer from perchloroethylene, but rats, rabbits, guinea pigs and monkeys do not.[38] Rats and humans develop cancer from Aflatoxin B_1, but mice and monkeys are relatively resistant[39] as described in Figure 14. Man gets cancer from β-naphthylamine, but most strains of rats[40] and hamsters do not[41], whereas dogs do. Thus, the extrapolation of the results of tests made on animals to humans always has uncertainties. That is, just because

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a chemical is an animal carcinogen does not prove that it is a human carcinogen.

To further complicate the picture and add to the confusion is the fact that a given carcinogen can affect entirely different organs in different mammalian species. For example, benzidine is said to cause bladder tumors in man, liver tumors in the hamster, and acoustic tumors in the rat while estrogens are reported to cause breast tumors in mice, uterine and abdominal tumors in the guinea pig, and kidney tumors in the hamster.[14]

Also arguments have persisted in scientific circles concerning the number of animal species which are needed for a reliable extrapolation to humans. Some say three, others say two and some including OSHA say one positive test in one animal species is sufficient to label a substance as a potential human carcinogen.

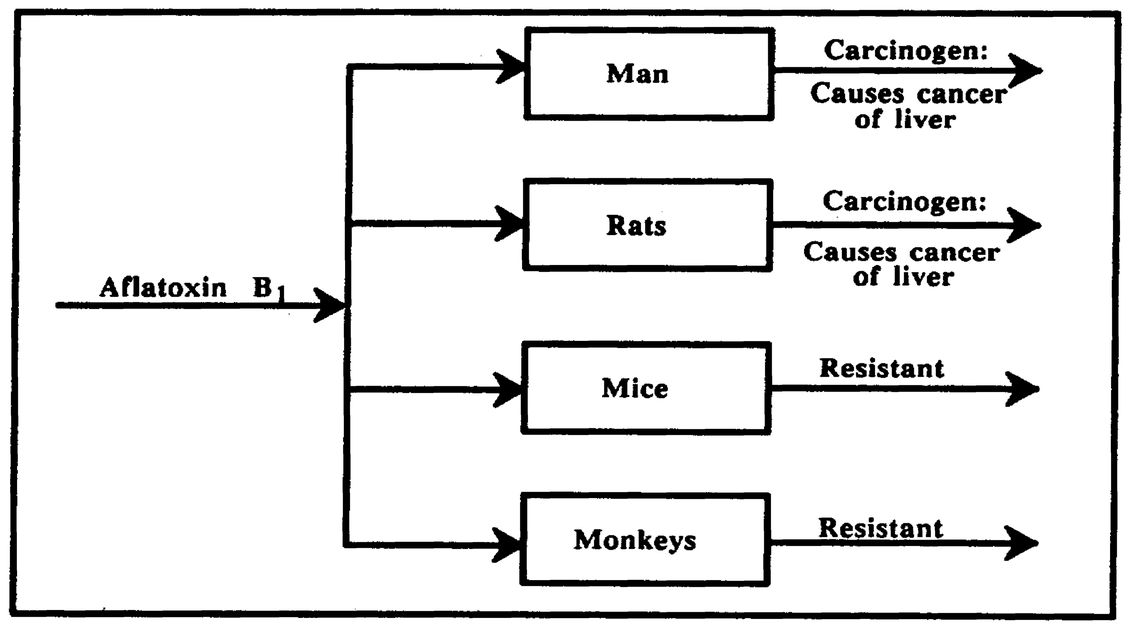

Figure 14. Aflatoxin B_1, a natural carcinogen found in grains and peanuts, has a different effect on different animal species.

Research Developments — Problems in Extrapolation from High-Dose Animal Tests to Low-Level Human Exposure

Human exposure to chemicals ordinarily occurs at low-dose levels. For animal tests at these correspondingly low levels of exposure to be meaningful, a prohibitively large number (millions) of animals would be required. The alternative of obtaining a significant number of tumors in a relatively small population by use of high doses has become standard procedure in animal tests. However, this approach leads to further difficulties because many chemicals are carcinogenic at high doses but not at low doses. Thus, the extrapolation from high-dose animal tests to low-dose human exposure becomes even more difficult as summarized in Figure 15.

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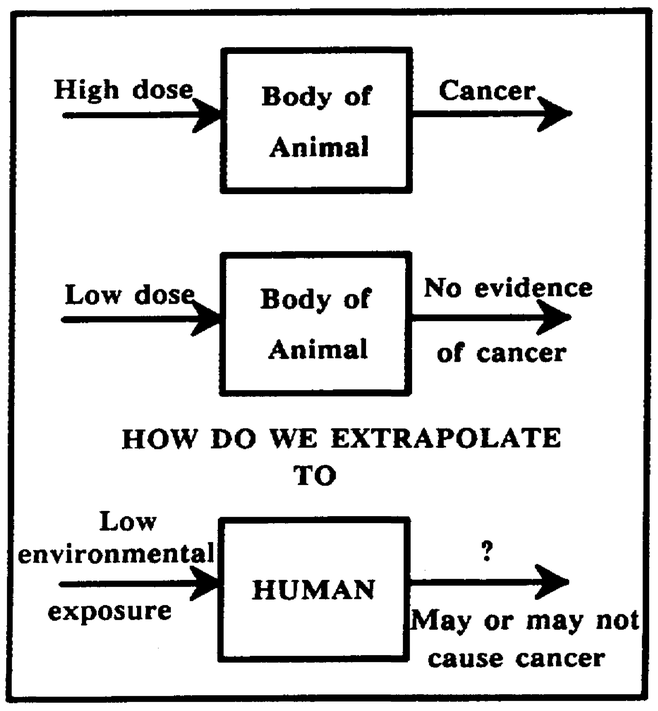

Figure 15. How do we extrapolate from high dose rates for animal tests to low exposure rates for humans?

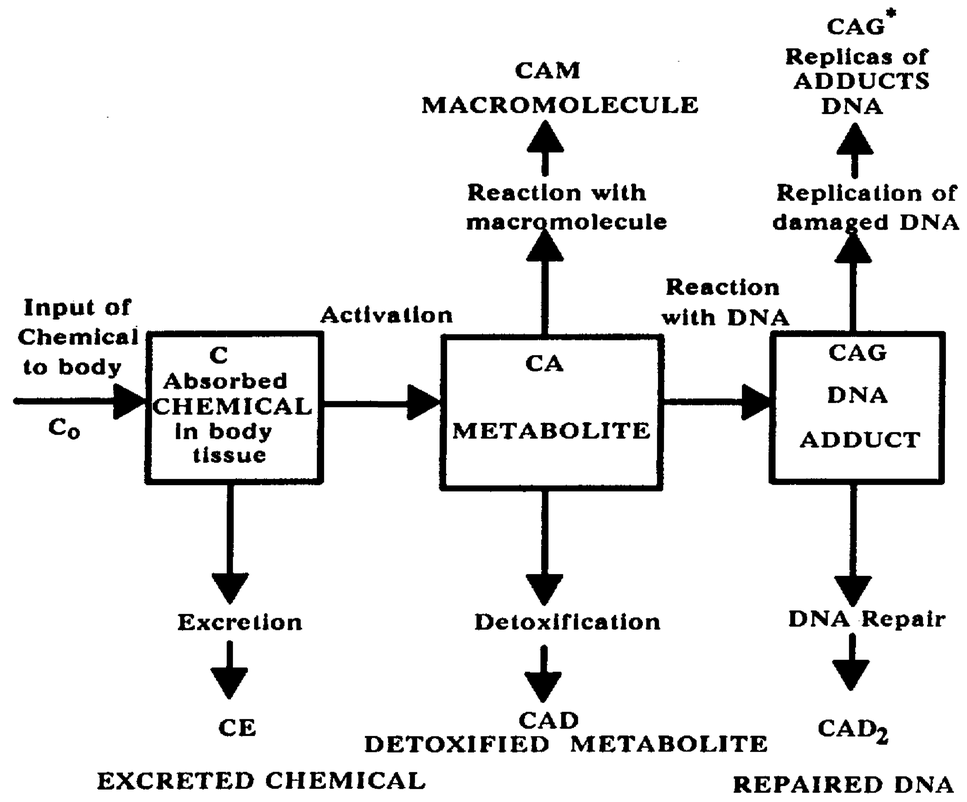

Figure 16. A Hypothetical model of the fate of a carcinogen in the body of a mammal. [From Gehring, P. J., and G. E. Blau, *J. Environ. Pathol. Toxicol.*, 1, 163(1977).]

Research Developments — The Theory that Cancer is Formed by a System of Competing Chemical Reactions within the Body is Gaining Acceptance

The difference in the results obtained from the animal tests at high doses and low doses can be visualized through the use of **Pharmacokinetics** models. **"Pharmacokinetics"** is derived from the Greek word *"pharmakon"* meaning drug or poison and the word "kinetics" which refers to the speed of a chemical reaction. The models were proposed and utilized in a series of papers by Gehring and Blau[42], and Reitz and Watanabe[43], Andersen et al.[44], and Clewell and Andersen.[45] These models consider both the reaction of mutagens with DNA to form DNA adducts, and the repair of DNA adducts.

The initial findings by Miller and Miller[26] have been formalized and expanded in a more recent article[46] in which they state that most chemical carcinogens are metabolized into a chemically active form called a metabolite, upon entering the body. The metabolite is capable of reacting with many cellular macromolecules such as DNA, RNA, and proteins.[46] Some carcinogens such as alkylating agents are in their ultimate form as administered, but most require metabolism to the active metabolite form.[46]

Of particular importance is the reaction of the carcinogen or the metabolite with the DNA, since this macromolecule contains the genetic information for the cell. The specific sites of reaction on the DNA with the given chemical are very important in determining

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the mutagenetic effect. Certain substitutions are more likely to result in miscoding than others. Furthermore, substitutions at some sites are much more readily excised from DNA than at other sites.

Experimental evidence that DNA repair is possible in metabolic systems was provided by Russel et al.[47,48] Ethylnitrosourea, the most potent mutagen for mice yet discovered, was administered to mice in two ways. In one test, one massive dose of 100 mg/kg (milligram of ethyl-nitrosourea per kilogram of mouse) was administered, and in the other test, doses of 10mg/kg were administered weekly over a 10 week interval. Although it was anticipated that the 10 weekly doses would give the same number of mutations as the single large dose of 100 mg/kg, this was not the case; the weekly doses produced only 13% as many mutations as did the massive dose. Russel et al. interpreted the reduced mutagenic effect of the small weekly doses to the repair process for the damaged DNA. The repair process appeared to have been overwhelmed when the chemical was given as a single large dose of 100 mg/kg. The hypothetical model shown in Figure 16 was used by Gehring and Blau and by Clewell and Andersen to represent the biochemical processes involved when a chemical carcinogen is introduced into the body of a mammal. After the chemical having concentration C_0 has entered the body, it is absorbed in the body tissue at the concentration C. Next , some of it may be excreted with concentration C_E and some of it bioactivated to a metabolite with concentration C_A. The bioactivated molecule then reacts to form detoxified chemicals, noncritical macromolecules, or it may react with genetic material DNA to form CAG (damaged DNA or DNA adducts). The DNA adducts may be repaired by reaction to the form CAD_2 or a replication of the damaged DNA may occur to give CAG*. In this model, the formation of CAG* is the crucial event in the initiation of carcinogenesis because replication of the damaged DNA may produce new molecules whose DNA no longer appears to be in need of repair by the repair systems of the body. This model of the reaction system may be represented by a system of differential equations and the parameters and rate constants appearing in these equations evaluated by use of *in vivo* and *in vitro* experiments as described by Clewell and Andersen.[45]

Research Developments — At High Doses of a Chemical Carcinogen, the Repair Mechanism for the Damaged DNA is Overwhelmed and Cancer Formation may Result

To demonstrate that the above model describes known metabolic behavior, a series of plausible values for the parameters and rate constants were selected and the resulting set of differential equations solved numerically by use of a computer. Solutions were obtained for a wide variation of initial concentrations C_0 ranging from 10^{-6} to 1 in moles/kg. The results are shown in Figure 17.

These graphs of the concentrations of the postulated compounds formed in the model are useful for the elucidation of the problem of high dose-low dose extrapolations. First observe that for initial doses greater than 10^{-4}, the replicated-damaged DNA begins to form faster than the other products. Also the ability of the system to repair damaged DNA in proportion to the initial dose C_0 begins to decrease at 10^{-2}, which implies that the repair system is becoming overwhelmed; damaged DNA is being produced faster than

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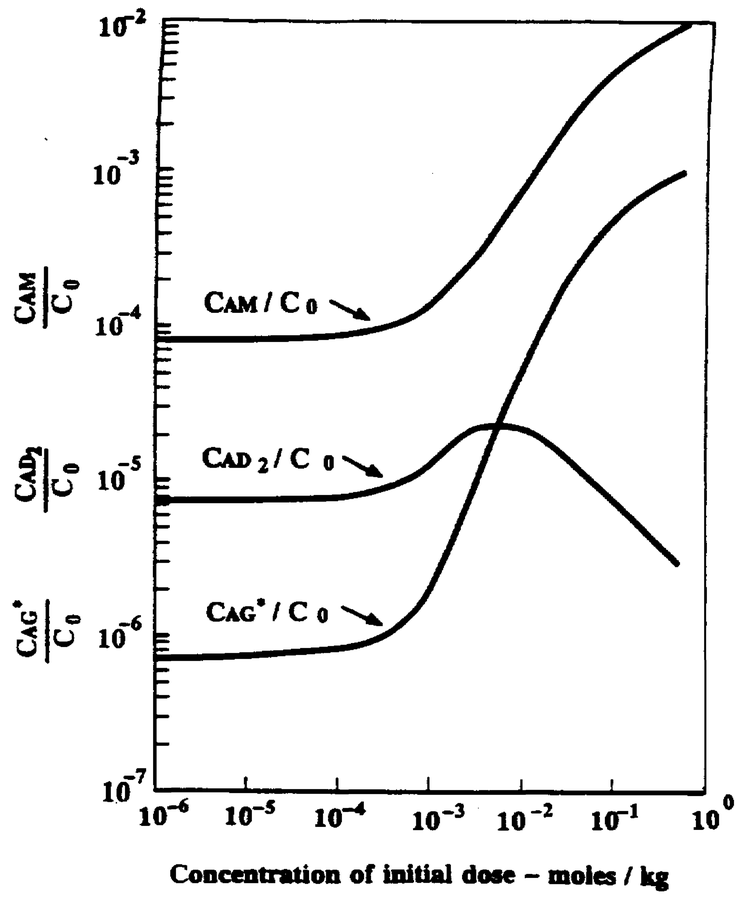

Figure 17. Variation of the products CAM, CAD_2, and CAG^* as a function of the initial concentration. CAM = concentration of macromolecules, CAD_2 = concentration of repaired DNA, and CAG* = concentration of replicated damaged DNA molecules; all concentrations are in g moles per Kg of animal. [From Gehring, P. J., and Blau, *J. Environ. Pathol. Toxicol.*, 1, 163 (1977).]

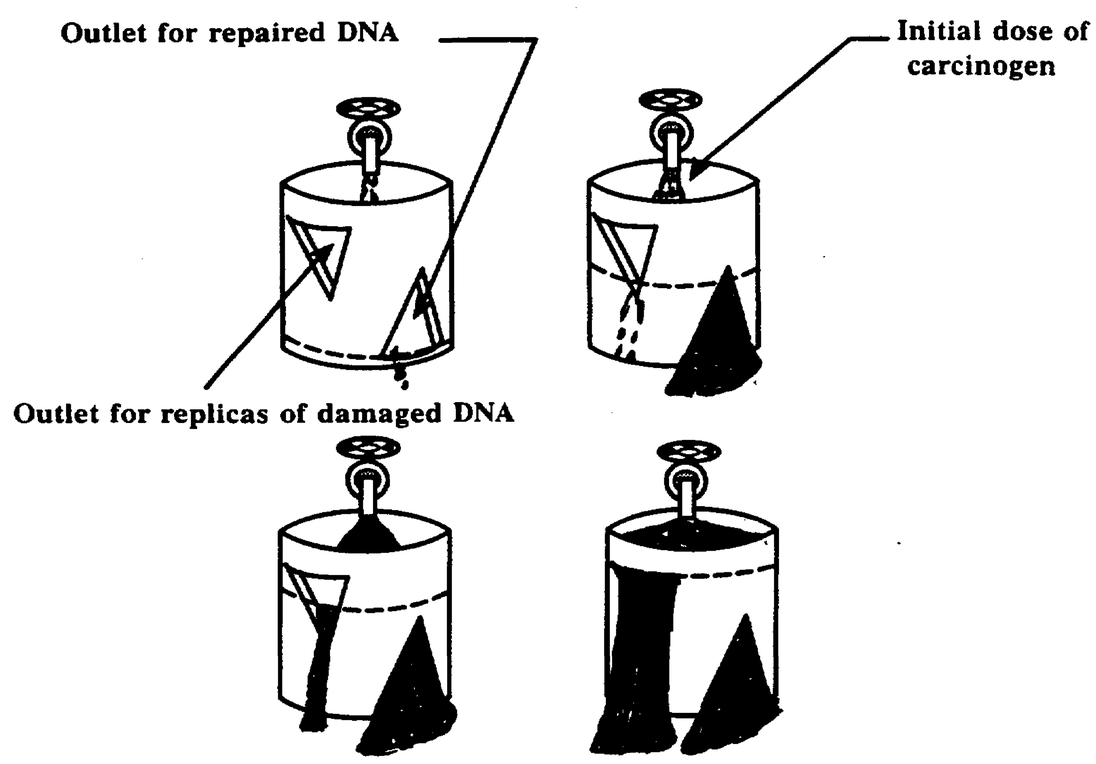

Figure 18. Hydraulic analogy of the overwhelming of the repair system for damaged DNA. [From Gehring, P. J., and G. E. Blau, *J. Environ. Pathol. Toxicol.*, 1, 163 (1977).]

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it can be repaired. Production of macromolecules CAM also increases as C_0 is increase but such molecules are not involved in the replication processes.

To illustrate the overwhelming of the system at high doses, the hydraulic analogy shown i Figure 18 (analogous to the one proposed by Gehring and Blau) is useful. For concentratio greater than $C_0 = 10^{-4}$, small increases in the initial concentration C_0 of the carcinoge give much larger increases in the concentration C_{AG^*} of the damaged adduct, which illustrated by the following numerical example.

Example. Suppose the concentration C_0 of the dose of the carcinogen is increased 10 fold from 10^{-4} to 10^{-2}. Use Figure 17 to find the concentration of the damaged DN adduct at 10^{-2}.

Solution :

From Figure 17, it follows that at $C_0 = 10^{-4}$,

$$\frac{C_{AG^*}}{C_0} = 7 \text{ x } 10^{-7}$$

and

$$C_{AG^*} = 7 \text{ x } 10^{-7} \text{ x } 10^{-4} = 7 \text{ x } 10^{-11}$$

At $C_0 = 10^{-2}$

$$\frac{C_{AG^*}}{C_0} = 7 \text{ x } 10^{-5}$$

and

$$C_{AG^*} = 7 \text{ x } 10^{-5} \text{ x } 10^{-2} = 7 \text{ x } 10^{-7}$$

Thus, as C_0 is increased by a factor of **100**, the concentration of damaged DNA adduc increases by a factor of **10,000**; that is,

$$\frac{7 \text{ x } 10^{-7}}{7 \text{ x } 10^{-11}} = 10^4 = \mathbf{10,000}$$

This example demonstrates the effect of overwhelming the DNA repair mechanisms by us of high doses of the carcinogen. It also suggests the possibility of overestimation of the ris through the use of linear extrapolation methods. Note, however, that this multiplicatio factor does not apply at low initial concentrations near the origin of the graph in Figu 17.

Use of Physiologically Based Pharmacokinetics (PB-PK) Model in Risk Assessment

When the transport of a chemical from its point of entry into the body to the reaction sit within the body is included in the model, the combined model is called a **Physiologically Based Pharmacokinetic** model. Both *in vivo* and *in vitro* data are generally needed i order to evaluate the parameters of this model. Reitz et al.[43] evaluated three types o

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model parameters [(1) physiological constants, (2) partition coefficients, and (3) metabolic rate constants] for methylene chloride by use of *in vivo* data from the literature and laboratory, and *in vitro* laboratory studies. The model was validated by comparing computer simulations with experimental data gathered in mice, rats, and humans.

In order to reduce the uncertainties in the estimation of human hazards by extrapolation from animal studies, Clewell and Andersen[45] propose the use of PB-PK models because they take into account actual physiological properties of the various species, the specific partitioning of different chemicals between tissues and blood, and the concentration dependent rates of metabolism. Because of the fundamentals upon which the PB-PK models are based, they are particularly useful for high dose–low dose extrapolations as well as dose-route and interspecies extrapolations. As an example of the use of PB-PK models to improve risk assessments, Clewell and Andersen[45] cite the estimations of Singh et al.[49] and Blancato et al.[50] By following the conventional default procedure for methylene chloride [without the use of PB-PK modeling data], Singh et al.[49] estimated that the risk for continuous exposure to a concentration of 1 microgram per cubic meter of methylene chloride inhaled in air was 4.1×10^{-6}. Subsequently this estimated risk was lowered by Blancato et al.[50] through the use of PB-PK modeling. Although a lower risk was obtained in this instance by PB-PK modeling than classical methods, this is not always the case.

For those chemicals which form metabolites that react with DNA, Hoel et al.[51] has proposed the use of the concentration of the resulting DNA adducts to follow tumor formation. There is evidence that tumor response is linearly related to the appropriate DNA adducts in the target organ. PB-PK models whose parameters have been accurately determined may be used to help predict the concentration of DNA adducts produced over a wide range of dose concentrations.

Sielken[52] also points out that the biologically reflective dose should be used instead of the administered dose because its use enables the modeler to incorporate known biology, physiology, and pharmacokinetics in the model. Use of the "Biologically Effective Dose" such as DNA adducts aids in more accurate extrapolations from high to low doses and from species to species. Sielken developed an Individual Response Model based on the biological effective dose (BED) which took several factors into account that are of great importance in risk assessment. The first of these is the difference in individual susceptibilities to potentially hazardous chemicals. Individual responses to a particular exposure differ because of many factors including background exposures, genetic traits, preexisting diseases, and behavioral traits. The individualized model also reflects the inter-individual variation in the susceptibility and background dose. In addition, the Individual Response Model is a dynamic model in that it may be used to predict the probability that a randomly selected individual from the population will have a carcinogenic response by time t from an administered dose of chemical carcinogen of concentration C_0 at time t_0 or a time-dependent concentration (administered dose) profile.

In summary, it is evident from the foregoing discussion that the quantitative description of the cancer process is following the same familiar course as that followed in the development in the field of chemical kinetics, catalysis and chemical reaction engineering. Although there are few reaction mechanisms that we understand on an atomic level well enough to compute the kinetic rate constants and mass transfer coefficients from theoretical

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considerations alone, we can nevertheless determine these parameters in the laboratory and pilot plants and use them in the design and control of chemical reactors. It should also be noted that the reaction engineering models, like the PB-PK models accurately predict the product response for a given change in the initial concentration of the chemical reactant.

Measures of Potency and a Ranking of Possible Hazards of Chemical Carcinogens

For the numerical description of the carcinogenic potency of chemicals in chronic-exposure animal experiments, Peto et al.[53] proposed the use of the index called the TD_{50} (the tumorgenic dose rate 50). The TD_{50} is defined as the chronic dose rate (in mg/kg of body weight per day) which would be required to give half of the animals tumors within the standard life span for the species (with appropriate adjustments being made for background and experimental procedures as described by Peto et al.[53])

Gold et al.[54] computed the TD_{50}'s for all of the tests reported by the NCI/NTP Bioassay Program and for all long-term experiments in the published literature. The TD_{50}'s for approximately 3000 laboratory animal experiments on 770 chemicals were published by Gold et al.[50] These results and the TD_{50}'s for additional tests (for a total of 900 chemicals) are listed in three installments of *Environmental Health Perspectives* from 1984 to 1987. The rules and conventions given by Gold et al.[54] were used in the estimation of these TD_{50}'s. As shown in Figure 19, the range of potency is more than 10 million fold. The definition of the TD_{50} is analogous to the definition of the LD_{50} (lethal dose 50; that dose of chemical which kills 50% of the animals). Other indices for the potency of chemical carcinogen have been proposed, and their relationship to the TD_{50} have been presented by Gold et al.[54]

Since the animal experiments for the determination of the TD_{50}'s are carried out at high-dose rates where many metabolic processes and other defense mechanisms are normally swamped, this index is generally a poor measure of the carcinogenicity of the chemical at low dose rates at which humans are usually exposed. However, to provide some measure of the potential hazard of human exposure, Ames et al.[55] utilized the information in the TD_{50} in the definition of an index which he called "HERP" [(Human Exposure/Rodent Potency dose)(100)]. More precisely,

$$\text{HERP} = \frac{\text{human exposure (mg/kg/day)}}{\text{TD}_{50}\text{ (mg/kg/day)}} \times 100$$

The units mg/kg/day denote the milligrams of the dose of chemical per kilogram of body weight per day. [This definition of HERP is called HERP(in %) by Ames et al.[53] in their tabulation of the TD_{50}'s for 975 chemicals in rodents.]

Because of the nonlinearity of the functions and the other problems, enumerated above, involved in the extrapolation from animals to humans, the HERP cannot be regarded as any measure of a risk assessment; nor was it proposed as one. Instead it may be used to raise the question of further consideration and possible experimental investigation.

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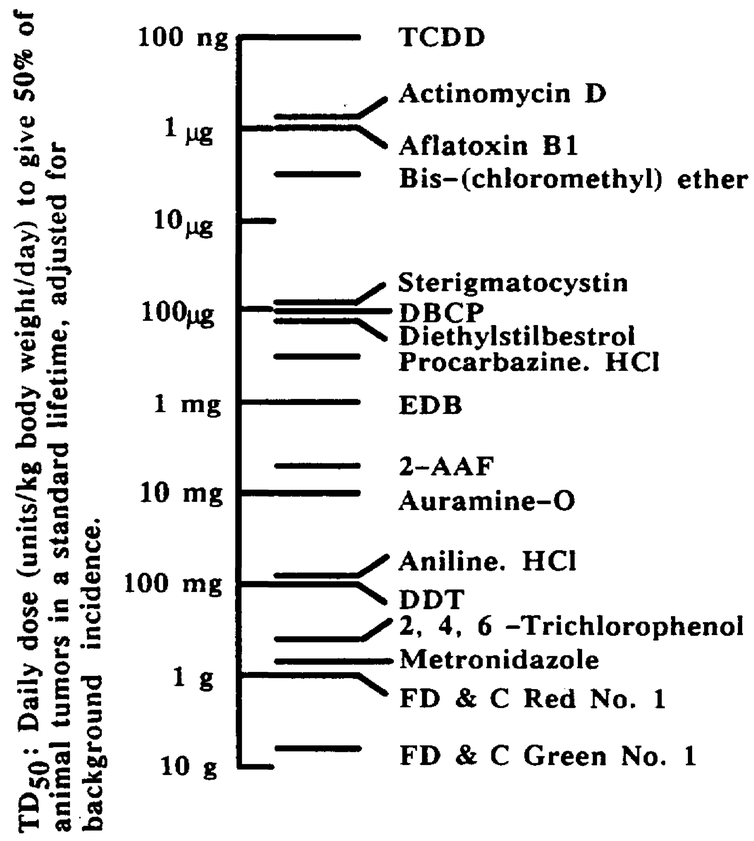

Figure 19. Range of carcinogenic potency in male rats as measured by the TD_{50} index . [From Gold, L. S. et al., *Environ. Health Perspect.*, 58, 9(1984).]

RELATIVE RANKING OF POSSIBLE HAZARDS

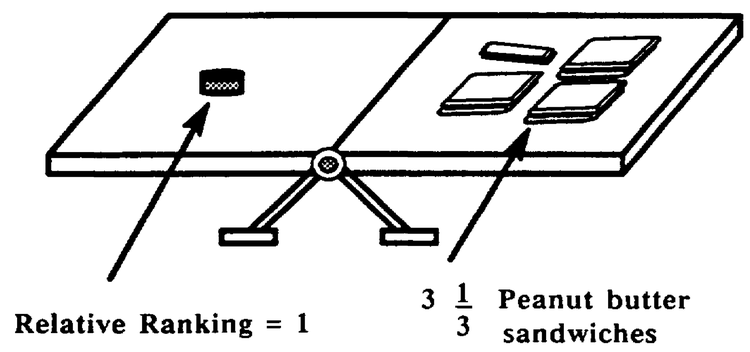

Figure 20. Three and one-third peanut butter sandwiches have a Relative Ranking of unity.

RELATIVE RANKING OF POSSIBLE HAZARDS

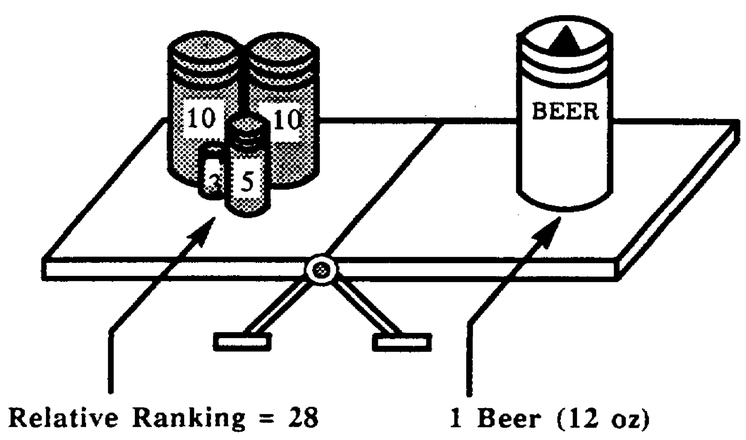

Figure 21. One twelve ounce beer has a Relative Ranking = 28.

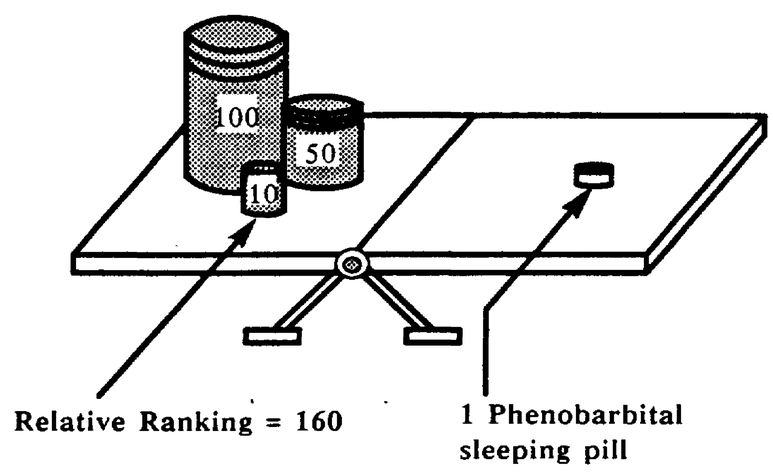

Figure 22. One phenobarbital pill has a Relative Ranking = 160.

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Instead of expressing the possible hazards of chemicals and other substances in terms of HERP's, they may be ranked relative to any one of the others. If, for example, the possible hazard of consuming one raw mushroom daily, or one basil leaf daily, or three and one-third peanut butter sandwiches daily [each of which corresponds to a HERP = 0.1], is taken as a basis, then the possible hazards of other substances may be ranked relative to these as illustrated in Figures 20–22.

In the same article, Ames et al.[55] called attention to the fact that nature is a far greater producer of chemical carcinogens than is man. Although we are exposed to a "sea" of natural carcinogens, this should not be taken as a license to make significant additions to our surroundings.

On the other hand we cannot, in fact, technically or otherwise, reduce the exposure to all chemical carcinogens to zero. Thus the level of reduction becomes a risk management problem which has been dealt with by Deisler in a series of papers.[56–60] The approach recommended by Deisler for dealing with this problem is to first select a set of very desirable but realistically achievable, quantitative risks, which broadly applied would achieve the following : "(1) cause the deployment of our resources and information so as to protect the most people from the risk of cancer as soon and as effectively as possible; (2) lay a foundation for continuing risk reduction and control; and (3) as a desired, long-term end point, to reduce the contribution of the incidence of cancer related to industrially derived agents to a total cancer level as near to insignificance as possible when compared with the total incidence of cancer from all causes, regardless of what the contribution is today".[55]

Cancer Mortality along the Upper Texas Gulf Coast

The determination of cancer mortality resulting from environmental exposure is difficult because of the many "confounding factors" such as occupational exposure, smoking, and migrating populations.

The "Ill Winds Study" by Molinari[61] attributed the higher cancer rates (than the U. S. average) in the Texas Gulf Coast Counties of Chambers, Jefferson, Galveston, and Harris to the fact that each contained or was downwind of one or more petrochemical plants.

While this study was faulted by its methodology and strongly criticized by epidemiologists, there is no question about the higher cancer death rate along the Upper Gulf Coast, but there is a question as to what is the cause. Smith et al.[62] cited one major epidemiological study for Harris County which showed that air pollution caused 3% of the cancer mortality in white males during the study period, 1981–1983. However, the principal investigator of this study, stated that neither of the "confounding factors", smoking and occupational hazards, was adequately controlled, and if they had been, it is likely that the contribution of air pollution would have dropped less than 1%. This is consistent with an EPA study, called "the 6 month study"[63], which estimated the potential of air pollution for causing cancer in the U.S. population at 1,700 to 2,000 cases per year or roughly 0.2%.

Because of the continued concern of the citizens over the health consequences of exposure to ambient air contaminants, the Texas Air Control Board (TACB) commissioned the "Gulf

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Table 10. Frequency of Detection of the 15 Chemicals at Each of the Monitoring Stations. [From Rogers et al.,[64] Final Report, Texas Air Control Board, March (1988).]

Chemical	Austin	Beaumont	West Orange	Cloverleaf	Cloverleaf	Q. A. Texas City
Acrylonitrile	○	○	○	○	○	○
Epichlorohydrin	○	○	○	○	○	○
Ethylene Oxide	○	○	○	○	○	○
Coronene	○	○	○	○	○	○
Dioxins	○	○	○	○	○	○
Furans	○	○	○	○	○	○
Vinyl Chloride	○	○	○	○	○	○
Arsenic	○	○	◒	○	○	○
Lead	○	◒	○	◒	○	◒
Benzo(ghi)perylene	○	◒	○	◒	◒	○
Benzo(a)pyrene	○	◒	◒	◒	◒	◒
Chrysene	◒	◒	◒	◒	◒	◒
Pyrene	○	●	●	●	◒	◒
Formaldehyde	○	●	●	●	—	●
Benzene	●	●	●	●	●	●

○ never detected ◒ detected infrequently ● detected frequently

Table 11. Summary of Annual Levels by Site. [From Rogers et al.[64]]

Chemical	Austin	Beaumont	West Orange	Cloverleaf	Cloverleaf	Q. A. Texas City	TACB Screening Value
Benzene	6	6	11	18	11	7	3
Formaldehyde	—	6	6	7	—	4	1.5
Lead	—	0.2	—	0.2	—	0.2	1.5
Benzo(a)pyrene	—	0.007	0.005	0.01	0.007	0.005	0.005
Benzo(ghi)perylene	—	0.007	—	0.007	0.006	—	0.005
Pyrene	—	0.018	0.018	0.014	0.013	0.017	0.005
Chrysene	0.006	0.009	0.007	0.009	0.01	0.01	0.005

Data are in micrograms per cubic meter.

Table 12. Comparison of Emissions of Study-Site Cities with Those of Other U. S. Cities. [From Rogers et al.[64]]

Substance	Annual Average at Study Sites	Annual Average in U. S. Cities
Benzene	6 - 18	2 - 20
Formaldehyde	1.5 - 6.9	4 - 6
Pyrene	0.01 - 0.02	0.0001 - 0.036
Chrysene	0.006 - 0.01	0.0006 - 0.01
Benzo(a)pyrene	0.005 - 0.014	0.0001 - 0.03
Benzo(ghi)perylene	0.005 - 0.007	0.003 - 0.01
Lead	0.22 - 0.24	0.05 - 3.4

Data are in micrograms per cubic meter.

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Coast Community Exposure Study" (GCCES). The Final Report of this study by Rogers et al.[64], released in 1988, presents the results of a quantitative study of the environmental contaminants detected at 6 Texas monitoring locations: Austin, Beaumont, West Orange, Cloverleaf (Harris County), Cloverleaf Q. A. (Harris County), and Texas City from October 9, 1985 through September 26, 1986.

The air samples collected at the monitoring stations were analyzed for the chemicals shown in Table 10. The quantitative results obtained are shown in Tables 11 and 12. The conclusion of the report was that "the data showed no indication of a health risk. The levels of all substances which were detected were within the range of levels considered typical of heavily-urbanized areas across the United States."[64]

The highest concentration measured for any pollutant was benzene, and at the measured upper bound of 18 micrograms per cubic meter which corresponds to a Relative Ranking of 0.0839, which is 12 times smaller than the Ranking of 1 for the human exposure to the natural carcinogens in a diet containing one basil leaf or three and one-third peanut butter sandwiches, daily.

This section is concluded by a brief description of the most recently published (October, 1988) epidemiological study of Harris County. In this ecological analysis, Buffler et al.[65] took several confounding factors into consideration which were not considered in earlier studies. Elevated lung cancer rates in Harris County compared with the other U.S. counties led to the initiation of this study which examined the air pollution-lung cancer mortality relation for white males in Harris County, Texas, 1979–1981. Factors taken into account were : median age, two social and demographic factors (family life cycle and migration), an age-dependent smoking index, and air pollution as measured by total suspended particulates. Air pollution was not demonstrated to be a strong determinant of lung cancer in that the presence of air pollution accounted for less than 5% of the total variation in intraurban lung cancer mortality. The relation between air pollution and lung cancer was highly dependent on which social and demographic factors were selected for inclusion in the analysis. Their final conclusion was that the hypothesis that air pollution is contributory to lung cancer cannot be tested until other stronger individual risk factors for lung cancer can be better measured and controlled in the studies.

The Houston Regional Monitoring Program

This is a continuing program which is supported by 35 companies. Samples are collected at 6 locations and analyzed for 173 chemical compounds. The results obtained thus far mirror those reported by the Texas Air Control Board in the Gulf Coast Community Exposure Study.[64]

Results of a Study of Liver Cancer Rates in Brazoria County

As a consequence of a study by Hoover and Fraumeni[66] in which a 10% excess mortality rates above those for the U.S. average was found for chemical-industry countries, a study by Buffler et al.[67] was sponsored by the National Cancer Institute. Thirty-nine cases of

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liver cancer were observed in white males, the group in which the carcinogenic effect of occupational exposure to chemicals would be expected to be observed. The actual number of cases observed was too small to establish an association with occupational chemical exposure, although the liver cancer mortality rates were slightly higher for Brazoria County than they were for the U. S.

The EPA TEAM Study (The Total Exposure Assessment Monitoring Study[68])

The results of an extensive five year study by EPA (reported in 1985) in which scientists concluded that, contrary to popular belief, people living in heavily industrialized areas containing petrochemical, paint and plastic processing plants are not subjected to greater exposures to the commonly identified toxic chemicals than are people living in less industrialized, or even rural areas. This conclusion was based on studying 355 people in the highly industrialized cities of Bayonne and Elizabeth, N.J., 25 people in the highly industrialized city of Greensboro, N.C., and another 25 in rural Devils Lake, N.D.

A further significant finding was that people are exposed to far greater concentrations of toxic chemicals indoors than they are outdoors, even in those cities where plants that manufacture or use these chemicals are located. This finding has also been confirmed by European studies. By use of both indoor and outdoor monitoring systems, the EPA investigators found indoor levels to be two to five times greater than outdoor levels for the commonly identified toxic chemicals. At highest exposures, the indoor levels were up to 70 times greater than outdoor levels.

Since people spend up to 95% of their time indoors where the pollution levels of toxic chemicals are far higher than they are outdoors, one comes to the obvious conclusion that our present method of basing exposure estimates on fixed-station monitors located outdoors does not accurately reflect the total human exposure.

Industrial Epidemiological Studies

Industrial epidemiological studies are characterized by the "healthy worker effect," which can be attributed to several factors. The conditions for employment require that people entering the work force be in good health. The nature of the employment leads to workers establishing a healthier life style [regular routine for work, rest, meals] than is followed by the general public. Workers also receive better medical care than does the general public.

Typical of the results of industrial epidemiological studies are those published by Dow's Epidemiological Department. These extensive studies, numbering over 60, involved many highly publicized chemicals, and except for those workers who had been exposed in the past to arsenic, asbestos, and vinyl chloride, total mortality rates as well as cancer mortality rates were less than those for the surrounding neighborhoods. Extensive studies, published by Union Carbide Department of Epidemiology found an excessive cancer mortality for only one subcohort of workers, a group which had worked in vinyl chloride plants that are no longer in existence.

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In a series of studies conducted by the Medical Department of Phillips Petroleum Company, no job associated cancers were detected in the carbon black production workers and no job associated leukemia was found in the refinery workers.

Typical of industry's efforts in the area of epidemiological studies is the following report from Exxon's Department of Medicine and Environmental Health : "Based upon 137,702 person-years of observation, the mortality experience of workers at Exxon's refineries and chemical plants in three areas of the United States was found to be generally lower than that of the U.S. population."

Du Pont established an epidemiological section in its Medical Division in 1956. Since that time, the company has conducted more than 80 special studies and published over 65 scientific papers. In addition, Du Pont maintains company wide registries for mortality, cancer incidence, coronary heart disease, and cerebrovascular accidents.

Shell Oil Company has performed several epidemiology studies of its work force. Overall, all cause and cancer mortality has been well below that for the U.S. population. Isolated specific cause of death elevations have been found at some locations; however no work related cause has yet been identified. Investigations of these mortality subgroups continue. Shell encourages publication of studies and routinely notifies employees as well as state and federal authorities of all study results. Shell also supports and participates in larger industry studies involving more than one company.

Although the lower mortality rates from all causes other than cancer can be attributed to the factors listed above (pre-employment screening, life style, and better medical care), the lower cancer mortality rates cannot (yet) be so explained. General forms of cancer cannot (yet) be screened for, and various comparisons for lifestyles have not (yet) turned up a reason why cancer, too, may not also exhibit the healthy worker effect.

Prioritizing our Efforts and Expenditures

Our problem is one of putting the reduction of cancer mortality caused by occupational hazards (4%) and environmental pollution (2%) in perspective. Our present rate of increase in industrial environment costs is depicted in Figure 23. We are all in favor of a clean livable environment; this may not be a uniquely risk-free or unaltered environment. Where do we stop — how much is enough? Even if we closed all chemical plants, eliminated all automobiles, alcohol, and all other possible sources of occupational hazards and environmental pollution, we would still be left with an estimated 90+% of the cancer mortality.

Instead of spending more and more of our national resources on the impossible goal of totally eliminating environmental risks, should we not move faster on a broad front, including an assault on the 90+% cancer mortality? If we can take measures to reduce risks and mortality 10-fold, should we wait to begin acting? Should we not spend more on cancer research?

Our future is brighter than many would have us believe. Although there is approximately a 20 year latency period effect, for cancer caused by use of tobacco and asbestos, a decline in cancer mortality can be expected because of the decreased exposure to these substances.

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Although good news has been long in coming regarding cancer cures and preventions, there is a reason for renewed hope. New developments and progress are being made in cancer research. For example, an Associated Press release of December 16, 1988 described a study by Wen-Hwa Lee, Professor of Pathology at the University of California, Davis, which suggests that a genetically engineered virus carrying a cancer suppressing gene is able to prevent the formation of malignant cells. Lee said the results suggest that this technique could be used to genetically convert malignant tumor cells, which are fast growing, to cells that grow normally. He said this technique holds promise for development within five years.

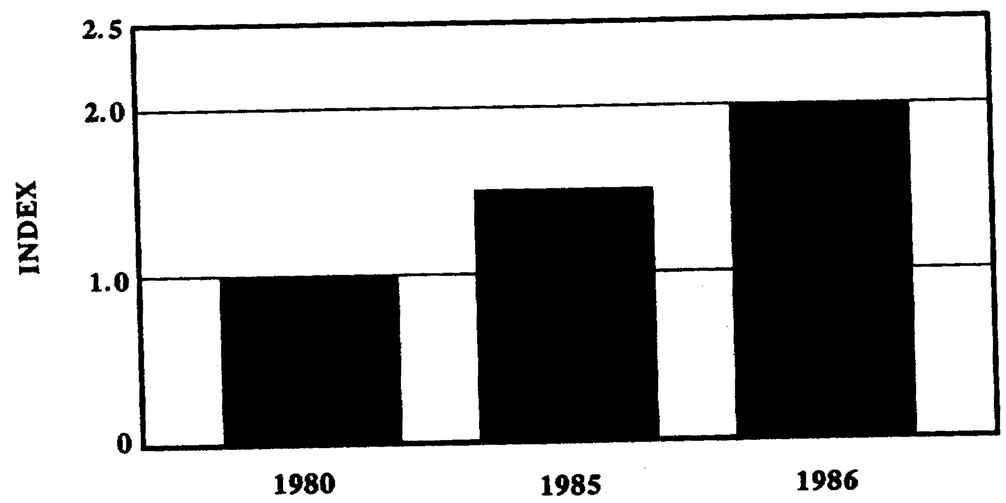

Figure 23. Environmental costs for Texas industries are 100 % above those of 1980. [Courtesy of Dow Chemical Company.]

Furthermore, the quantification of the cancer process through the use of pharmacokinetics, introduced in 1973 by Teorell[69], is a rapidly advancing field in which the well-known principles of chemical kinetics are applied to describe the effect of chemicals on animals and humans. Recent advances in this area by numerous authors including Gehring and Blau[42], and Andersen et al.[44] give renewed hope for a better understanding and quantification of the cancer process in the not too distant future.

We must find a way to strike the proper balance between a clean, livable environment and an unaltered environment. The balance is between achieving and maintaining a livable, viable environment and the existence of an industry of the magnitude required to provide the necessary benefits for a populated world. Neither super safety, nor environmental carelessness is the answer. To solve these problems will require the sincere cooperation of the general public, industry, and government.

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APPENDIX A

CALCULATION OF AGE–STANDARDIZED RATES

Age–standardized death rates used by Doll and Peto[1] and most other authors is defined as the weighted average of the 18 separate *age-specific rates.* The *age-specific* death rate is defined as the death rate from some particular type of cancer (say, lung cancer) in a particular population (say U. S. males) in a particular narrow range of ages. The *18 five-year age ranges* used by Doll and Peto are: 0–4, 5–9, 10–14, 15–19, . . . , 75–79, 80–84, and 85 and over.

The age standardized rate computed obviously depends on the weights selected. For example, if positive weights were assigned to the first 13 age-specific male stomach cancer rates (those for 0–4, 5–9, . . . , 60–64) and zero weights for the last five ranges (65–69, 70–74, . . . , 80–84, and 85 and over), then one would obtain the age standardized stomach cancer rates among men under 65 years of age. To demonstrate the age-standardization procedure, the male cancer rates for the year 1977 are age-standardized to the U. S. 1970 population. The U. S. male cancer deaths for each age group for 1977, the male population for 1977, and the 1970 population distribution per 1,000,000 people (all taken from the U. S. census) is shown in Table A-1.

From column 3 of Table A-1, it is evident that for any age range i, the 1977 male cancer death rate is age-adjusted to the 1970 census (per 100 million people) as follows:

$$\bar{d}_{i,1977} = \left(\frac{d_{i,1977}}{n_{i,1977}}\right) w_{i,1970}$$

where

$d_{i,1977}$ = number of U. S. male cancer deaths in range i in 1977.

$\bar{d}_{i,1977}$ = number of U. S. male cancer deaths in age range i in 1977 after age-adjusting to the 1970 U. S. census (per 100 million people).

$n_{i,1977}$ = number of U. S. males in age range i in 1977.

$w_{i,1970}$ = weight for range i = number of people in range i per 100 million population.

The total U. S. male deaths due to lung cancer in 1977 [age-adjusted to 1970 per 100 million people] is the sum of the entries of column 3 of Table A-1, or the sum of the $\bar{d}_{i,1977}$'s. Since there are approximately 110 million males and 110 million females in the U. S. (with 10 million of each being over 65), the basis of 100 million people for the 1970 weight factor is a convenient basis because the resulting weighted cancer deaths are approximately equal to the actual number of cancer deaths.

The age-adjustment of the deaths for different years relative to one particular year, such as 1970, makes the comparison of trends more meaningful as discussed by Doll and Peto. The point on the curve in Figure 2 for cancer mortality of men for the year 1977 may

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be obtained from Table A-1 by converting the weighted sum of 68,643.9 deaths per 100 million to deaths per 100,000; that is,

$$68,643.9 \left(\frac{\text{deaths}}{100 \text{ million}}\right) = 68.6439 \left(\frac{\text{deaths}}{100,000}\right)$$

Table A-1. Calculation of the 1977 U. S. Male Cancer Deaths, Age-adjusted to the 1970 U. S. Census.

$$④ = \frac{①}{②} \times ③$$

Age Range No.	Age Range Years	① U. S. Male, Lung Cancer Deaths in 1977	② U. S. Male, Population in 1977	③ Weights, 1970 U. S. Census, per 100 million people	④ 1977, Deaths per 100 Million Age-adjusted to 1970
1	0–4	2	8.090×10^6	8.4416×10^6	2.1
2	5–9	5	9.051×10^6	9.8204×10^6	5.4
3	10–14	2	9.922×10^6	10.2304×10^6	2.1
4	15–19	10	10.896×10^6	9.3845×10^6	8.6
5	20–24	24	10.404×10^6	8.0561×10^6	18.6
6	25–29	55	9.378×10^6	6.6320×10^6	38.9
7	30–34	147	8.016×10^6	5.6249×10^6	103.2
8	35–39	423	6.397×10^6	5.4656×10^6	361.4
9	40–44	1, 119	$5,740 \times 10^6$	5.8958×10^6	1, 149.4
10	45–49	2, 921	5.879×10^6	5.9622×10^6	2, 962.3
11	50–54	5, 780	5.863×10^6	5.4643×10^6	5, 386.9
12	55–59	8, 607	5.425×10^6	4.9077×10^6	7, 786.3
13	60–64	11, 495	4.500×10^6	4.2403×10^6	10, 831.6
14	65–69	12, 987	3.704×10^6	3.4789×10^6	12, 063.5
15	70–74	11, 479	2.592×10^6	2.6789×10^6	11, 863.8
16	75–79	7, 972	1.648×10^6	1.8871×10^6	9, 128.6
17	80–84	4, 241	1.018×10^6	1.1241×10^6	4, 683.0
18	85–89	2, 038	0.674×10^6	0.7435×10^6	2, 248.1
		69, 307	109.197×10^6	100.0000×10^6	68, 643.9

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APPENDIX B

PHARMACOKINETICS–DEVELOPMENT OF EQUATIONS

The following development is presented for the purpose of providing an introduction to the field of pharmacokinetics and to demonstrate its similarity to the well-known field of chemical kinetics and catalysis.

"Pharmacokinetics" is a combination of the Greek word "*pharmakon*" meaning drug or poison and the word "kinetics". Thus, it means the application of the principles of the well-known fields of chemical kinetics and catalysis to the reaction of chemicals within the body or biochemical reactions.

Rates of Chemical Reactions

The rate of reaction of compound A may be defined as the moles of A disappearing by reaction per unit time per unit of volume occupied by A or more precisely

$$r_A = \frac{g\ moles\ of\ A\ disappearing}{(sec)(liter)} \tag{B-1}$$

or the rate of reaction may be defined in terms of appearance,

$$r_A = \frac{g\ moles\ of\ A\ appearing}{(sec)(liter)} \tag{B-2}$$

Generally, it is convenient to define the rate of reaction for reactants in terms of disappearance and products in terms of appearance.

For a batch reaction system of constant volume, it is readily shown[70]† by material balance on a batch reactor that,

$$r_A = -\frac{dC_A}{dt} \tag{B-3}$$

where r_A is defined in terms of disappearance, and C_A is equal to the concentration of A at any time t in gram mole per liter of reacting mixture.

If the rate of reaction r_A is defined in terms of appearance, then the material balance yields

$$r_A = \frac{dC_A}{dt} \tag{B-4}$$

† Additional references are listed at the end of each appendix.

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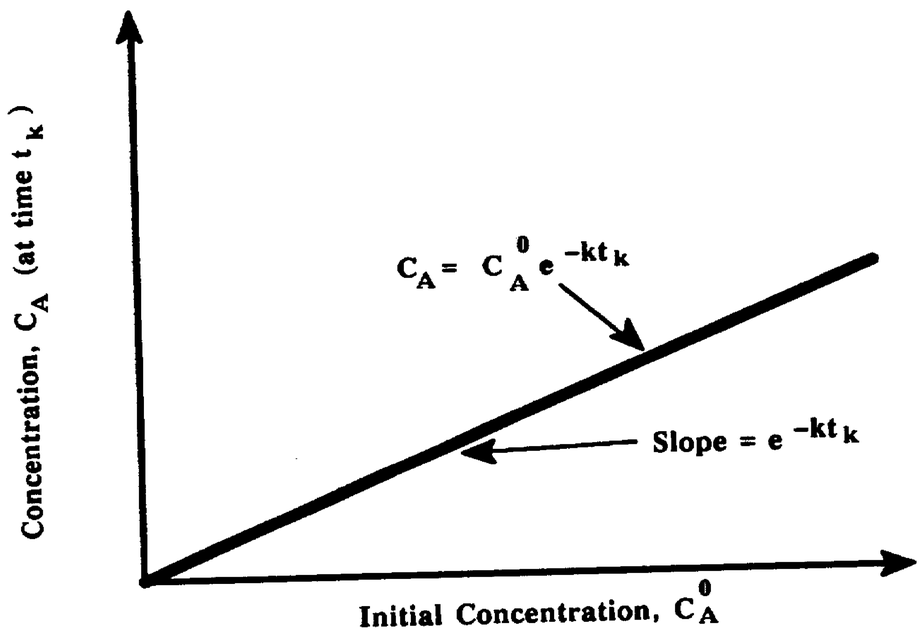

Figure B-1. At any time $t = t_k$, the concentration C_A given by Equation (B-8) is a linear funct of the initial concentration C_A^0.

Example of a Linear System

Consider the case where compound A reacts unidirectionally to form compound B

$$A \xrightarrow{k} B \tag{B-5}$$

Further suppose that from experiment it is found that the rate of reaction of A to B is proportional to the concentration of A raised to the first power (or the rate of reaction is first order with respect to the concentration of A) ; that is,

$$r_A = kC_A \tag{B-6}$$

where k is a proportionality constant at any given temperature.

Elimination of r_A from Equations (B-3) and (B-6) yields

$$-\frac{dC_A}{dt} = kC_A \tag{B-7}$$

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Equation (B-7) is an ordinary linear differential equation with constant coefficients which is readily solved by separation of the variables followed by integration to give :

$$C_A = C_A^0 e^{-kt} \tag{B-8}$$

where C_A^0 is the concentration of A at time t=0. At any time $t = t_k$, Equation (B-8) is a linear function of the initial concentration C_A^0. A plot of C_A versus C_A^0 at any time $t = t_k$ yields a straight line as shown in Figure B-1. Thus, one could extrapolate from any one point on this line to any other point by linear extrapolation, and obtain the correct answer since C_A is a linear function of C_A^0. This is the basis for the concept of linear extrapolation commonly employed to extrapolate from high initial doses (high values of C_A^0) to low doses (low values of C_A^0) corresponding to human exposures. Obviously, this technique gives the correct result if and only if C_A is a linear function of C_A^0 as in the example shown in Figure B-1.

Saturable Processes : Development of the Michaelis-Menten Rate Expression

Instead of the linear variation of C_A with respect to C_A^0, other reactions have mechanisms in which C_A is not a linear function of the initial concentration as in the case of saturable processes described by the Michaelis-Menten rate expression. To develop this rate expression consider the case where the product B is formed from A by the reaction mechanism

$$A + S \underset{k_1'}{\overset{k_1}{\rightleftharpoons}} AS \overset{k_2}{\rightarrow} B + S \tag{B-9}$$

S is a compound in the system which is consumed to produce AS, the active intermediate, and produced when B is formed from AS. Compound B is produced at the rate $r_2 = k_2 C_{AS}$, and thus,

$$\frac{dC_B}{dt} = k_2 C_{AS} \tag{B-10}$$

In the development which follows, expressions for C_{AS} and C_B in terms of C_A are needed. These expressions are obtained by use of the well-known "pseudo steady state assumption" from chemical kinetics. This assumption consists of the supposition that the active intermediate AS reacts to form B at approximately the same rate at which it is formed from A, which may be expressed as

$$\frac{dC_{AS}}{dt} \cong 0 \tag{B-11}$$

From the mechanism given by Equation (B-9), it follows that

$$\frac{dC_{AS}}{dt} \cong 0 = k_1 C_A C_S - k_1' C_{AS} - k_2 C_{AS}$$

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which is readily solved for C_{AS} to give

$$C_{AS} = \frac{k_1 C_A C_S}{k_1' + k_2} \tag{B-12}$$

By material balance

$$C_S = C_S^0 - C_{AS} \tag{B-13}$$

where C_S^0 denotes the initial concentration of S, and initially $C_{AS}^0 = 0$.

Elimination of C_S from Equations (B-12) and (B-13) yields

$$C_{AS} = \frac{C_S^0 C_A}{(\frac{k_1' + k_2}{k_1}) + C_A} \tag{B-14}$$

Substitution of this expression for C_{AS} into Equation (B-10) followed by rearrangement yields

$$\frac{dC_B}{dt} = \frac{k_2 C_S^0 C_A}{(\frac{k_1' + k_2}{k_1}) + C_A} = \frac{V C_A}{K + C_A} \tag{B-15}$$

where V and K are the Michaelis-Menten parameters whose definitions follow immediately from Equation (B-15).

Next dC_B/dt is related to dC_A/dt by first observing that by material balance

$$C_A = C_A^0 - C_{AS} - C_B \tag{B-16}$$

where it has been assumed that $C_{AS}^0 = C_B^0 = 0$. Explicit differentiation yields

$$\frac{dC_A}{dt} = -\frac{dC_{AS}}{dt} - \frac{dC_B}{dt} \tag{B-17}$$

Then by use of the pseudo steady state assumption, Equation (B-11), it follows that

$$\frac{dC_A}{dt} \cong -\frac{dC_B}{dt} \tag{B-18}$$

and thus, the Michaelis-Menten equation is obtained by combining Equations (B-15) and (B-18) to give

$$-\frac{dC_A}{dt} = \frac{V C_A}{K + C_A} \tag{B-19}$$

Consequently, the rate expression for the rate of reaction of A is given by

$$r_A = \frac{V C_A}{K + C_A} \tag{B-20}$$

where r_A is defined in terms of disappearance.

Characteristics of the Michaelis-Menten Rate Expression

Unlike the rate expression given by Equation (B-6), the one given by Equation (B-20) for r_A varies in its dependence on the concentration C_A. At small values of $C_A \ll K$, Equation (B-20) reduces to

$$r_A \cong \frac{V}{K} C_A \tag{B-21}$$

which is seen to be of the same form as Equation (B-6), first order with respect to A. As C_A^0 is increased, the value of C_A increases at any time t_k. The rate of reaction at any time t_k increases as C_A increases until it approaches its asymptotic maximum value which is shown as follows :

$$\lim_{C_A \to \infty} r_A = \lim_{C_A \to \infty} \frac{VC_A}{K + C_A} = \lim_{C_A \to \infty} \frac{V}{1 + \frac{K}{C_A}} = V \tag{B-22}$$

Also of interest is the fact that at $C_A = K$, r_A is equal to one-half its maximum value

$$r_A|_{C_A = K} = \frac{VK}{K + K} = \frac{V}{2} \tag{B-23}$$

A graph of r_A versus C_A is shown in Figure B-2.

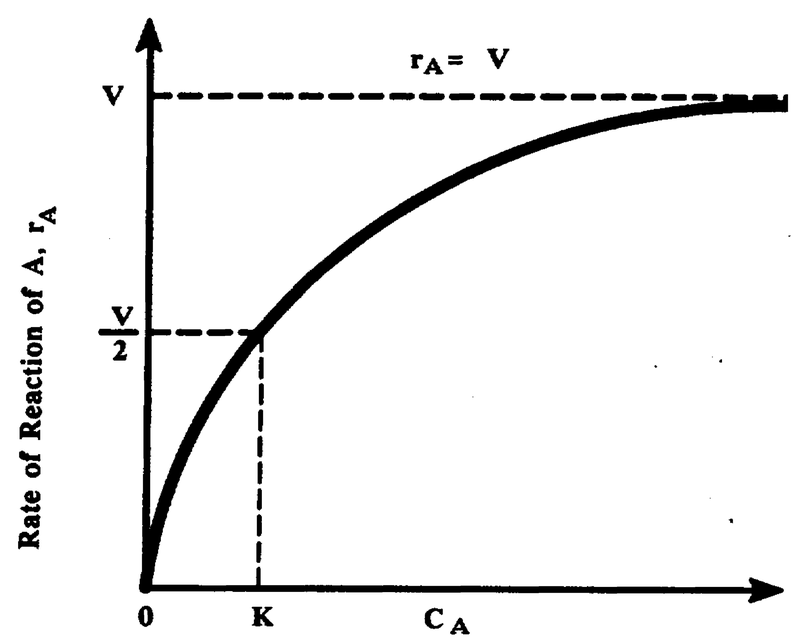

Figure B–2. The rate of reaction of A approaches its maximum value asymptotically as C_A is increased indefinitely.

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Equation (B-19)is readily solved by separation of the variables followed by integration from t=0 to t=t. At t=0, the initial value (or dose concentration) is denoted by C_A^0. That is,

$$-\int_{C_A^0}^{C_A}[\frac{K+C_A}{VC_A}]dC_A = \int_0^t dt \tag{B-24}$$

Thus

$$ln\frac{C_A}{C_A^0} + \frac{1}{K}[C_A - C_A^0] = -\frac{V}{K}t \tag{B-25}$$

which is readily rearranged to the exponential form

$$C_A = C_A^0 e^{\frac{1}{K}(C_A^0 - C_A)} e^{-\frac{V}{K}t} \tag{B-26}$$

Of importance is the behavior of the concentration C_A as the initial concentration C_A^0 (or dose) is increased. In this analysis, let the time be fixed at $t = t_k$ for all choices of C_A^0. At values of $C_A^0 \ll K$, Equation (B-26) reduces to

$$C_A = C_A^0 e^{-\frac{V}{K}t_k} \tag{B-27}$$

Observe that C_A is always less than C_A^0, and if $C_A^0 \ll K$, then

$$e^{\frac{1}{K}(C_A^0 - C_A)} \cong 1$$

A plot of C_A versus C_A^0 is shown in Figure B-3. The approximately linear portion of the curve is given by Equation (B-27). As C_A^0 is increased, the relationship

$$e^{\frac{1}{K}(C_A^0 - C_A)} > 1$$

always holds for intermediate values of C_A^0 because C_A^0 is always greater than C_A and thus $C_A^0 - C_A > 0$. Consequently, for all choices of finite values C_A^0, the value of C_A given by Equation (B-26) will always be less than C_A^0 and the resulting curve is of the form shown in Figure B-3. However, in the limit as C_A^0 approaches ∞, C_A likewise approaches infinity, and $C_A^0 - C_A$ is of the indeterminant form $\infty - \infty$. However, the variation of C_A versus C_A^0 in this region may be found by returning to Equation (B-22). At large values of C_A, $r_A \cong V$. Thus, as C_A becomes very large, $-dC_A/dt = V$ is readily solved to give

$$C_A = C_A^0 - Vt \tag{B-28}$$

Thus, at any $t = t_k$, the curve of C_A versus C_A^0 becomes linear at exceedingly high values of C_A^0 and its slope approaches unity. Also, it can be shown that the second derivative of C_A with respect to C_A^0 is always positive, which does not permit a point of inflection, and thus the curve of C_A versus C_A^0 is of the form shown in Figure B-3.

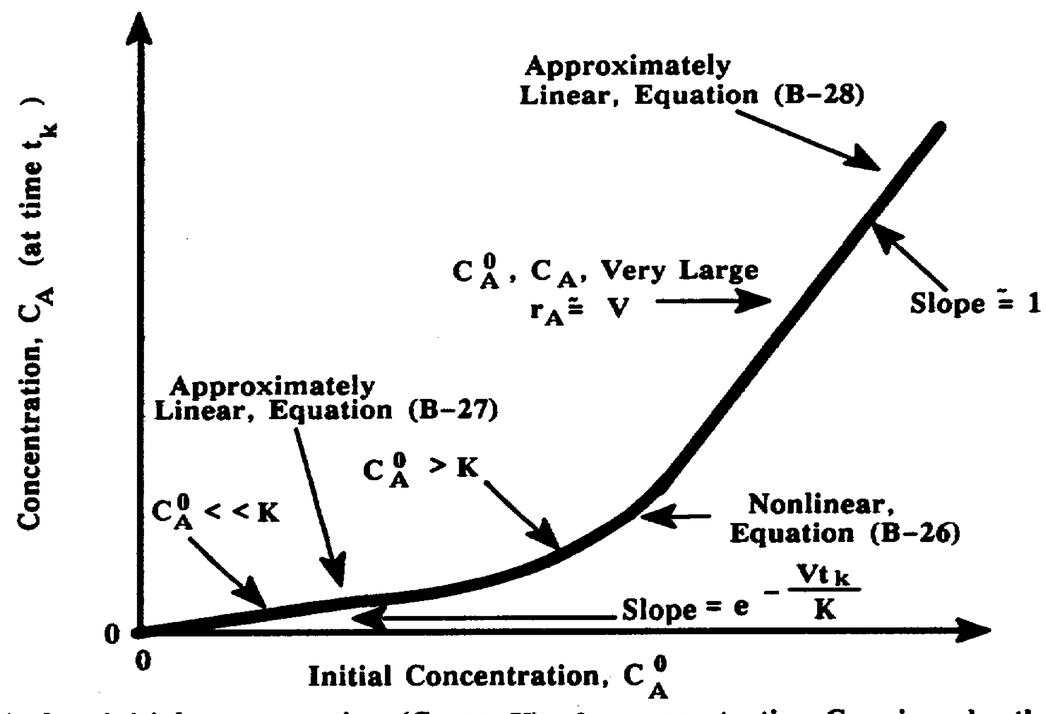

Figure B-3. At low initial concentration (C_A << K), the concentration C_A given by the Michaelis-Menten expression is approximately a linear function of C_A^0, but becomes nonlinear as the initial concentration C_A^0 is increased.

The parameters K and V of Equation (B-19) may be determined in a number of ways. One could use the relationships displayed in Figure B-2 or one could simply rearrange Equation (B-19) to the linear form :

$$-\frac{1}{dC_A/dt} = (\frac{K}{V})\frac{1}{C_A} + \frac{1}{V} \tag{B-29}$$

The instantaneous rate dC_A/dt may be approximated from experimental data by noting that

$$\frac{dC_A}{dt} \cong \frac{C_A(t_2) - C_A(t_1)}{t_2 - t_1} = \frac{\Delta C_A}{\Delta t}$$

where $t_2 > t_1$ and $C_A(t_2), C_A(t_1)$ denote the values of C_A at times t_2 and t_1. For small values of Δt, Equation (B-29) may be restated in the approximate form :

$$-\frac{1}{\Delta C_A/\Delta t} = (\frac{K}{V})\frac{1}{C_A(t_{av})} + \frac{1}{V} \tag{B-30}$$

where $t_{av} = (t_2 + t_1)/2$. The straight line has the slope $\frac{K}{V}$ and the intercept $\frac{1}{V}$, from which K and V may be obtained.

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Linear Extrapolation Involving the Michaelis-Menten Model

Since C_A is approximately a linear function of the initial concentration C_A^0 at small values of C_A and C_A^0 ($C_A < C_A^0 \ll K$) at any time t_k, it follows that approximately the correct value of C_A may be obtained by linear extrapolation between different initial concentrations C_A^0 in this region of concentrations as indicated by Figure B-3. However, as shown in this figure, the concentration C_A is no longer linear with C_A^0 throughout the entire range of higher concentrations. Hence, linear extrapolation would generally fail to give the correct result at the higher concentrations or from higher concentrations to lower concentrations.

In fact, the mechanism given by Equation (B-9) which leads to a linear variation of C_A versus C_A^0, at low values of C_A, and nonlinear at relatively high values of C_A^0 should not be taken to mean that this is a general result for all mechanisms. When the mechanism is expanded to include several subsequent consecutive, simultaneous, and competing reactions, the nature of the variation of C_A versus C_A^0 can be expected to change and most likely C_A will become nonlinear with C_A^0 for both low and high values of C_A^0.

Also, a slightly different mechanism than the one given by Equation (B-9) could lead to a different form of the curve of C_A versus C_A^0. Suppose for example, that the rates given by Equation (B-9) are all second order with respect to C_A instead of first order; that is, suppose the mechanism is given by

$$2A + 2S \underset{k_1'}{\overset{k_1}{\rightleftharpoons}} 2AS \overset{k_2}{\rightarrow} 2B + 2S \tag{B-31}$$

Instead of Equation (B-10), one obtains

$$\frac{dC_B}{dt} = k_2 C_{AS}^2 \tag{B-10$'$}$$

Thus,

$$\frac{dC_{AS}}{dt} \cong 0 = k_1 C_A^2 C_S^2 - k_1' C_{AS}^2 - k_2 C_{AS}^2$$

and,

$$C_{AS} = \frac{C_A C_S}{\sqrt{\frac{k_1' + k_2}{k_1}}} \tag{B-12$'$}$$

Since $C_S = C_S^0 - C_{AS}$, Equation (B-14) becomes

$$C_{AS} = \frac{C_S^0 C_A}{\sqrt{\frac{k_1' + k_2}{k_1}} + C_A} \tag{B-14$'$}$$

and by following the same steps as before,

$$r_A = -\frac{dC_A}{dt} = \frac{VC_A^2}{[K + C_A]^2} \qquad \text{(B-20}'\text{)}$$

where

$$V = k_2(C_S^0)^2$$

$$K = \sqrt{\frac{k_1' + k_2}{k_1}}$$

Thus, for $C_A \ll K$

$$r_A \cong \frac{VC_A^2}{K^2} \qquad \text{(B-21}'\text{)}$$

and

$$\lim_{C_A \to \infty} r_A = V \qquad \text{(B-22}'\text{)}$$

Also,

$$r_A|_{C_A=K} = \frac{VK^2}{(2K)^2} = \frac{V}{4} \qquad \text{(B-23}'\text{)}$$

The integrated forms of Equations (B-21′), (B-20′) and (B-22′) are, respectively,

$$C_A = \frac{C_A^0}{1 + C_A^0 \frac{V}{K^2} t} \qquad (C_A \ll K) \qquad \text{(B-27}'\text{)}$$

$$K^2[\frac{1}{C_A^0} - \frac{1}{C_A}] + 2Kln\frac{C_A}{C_A^0} + (C_A - C_A^0) = -Vt \qquad (\textit{intermediate values of } C_A^0) \qquad \text{(B-25}'\text{)}$$

$$C_A = C_A^0 - Vt \qquad (\textit{very large values of } C_A^0) \qquad \text{(B-28}'\text{)}$$

Thus, in this case the curve of C_A versus C_A^0 is nonlinear at small as well as intermediate values of C_A^0 and linear at large values of C_A^0, as shown in Figure B-4. Also, it can be shown that the first derivative of C_A with respect to C_A^0 is always positive, approaches unity at the origin, is less than unity for $0 < C_A^0 < \infty$, and approaches unity as C_A^0 approaches infinity. Thus, the curve has a point of inflection, which is confirmed by showing that the second derivative has a zero in the interval $0 < C_A^0 < \infty$. The curves shown in Figures B-3 and B-4 have the shape of a hockey-stick which has been observed experimentally as discussed by Hoel[51], Sielken[52], and Menzel.[71]

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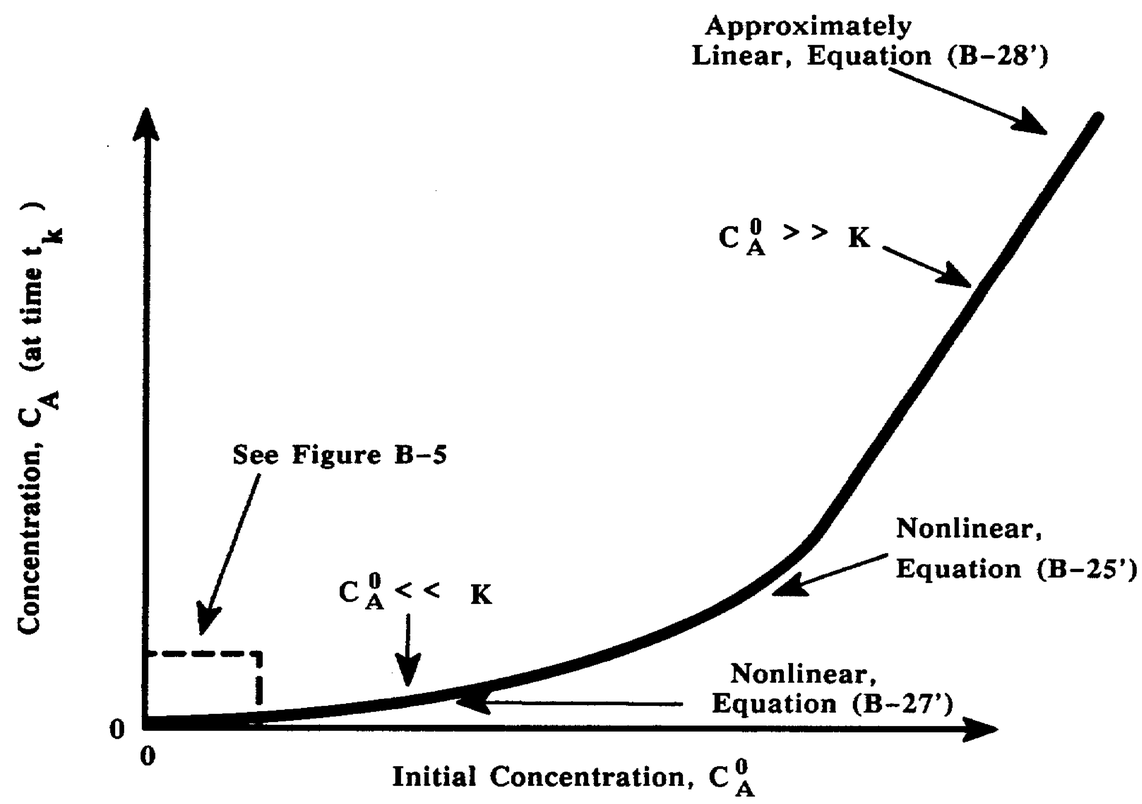


Figure B-4. From low to relative high initial concentrations, C_A versus C_A^0 is nonlinear, and at very high initial concentrations it becomes approximately linear. [See Equations (B-25'), (B-27') and (B-28').]

The blown-up section of the curve shown in Figure B-4 is presented in Figure B-5. The larger scale of Figure B-4 completely masks the shape of the curve near the origin.

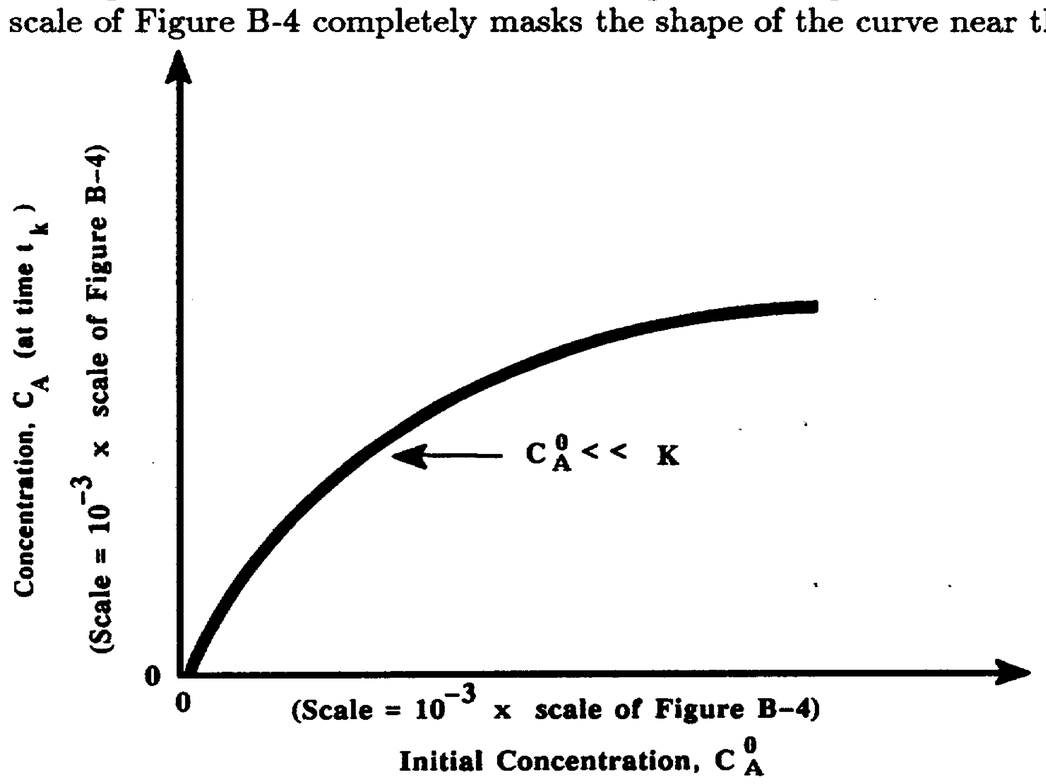
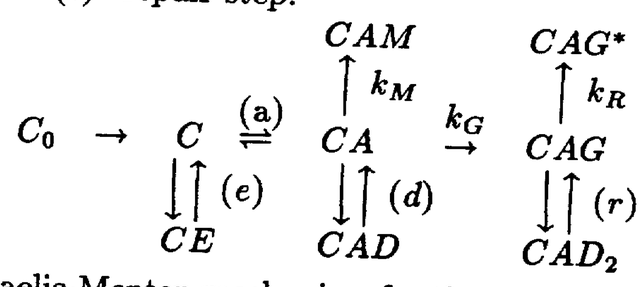


Figure B-5. Blown-up graph of the function near the origin in Figure B-4. As C_A^0 approaches zero, the slope approaches unity.

Formulation of the rate Expressions for the Cancer Mechanism Shown in Figure 16

In the following mechanism proposed by Gehring and Blau[42], the steps shown as equilibrium reactions are a short hand for the Michaelis-Menten mechanism. The absorbed chemical is denoted by C and its concentration is also denoted by C; the excreted chemical by CE and its concentration by C_E; the activated metabolites by CA and their concentration by C_A; the damaged DNA adducts by CAG and their concentration by C_{AG}; the detoxified metabolites by CAD and their concentration by C_{AD}; the macromolecules by CAM and their concentration by C_{AM}; the repaired DNA adducts by CAD_2 and their concentration by C_{AD_2}; and the replicates of the damaged DNA by CAG* and their concentration by C_{AG^*}. Also, k_M, k_G, and k_R are the rate constants shown, the letters enclosed by parenthesis have the following meanings : (e)=excretion, (a)=activation, (d)=detoxification, and (r)=repair step.



To illustrate the Michaelis-Menten mechanism for the steps shown as equilibrium reactions, the complete mechanism for the reaction of C to CE and to CA is presented; namely,

$$C + S \rightleftharpoons CS_e$$

$$CS_e \rightleftharpoons SE + S_e$$

$$C + S_a \rightleftharpoons CS_a$$

$$CS_a \rightleftharpoons CA + S_a$$

where S_e and S_a denote the respective substrates involved in the respective reactions. In a manner analogous to that demonstrated in the development of the Michaelis-Menten relationship given by Equation (B-19), it is readily shown that

$$-\frac{dC}{dt} = \frac{V^e C}{K^e + C} + \frac{V^a C}{K^a + C}$$

Similarly,

$$-\frac{dC_A}{dt} = \frac{V^d C_A}{K^d + C_A} - \frac{V^a C}{K^a + C} + k_M C_A + k_G C_A$$

$$-\frac{dC_{AG}}{dt} = \frac{V^r C_{AG}}{V^r + C_{AG}} + k_R C_{AG} - k_G C_A$$

The rates of appearance of CE, CAD, CAM, CAD_2, and CAG^* are given by

$$\frac{dC_E}{dt} = \frac{V^e C}{K^e + C}$$

$$\frac{dC_{AD}}{dt} = \frac{V^d C_A}{K^d + C_A}$$

$$\frac{dC_{AM}}{dt} = k_M C_A$$

$$\frac{dC_{AD_2}}{dt} = \frac{V^r C_{AG}}{K^r + C_{AG}}$$

$$\frac{dC_{AG^*}}{dt} = k_R C_{AG}$$

For an initial concentration of the chemical C and a set of values for all of the V and K parameters as well as the rate constants k, the above set of ordinary differential equations may be solved simultaneously as a function of time. From the results so obtained, graphs similar to those shown in Figure 17 may be prepared.

ADDITIONAL REFERENCES

68. Holland, C. D., and R. G. Anthony, *Fundamentals of Chemical Reaction Engineering*, 2nd. Edition, Prentice-Hall, Inc., Englewood Cliffs, New Jersey (1989).
69. Menzel, D. B., "Physiological Pharmacokinetic Modeling," *Environ. Sci. Technol.*, *21*, (10) (944).1987

APPENDIX C

THE BIOCHEMICAL BASIS FOR CELL REPLICATION

This appendix consists of an abbreviated summary of some of the fundamentals and developments of biochemistry which form the basis of the present theory of cell replication within the human body. Excellent treatments of the concepts summarized in this appendix are given by Ham[72], Bailey and Ollis[73], and Aiba et al.[74]

According to Ham[72], a cell may be defined as the smallest unit of living material which is capable of existing independently in a non-living environment by synthesizing substances which it needs from nutrients absorbed from its environment. Most cells also have the capacity to reproduce themselves. The condition of a non-living environment rules out viruses because they can exist only in a living environment.

Cells have also been defined in terms of their structure; namely, as a minute body of *protoplasm* surrounded by a limiting *membrane* and containing a *nucleus*. Protoplasm was defined by Huxley as the physical basis of life. It is now generally accepted that the manifestations of life are dependent upon the continuance of a vast number of chemical reactions which occur in and also with the protoplasm. Since protoplasm is consumed by chemical reaction it must be produced by other chemical reactions from the surrounding nutrients.

The sum total of all chemical reactions occurring in a cell and conferring on it the properties of life is called its *metabolism*. Most of the chemical reactions which occur within a cell are catalyzed by *enzymes* which consist of proteins that are synthesized from amino acids. Proteins also serve a number of other purposes in cells, such as membranes.

There are three fundamental classes of cells; namely, (1) those which never reproduce themselves such as nerve cells, (2)those which constantly reproduce themselves (called stem cells) to replace those that are lost, and (3) those which do not ordinarily reproduce themselves but have the capacity to do so, such as liver cells.

Structures of DNA and RNA

A benchmark in the understanding of cell behavior was provided by the structural concept of the DNA (deoxyribonucleic acid) proposed in 1953 by Watson and Crick.[75] The biopolymer DNA contains all of the cell's hereditary information. The DNA nucleotide is composed of three components : (1) phosphoric acid, (2) deoxyribose sugar, and (3) the nitrogen bases, thymine (T), cytosine (C), adenine (A) , and guanine (G). The biopolymer RNA has a structure similar to DNA, and it assists in the replication of DNA. Instead of the deoxyribose sugar and thymine, RNA contains d-ribose sugar and the nitrogen base uracil.

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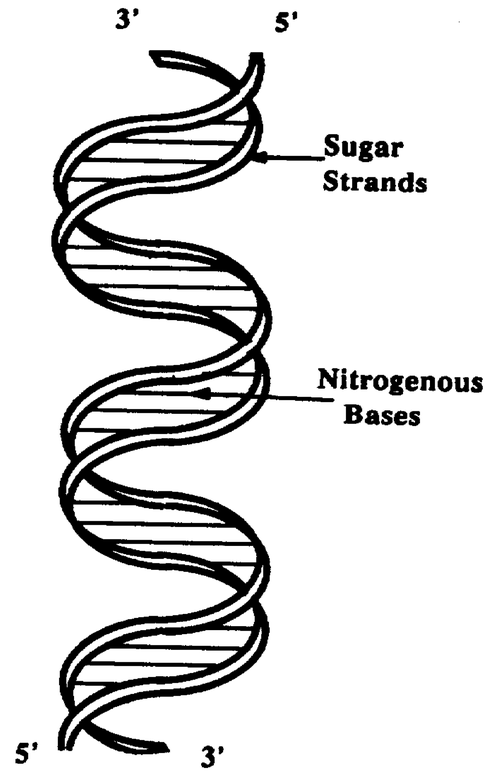
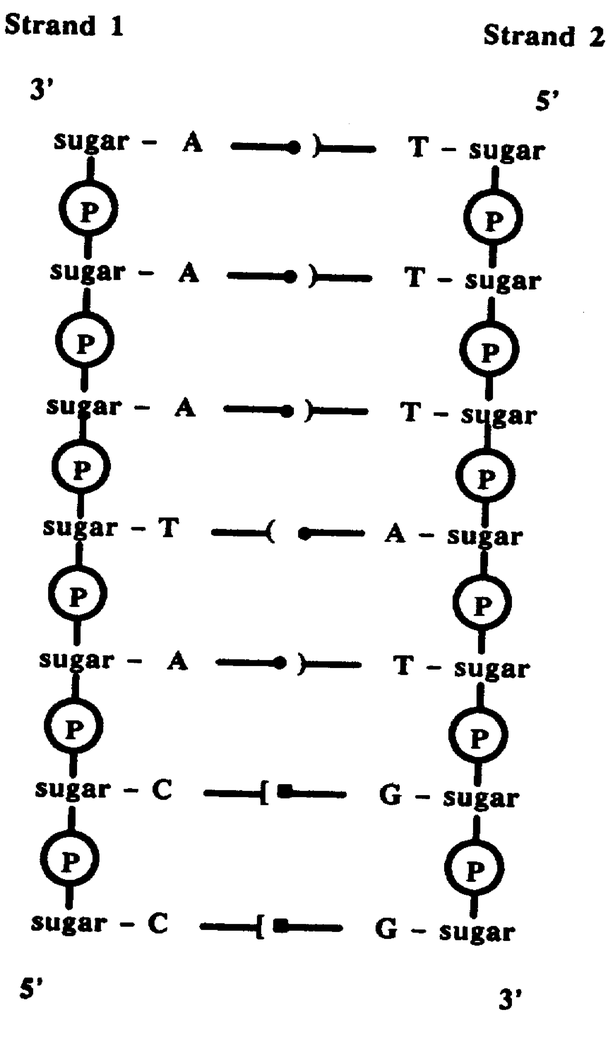


DNA Double-helical



P = phosphatediester bonds

—•)— = two hydrogen bonds connecting A and T

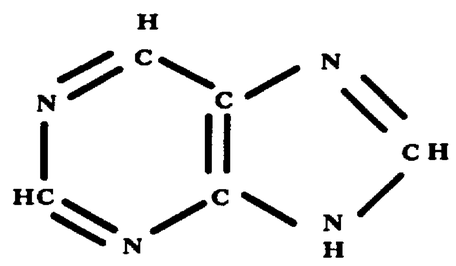
—■]— = three hydrogen bonds connecting G and C

A = adenine, T = thymine, G = guanine, C = cytosine

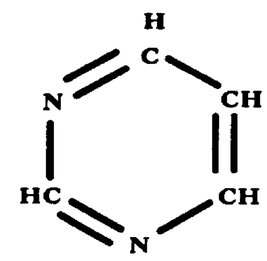
Figure C–1. The strands of the DNA molecule consist of the sugar deoxyribose connected by the phosphatediester bonds, and the strands are cross-connected by the nitrogen bases [adenine, thymine, cytosine, and guanine].

A diagram of a portion of the double helix DNA molecule is shown in Figure C-1. The sugars, deoxyribose and d-ribose which make up the nucleotides of the strands of DNA

of the DNA and the d-ribose of the RNA are usually derived from either purine or pyrimidine.

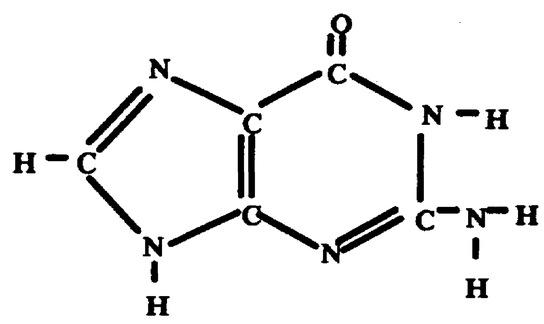


Purine

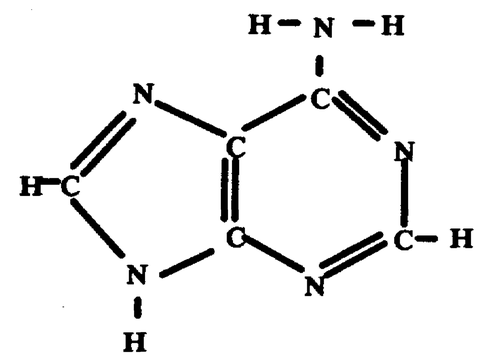


Pyrimidine

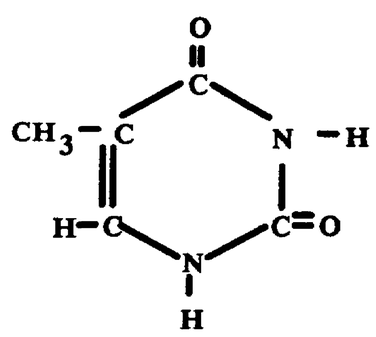
The nitrogenous bases shown in Figure C-3 are attached to each other in the crosslinks of the DNA by hydrogen bonds. Adenine and thymine are connected by two hydrogen bonds, while guanine and cytosine are thought to be connected by three hydrogen bonds as shown by Spencer.[76] The adenine-thymine hydrogen bonding is shown in Figure C-4. Also as indicated in Figure C-1, thymine is always paired with adenine, and cytosine is always paired with guanine. These strands may be as long as 40 million molecules, and the molecular weights of some DNA molecules range up to 2.8 billion.[73]



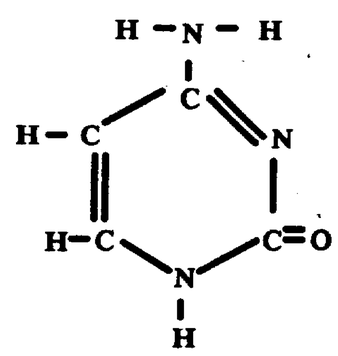
Guanine (in both DNA and RNA)



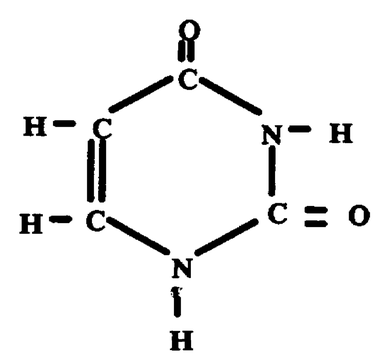
Adenine (in both DNA and RNA)



Thymine (in DNA only)



Cytosine (in both DNA and RNA)



Uracil (in RNA only)

Figure C–3. The nitrogen bases in DNA and RNA.

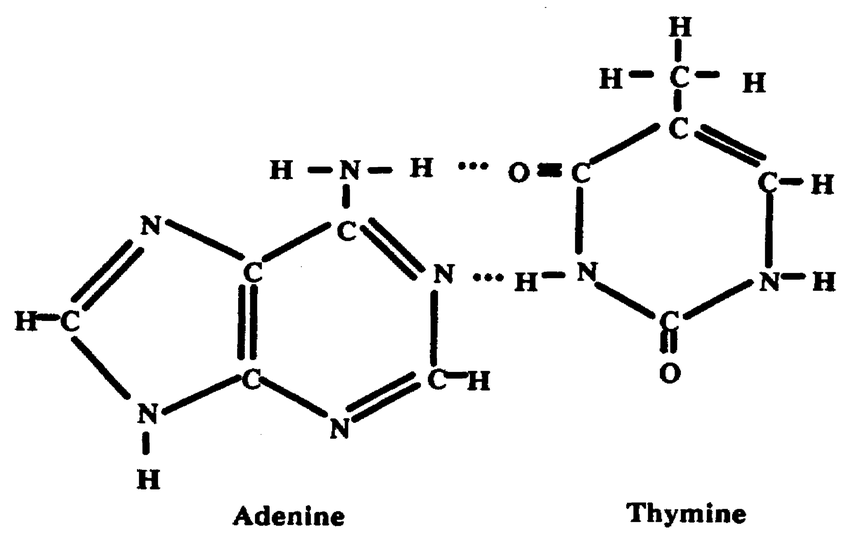


Figure C–4. Hydrogen bonding of the Adenine–Thymine in the DNA crosslinks.

Duplication of DNA Molecules

The first step in the duplication of a DNA molecule is the separation of the two strands by the breaking of the weak hydrogen bonds connecting the nitrogenous bases as shown in Figure C-5. After the two strands have been separated, a new strand is synthesized beside each of the original strands, and the two new molecules of DNA formed are exact replicas of the original DNA molecule as shown in Figure C-5.

Biological Implication of the DNA Duplication Process

The duplication process illustrated in Figure C-5 corresponds to cell division which in turn corresponds to the division of the *nucleus* of the cell. Prior to cell division, the nucleus appears as a well defined mass in the cell. During the division process, known as *mitosis*, the *nucleus* membrane dissolves and the chromosomes of the *nucleus* become rodlike in form. The chromosomes consist of DNA molecules and proteins, which had been identified by use of a microscope long before the structure of DNA was proposed by Watson and Crick[75] in 1953. The chromosomes carry all the hereditary information in the form of genes. Although much effort had gone into the visual identification of the genes prior to the proposed structure of DNA, it met with no success because it has now been established that genes are not physical entities but consist merely of the ordering of the nitrogenous bases connecting the DNA strands of sugars. This sequencing of the bases controls the synthesis of proteins. In particular, the genes consist of the sequences of "three letter

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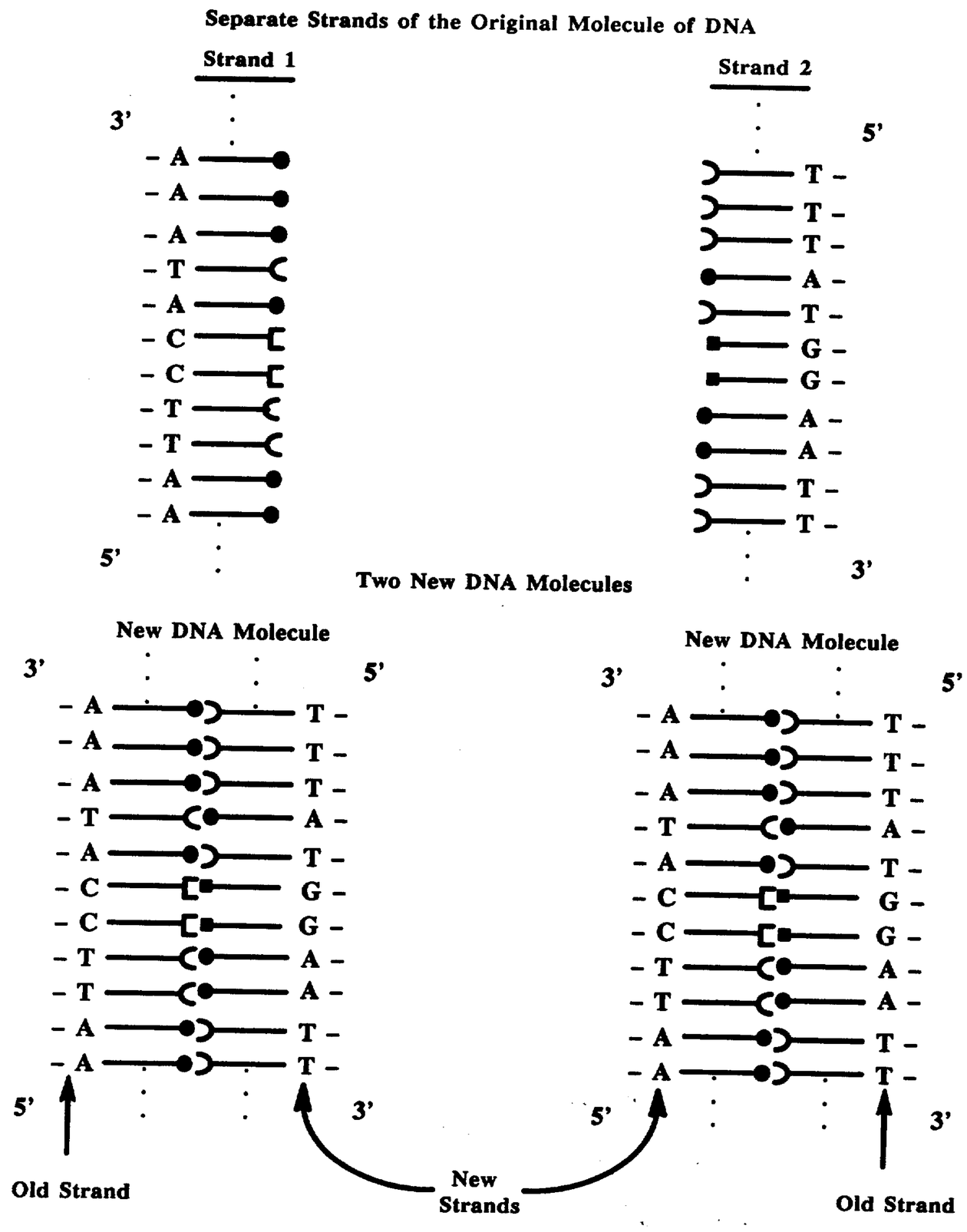


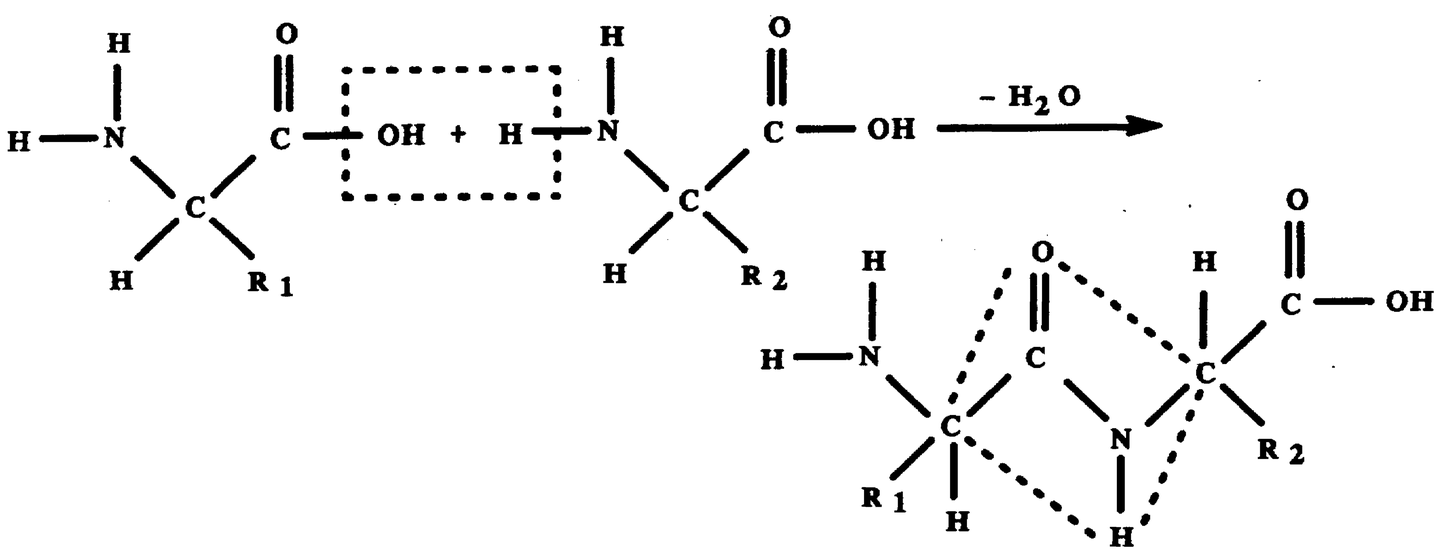
Figure C–5. Division of the DNA Molecule followed by Duplication.

words" selected from the four letters T, A, C, G corresponding to the four nitrogenous bases. Only code words of three letters each are needed to specify the 22 amino acids required for the synthesis of the appropriate proteins. The amino acids listed in Table

TABLE C-1. THE AMINO ACIDS INVOLVED IN THE SYNTHESIS OF PROTEIN ENZYMES.

Name	Abbreviation	Name	Abbreviation
Alanine	Ala	Leucine	Leu
Arginine	Arg	Lysine	Lys
Asparagine	Asn	Methionine	Met
Aspartic Acid	Asp	Phenylalanine	Phe
Cysteine	Cys	Proline	Pro
Glutamic acid	Glu	Serine	Ser
Glutamine	Gln	Threonine	Thr
Glycine	Gly	Tryptophan	Trp
Histidine	His	Tyrosine	Tyr
Isoleucine	Ile	Valine	Val

C-1 are those which are involved in the formation of protein enzymes by condensation. As an example, consider the formation of a simple protein polymer by condensation of two amino acids (containing the R_1 and R_2) as follows :



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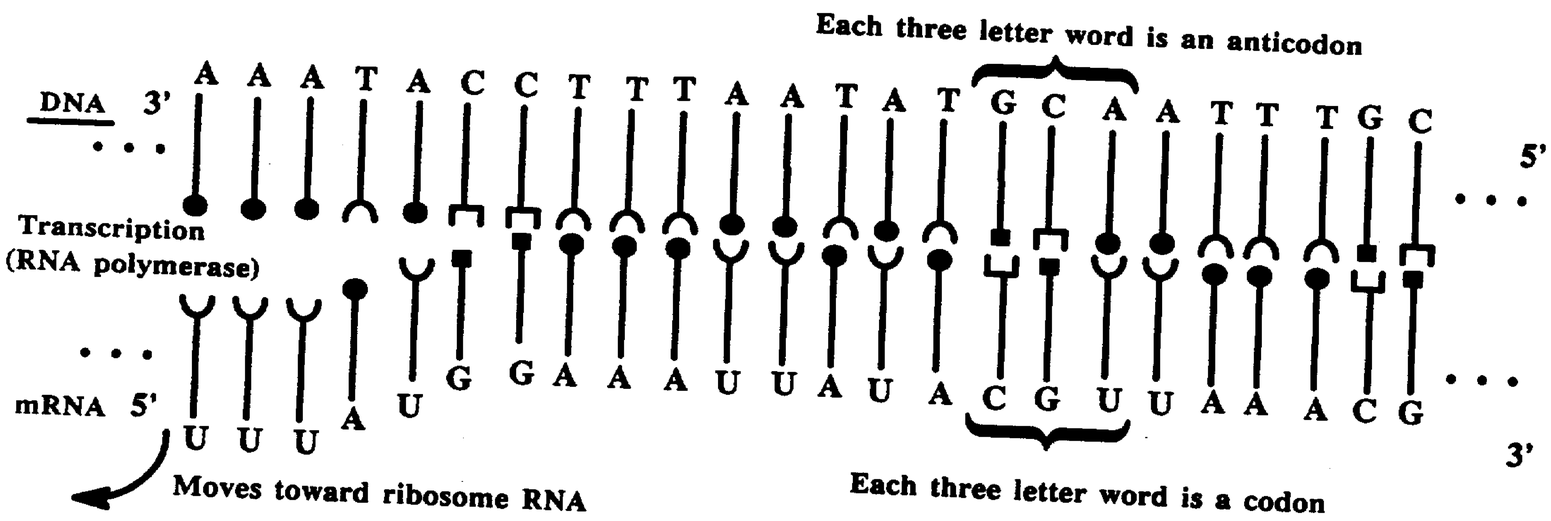
The peptide bond containing the six atoms shown by the dashed-box region constitute the peptide bond. In a similar manner, another amino acid may be added to this protein by a peptide bond.

Roles of RNA

There are at least three types of RNA and all three types are involved in the mechanism whereby information stored in the DNA chromosomes is made active at the sites of protein synthesis. The three types are called: (1)messenger RNA, (2)ribosomal RNA, and (3) transfer RNA, which are denoted by mRNA, rRNA, and tRNA, respectively. All of these are formed in the nucleus. The constituents of RNA are the same as those of DNA except as noted above; namely, RNA has the base uracil instead of thymine and the sugar, d-ribose, instead of deoxyribose. Also, most kinds of RNA are single-stranded, although some may be double-stranded.

As the strands of DNA begin to separate, the first step in the synthesis of the protein enzymes from amino acids is initiated by the synthesis of messenger RNA (mRNA) from polymerase in an order specified by the template (the 3′ to 5′ strand of DNA) as shown below.



The synthesis of messenger RNA is called *transcription* because the mRNA carries the inscription of the 3 letter words formed from A, C, G, and U. The molecular weight of mRNA ranges from 25,000 to 1,000,000 according to Bailey and Ollis.[73]

Perhaps the most amazing aspect of the protein production process depicted above is the fact that the code for the specification of each amino acid by a 3 letter word of the mRNA *(codons)* has been broken. This code is presented in Table C-2, which was taken from Bailey and Ollis.[73] Observe that from this table, the codon AUG of the mRNA has two functions, one to start the process of condensation of the amino acids and the second to specify that the first acid in the protein chain is to be methionine (Met). The next codon GAA specifies that the second amino acid is to be glutamic acid (Glu). Finally, the last

TABLE C-2. CODE OF THE CODONS OF mRNA.
[Taken from *Biochemical Fundamentals*, 2nd Ed. by Bailey, J.E., and D.F.Ollis, McGraw-Hill Book Company (1986).]

first	second: U	C	A	G	third
U	Phe	Ser	Tyr	Cys	U
	Phe	Ser	Tyr	Cys	C
	Leu	Ser	Stop	Stop	A
	Leu	Ser	Stop	Trp	G
C	Leu	Pro	His	Arg	U
	Leu	Pro	His	Arg	C
	Leu	Pro	Gln	Arg	A
	Leu	Pro	Gln	Arg	G
A	Ile	Thr	Asn	Ser	U
	Ile	Thr	Asn	Ser	C
	Ile	Thr	Lys	Arg	A
	Met	Thr	Lys	Arg	G
G	Val	Ala	Asp	Gly	U
	Val	Ala	Asp	Gly	C
	Val	Ala	Glu	Gly	A
	Val	Ala	Glu	Gly	G

codon UAA has the single function of stopping the process.

As the mRNA is formed it begins to move away from the DNA strand in the direction of the ribosome RNA (rRNA). The ribosome RNA and transfer RNA are synthesized in a manner similar to that shown for mRNA, by using a portion of a DNA strand as a template. Bailey and Ollis[73] state that the molecular weights of rRNA and tRNA range from 35,000 to 1,000,000 and 23,000 to 30,000, respectively. The sequencing of the bases attached to tRNA is the same as that of the bases attached to the 3′ to 5′ DNA strand where, of course, uracil replaces thymine. Each 3 letter word of tRNA attaches itself to one specific amino acid from among the 20 different types of amino acids in the neighborhood of the messenger mRNA and rRNA. The rRNA acts as a site for the mRNA as it participates in the formation of the protein enzymes. This process may be visualized as shown in the following diagram.

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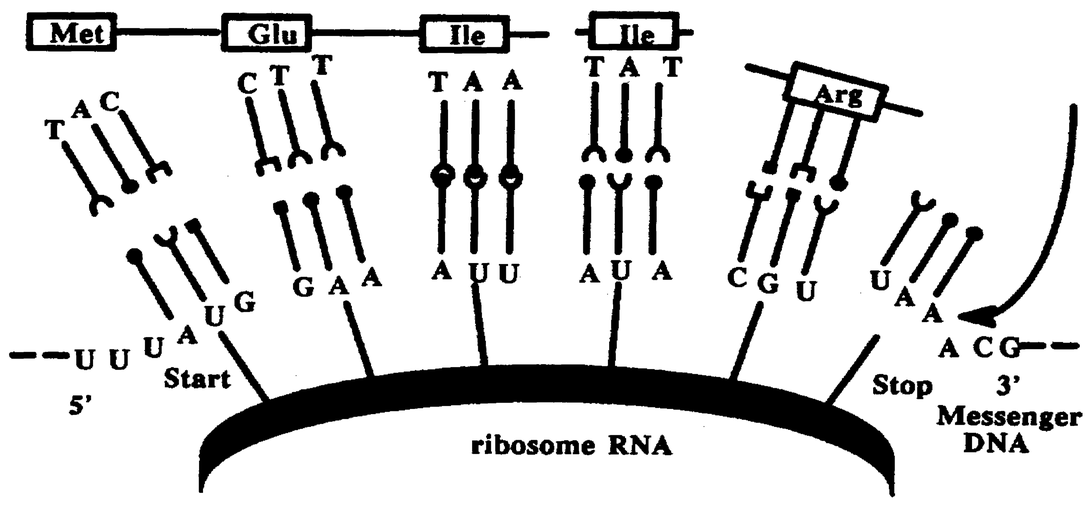

Some Mechanisms of DNA Damage and Repair

From the foregoing discussion of the roles of DNA and RNA in cell replication, it is evident that the most critical step in the utilization of the information carried by the DNA (the chromosomes and the associated genes) is the formation of protein enzymes from the amino acids. Since these enzymes catalyze the chemical reactions required to produce the products utilized in cell replication, the importance of producing the unique enzymes is of primary importance in the true replication of cells. In view of the complexity of this process, spontaneous errors in the replication of DNA, called *mutations*, do occur. The rate is relatively low, however, of the order of 1 error for every 10^6 gene duplications.

As an example, the alteration of a codon for one amino acid can lead to the substitution of a different amino acid in the protein chain, thereby forming an abnormal protein. Sickle-cell anemia in humans is caused by an abnormal protein which contains valine (Val) instead of glutamic acid (Glu). This substitution is caused by replacing the codon GAA or GAG for Glu by the codon GUA or GUG for Val.

Another postulated mechanism for spontaneous mutation is based on the fact that the nucleotide bases of DNA have several structural forms known as *tautomers*. Although the equilibrium is believed to favor the forms shown in Figure C-3, shifts to other tautomeric forms could cause errors in the base pairings.

Errors in transcription can also result from the incorporation of analogs of the correct bases in the DNA crosslinks. Damage to the DNA bases can be induced by a host of classical reactions such as alkylation. These mechanisms can lead to a number of different

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types of damage to the DNA such as : (1) single and double breaks of the DNA strands, (2) base alterations and strand alterations by chemical reactions, (3) intra-strand crosslinks of DNA, (4) crosslinks of the proteins, (5) crosslinks between proteins and a DNA strand, and (6) base substitution by analogs. A tremendous research effort has been made in the identification of these mechanisms of damage, and the literature is voluminous. The major references used in the preparation of this report are listed in the books given by references 77 through 80. Although these books constitute a small percentage of the total publications in this area, each of them contains hundreds of references.

Of all the possible mechanisms for DNA damage, the free radical mechanism is generally conceded as contributing the greatest variety of DNA damage. Many biological reactions occur by the free radical mechanism in a manner analogous to that seen in petrochemical polymerization reactions. Free radicals may be formed in a number of ways. Ionizing radiations such as x-rays and γ-rays , generate ions which generally lead to free radical formation. Also, many compounds including some beneficial drugs are capable of generating free radicals.

In view of all the possible mechanisms for damaging the DNA, the situation might appear hopeless, but that the bio-systems have a remarkable capacity for the repair of damage. Wilson (see pg. 1, Ref. 78) attributes the survival of the species to the inhibitory, scavenging and biochemical repair systems.

Free radical formation from sources other than radiation are prevented by the cell compartmentalized structure, the presence of catalase and glutathione peroxidase which are able to decompose hydrogen peroxide, and to the presence of the macromolecules transferrin and ferritin which are able to bind iron.

In the event that free radicals are formed, they are generally scavenged immediately by nearby organic material. If however, some do reach vital molecules and oxidize them, there is still the opportunity for free radical repair. The concept of free radical repair by reduction or hydrogen transfer arose in the early 1950's in an attempt to partly explain the protective actions of diphenyl picryl hydrazl and octyl mercaptan on radiation-induced decomposition of some polystyrene polymers.

Free Radicals Produced from Chemical Carcinogens and Their Significance in Carcinogenesis

Almost all classes of chemical carcinogens are able to generate free radicals by enzymatic reactions. On the other hand, noncarcinogenic compounds are relatively stable and difficult to convert to free radicals under the same conditions. As pointed out by Nagata (see pg. 58, Ref. 80), these results suggest a role of free radicals in chemical carcinogenesis. In fact, one of the general theories of cancer asserts that it is caused by uncontrolled free radical reactions. An extension of this theory is that the active forms of most chemical carcinogens are free radicals. Most of the studies support these concepts but do not constitute a proof that free radicals initiate the reactions which causes malignant transformation.[80] Also, the experimental evidence points to a role of free radicals in the rate of cell division.

Although the story of cancer formation and growth is not complete and direct proofs

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