THE UNIVERSITY OF KANSAS
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THE PHILIP NEWMARK MEMORIAL LECTURE:
Bivalent Sulfur in the Preservation of Life

By

Irving Goodman

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Philip Newmark

When Philip Newmark arrived in Boulder to begin his predoctoral studies at the University of Colorado, his warm personality, his humanism and his superb intellect were evident from the start. Dr. Newmark came to the University in the fall of 1946 to join our group engaged at that time in a research program involving the synthesis and biochemical study of pyrimidine nucleoside analogues as potential metabolic inhibitors.

Dr. Newmark plunged into this new venture with tremendous energy and enthusiasm and with remarkable thoroughness. He devoted endless hours, often deep into the night, to the complex problems of adapting the protozoan organism, *Tetrahymena geleii* W, to the study of metabolite antagonists, using the techniques developed by Dr. George Kidder of Amherst as a model. From the minutest detail to broadest basic concept, all aspects were examined with his characteristic intensity.

In April, 1949, after hundreds of *Tetrahymena* experiments, Dr. Newmark reported, ". . . The testing program with *T. geleii* W has just begun to prove fruitful. . . . The lack of activity of the synthetic nucleosides tested seems to be caused by the pyranose form of the sugar as compared with the furanose structure found in naturally occurring nucleosides. Our results show that *Tetrahymena* cannot readily hydrolyze the glycosidic linkage between the pyrimidine and pyranose sugar moeities. . . ." (It was Mrs. Newmark who established the pyranose structures of the synthetic nucleosides used in these experiments.)

The graduate years were beset with seemingly endless crises. Having come from a family of very modest income, Dr. Newmark was entirely dependent upon his own resources for support. Along with the customary annual problems of fellowship and scholarship applications and reports came a series of home crises culminating in the death of his mother in 1949 as a consequence of nitrous oxide anesthesia in an operation. Home difficulties continued to mount with the decline of his father, who, up until Phil's last days, was a source of grave concern.

It was during the Colorado period, in 1947, that Phil met and married a fellow graduate student, Marjorie Zeiger, now Dr. Marjorie Z. Newmark.

Dr. Newmark had no difficulty convincing the appropriate agencies of his ability and competence, for throughout his entire predoctoral career and during his years as a post-doctoral fellow he was awarded a continuous series of scholarships, fellowships and grants from various agencies including the U.S. Public

analytical thought showing broad comprehension and indicating long range benefits to science. In a research proposal submitted to the National Science Foundation, Dr. Newmark introduced his discussion with these terse observations:

"Most studies on the biosynthesis of nucleic acids and proteins have been carried out on microbial and animal tissue cell systems. Far less work has been done on plant systems. Viruses, especially the structurally simpler mosaic viruses that infect green plants, offer particularly advantageous experimental material. In the replication of one of these viruses in the living host cell we have an opportunity to observe the simultaneous synthesis of the specific ribose nucleic acid (RNA) and protein components of the virus."

Among Dr. Newmark's friends and colleagues was the full spectrum of scientists, from the young undergraduate student to the eminent Nobel laureate. With all, he was at ease. His home was always open to students, colleagues and friends from near and far. His activities as teacher and scholar were blended with a keen interest in the intellectual progress of his students. Throughout his career he maintained a prolific personal correspondence; his warm and frequent letters to friends around the world were a source of great pleasure and inspiration.

Philip Newmark came from a humble background. He was born in New York City in 1919, the only child of Samuel and Ethel Newmark. His father worked for many years as a window decorator on a very modest income. Phil attended P.S. No. 50 (1925-1930), Herman Ritter Junior High School (1930-1932), and DeWitt Clinton High School in New York and proceeded to the City College of New York where he received the B.S. degree in January 1939. Following graduation from CCNY, prospects for financing a graduate career in science appeared very poor; in his search for alternative opportunities Phil turned to the field of Civil Service where after passing numerous examinations he was accepted in April 1939 as Junior Messenger in the Anti Trust Division of the U.S. Department of Justice (at $600.00 per annum). He rose rapidly to Assistant Messenger and Messenger, but when in 1941 he was appointed Laboratory Assistant in the Department of Biology at CCNY the outlook for a career in science began to brighten, and he resigned from the Department of Justice. He started pre-doctoral courses at City College and at Brooklyn College, taking courses in the evenings until June 1942. During World War II, from 1942 to 1945, Dr. Newmark was a Research Associate engaged in work on a national defense project at the Underwater Explosives Research Laboratory, Woods Hole Oceanographic Institution, where his contribution was considered of highest merit.

Despite his modest means, Phil had a rich cultural background as a youth. His time was perpetually occupied with reading, music and science hobbies. He was an excellent photographer, mineralogist, geologist and was familiar in great detail with wildlife and natural phenomena. It was always a matter of great delight to travel in his company for Phil was sure to make many interesting observations of wildlife or natural formations along the road. He was a lover of books and music and enjoyed playing the violin in his spare moments.

Much of Phil's achievement in science was made possible by the constant and devoted companionship of his wife, Marjorie Zeiger Newmark. Their mutual

devotion, their charming family and their home have been sources of great inspiration and beauty.

Throughout his brief career, Dr. Newmark was unwavering in his defense of civil rights. When in 1952 he had been awarded a post-doctoral fellowship by The National Foundation for Infantile Paralysis for work with Professor Wendell M. Stanley of the Virus Laboratory, University of California, he was genuinely elated. But that was "the time of the oath" and Phil received a letter requesting that he sign the California loyalty oath. He decided to join the hundreds of University of California faculty members who considered this a violation of their civil rights, and who were later completely vindicated. Dr. Newmark risked the loss of his post-doctoral appointment, and wrote to Dr. Stanley, "I will not sign this oath as a matter of principle." Dr. Stanley replied, "Disregard the oath," and he explained that an administrative shift had made it possible to waive the oath requirement. Phil was a great admirer of the late A. J. Carlson, eminent physiologist of the University of Chicago. It was Ajax (as Carlson was affectionately called) who before World War II called attention to the Fascist law which required that all university professors in Italy must swear an oath of allegiance to Mussolini.

Always a genuine scholar and humanist, Dr. Newmark was profoundly concerned over continued nuclear weapons testing, radiation hazards, the threat of chemical and biological warfare and the abuses of chemotherapy. He was firmly convinced that scientists have a social responsibility to attempt to insure that science will be used in promoting human welfare. Dr. Newmark supported a variety of causes which were devoted to the elimination of war and to the promotion of civil rights.

The memory of Philip Newmark will be cherished by all who had the privilege of knowing him.

Today's science award is a fitting monument to a career, prematurely terminated, but fully committed to the promotion of science and human well-being.

Irving Goodman

THE PHILIP NEWMARK MEMORIAL LECTURE:

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By

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HAZARDOUS CHEMICALS IN MAN'S ENVIRONMENT

That we live today in an environment containing a variety of poisons is no longer a matter for debate or alarm, but an urgent matter for sober concern and intelligent control.

Only recently the Food and Drug Administration[12] after prolonged study and controversy established that the insecticide Endrin had contaminated stocks of canned oysters shipped from Louisiana and Mississippi. Massive fish kills along the shores of the lower Mississippi River over the past four years have also been attributed to Endrin. This insecticide has been widely used in the South to control insects in the sugar cane and cotton fields, and industrial wastes from its manufacture have been poured into the rivers and streams. It has been found in water supplies affecting at least one million Mississippi and Louisiana water consumers.

We live surrounded by a great variety of poisons, and most of them are man-made. Some are very obvious poisons such as the insecticides and fungicides whose dangers were so dramatically described by the late Rachel Carson.[10] There are others such as the radioactive wastes which, despite the recent Test-Ban Treaty, have been reaching alarming concentrations in our atmosphere and in our foods and even now surround us and invade our cells and tissues.

Quite recently a group of inhabitants of Newport, Indiana, became much alarmed over the discovery that vast quantities of nerve gases (probably compounds related to di-isopropylfluorophosphate) were being manufactured and stored in their neighborhood as potential weapons of war.[21] That these and other lethal weapons of biological and chemical warfare have been in large scale production has been public knowledge for many years.[23]

But these are the obvious poisons; what of the less obvious but perhaps even more pernicious ones.

On June 7, 1962 the Surgeon General of the U.S. transmitted to Congress a report entitled, "Motor Vehicles, Air Pollution and Health."[27] In this comprehensive report, air pollution was found linked in various ways to respira-

tory disease, lung cancer, damage to crops and animals, illness and death. Estimates of crop damage in California alone went from $500,000 in 1939 to $3,000,000 in 1953, and to over $8,000,000 in 1958. Plant damage in the nation as a whole is estimated between 150 to 500 million dollars annually. It would be meaningless to attempt to place a dollar value on the loss of human health and life. The problem of air pollution is so acute that Congress and the States have allocated millions of dollars for research aimed at its control and elimination.

We live in a world permeated with substances inimical to "the preservation of life." Table 1[27] summarizes the major classes of components of automotive exhaust produced annually in the combustion of hydrocarbon fuels by internal combustion engines. A great many industrial plants contribute to

Table 1. Annual discharge of air contaminants by motor vehicles (U.S.A.).

	Tons
Carbon monoxide	90,000,000
Hydrocarbons	12,000,000
Nitrogen oxides	9,000,000
Aldehydes	150,000
Sulfur compounds	225,000
Organic acids	60,000
Ammonia	60,000
Solids	9,000

our environmental pollution in the form of smoke and waste residues poured into the atmosphere and into our lakes and rivers. Some of the compounds isolated from exhaust gases are highly potent carcinogenic agents (Table 2)[27]; many are extremely toxic and irritating to skin and mucous membranes.

The U.S. Department of Health, Education and Welfare has published a report on a closely related challenge to the preservation of life entitled, "Smoking and Health."[28] The dangers and consequences of smoking have been set forth in unequivocal terms. Several hundred components of tobacco

Table 2. Examples of toxic compounds found in automotive exhaust.

Benzopyrenes*	Anthracene
Dibenzanthracenes*	Formaldehyde
Fluoranthrene	Acetaldehyde
Crotonaldehyde	Chrysene*
Lead compounds	Pyrene
Carbon monoxide	Acrolein
Nitrogen oxides	Nitro-olefins
Sulfur oxides	Ozone

* Carcinogenic

smoke have been isolated and studied in detail. The carcinogens found in automotive exhaust are also found in tobacco smoke (Table 3).[28]

TABLE 3. Carcinogenic polycyclic compounds isolated from cigarette smoke.

Benzo(a)pyrene	Dibenz(a,j)acridine
Dibenzo(a,i)pyrene	Dibenz(a,h)acridine
Dibenzo(a,h)anthracene	7-H-Dibenzo(c,g)carbazole
Benzo(c)phenanthrene	

The list of actual or potential toxic products in our surroundings could be greatly expanded. It includes household chemicals, food additives, paints, dyes, cosmetics, drugs, etc.[2, 26] I should like to discuss in more detail the toxic aspects of chemotherapeutic agents.

POTENTIALLY HARMFUL CONSEQUENCES OF CHEMOTHERAPEUTIC AGENTS

That a great many chemotherapeutic substances are indeed toxic compounds is well known. Toxicologists have attempted to classify drugs in terms of relative toxicities and have assigned empirical values to a great many compounds on the basis of the scale illustrated in Table 4.[15] A few of these toxicity ratings are listed in Table 5.[15]

Most insecticides fall in toxicity classes 6 to 4. For example, parathion, 6; endrin, 5; chlordane, 4; DDT, 4; heptachlor, 4; lindane, 4; malathion, 4. Antihistamines are in class 5.

By its very nature such a list cannot be complete, nor can it present a complete picture of potential drug damage to living systems, for in presenting acute toxicity data it cannot adequately express such factors as carcinogenic action, long term cumulative effects, hypensensitivity reactions, etc. Among the numerous drugs which have been linked with hypersensitivity reactions are the penicillins, barbiturates, salicylates, organic mercurials, iodinated contrast media and others.[24] Penicillin is an example of a drug which shows extremely low toxicity by all traditional criteria; yet it is currently responsible for an estimated 1000 deaths annually, and it is estimated that over 3,000,000

TABLE 4. Toxicity rating chart.

Toxicity rating	Probable lethal dose (Human) mg/kg
(6) Super-toxic	less than 5
(5) Extremely toxic	5 to 50
(4) Very toxic	50 to 500
(3) Moderately toxic	500 to 5 g
(2) Slightly toxic	5 g to 15 g
(1) Non-toxic	above 15 g

Table 5. Examples of drug toxicities.

Drug (Common name)	Toxicity rating
Acetanilide	4
Phenacetin	4
Aspirin	4
Aloin	4
Benzedrine	5
Atropine	6
Phenobarbital	4
Librium	3
Chlorpromazine	4
Codeine	5
Colchicine	6
Demerol	5
Digoxin	6
Ephedrine	5
Disulfiram (antabuse)	3

persons have had allergic reactions to it. It has replaced the therapeutic serums as the most frequent cause of anaphylaxis.[24]

It is safe to say that by far the great majority of drugs are toxic compounds. In fact, it has become almost a rule of thumb in the pharmaceutical industry that non-toxic compounds are unlikely to have useful chemotherapeutic activity. In a recent monograph on iatrogenic diseases, the eminent pathologist, Dr. David Spain, cites hundreds of papers reporting toxic effects of chemotherapeutic agents,[24] including drug-induced hepatitis, hemorrhage, anemia, leukopenia, Parkinsonism, phocomelia, renal disease, heart arrhythmias, fetal damage, and others.

The list of potentially harmful effects of drugs could be vastly expanded—I need only mention episodes such as those involving triparanol (the anti-chloesterol drug) or thalidomide (the tranquilizer) to call to mind some of the horror stories involved.

To recognize the possible dangers involved in the clinical use of certain chemotherapeutic agents should in no way minimize the full recognition of the tremendous positive achievements of modern science in the chemical control of human disease. On the contrary, such recognition should lead to new information on the chemical mechanisms of drug action and to the ultimate elimination of the dangers involved.

Background of Modern Chemotherapy

From the standpoint of perspective it is perhaps illuminating to consider briefly the background of modern chemotherapy. Science has developed an unprecedented number of chemical agents capable of controlling human dis-

ease; nevertheless the quantities and varieties of chemical hazards to life have never been greater.

Man's perpetual search for happiness in a world of adversity has for centuries occupied the minds of philosophers, scientists, and literary writers. Man, inadequately equipped to meet the challenge of life which often brings grief, illness, and disappointment, and frequently imposes impossible tasks, is frustrated in his constant efforts to achieve physical, economic and social stability. Freud recognized this "human condition" and in his work on "The Pleasure Principle"[14] held that human behavior is governed to a large extent by the desire to avoid or abolish pain, fear and other disagreeable sensations. Thus to eliminate disease, pain, fear and unpleasant sensations man resorts to various devices.

The use of drugs, or in Freud's term, "care killers," in alleviating the human predicament and in the control of disease is as old as the history of man. From earliest recorded history human distress was treated with palliatives, many containing narcotics and intoxicants. The Chinese have practiced chemotherapy for over 3000 years as evidenced by the great many remedies recorded in their ancient pharmacopoeias and Books of Herbs,[20] collections which even today are being searched assiduously by scientists of Communist China for leads to "new" drugs.

Many of the ancient remedies are now known and used in more purified form, and many are highly toxic substances; for example, ephedrine, chaulmoogra oil, santonin, emetine, and quinine are all of rather ancient herbal origin.

The pharmacopoeia of the Galenical school contained chiefly natural plant and animal products such as musk, rhubarb, castorium, camphor, tamarinds, ginger, etc.; Galen had banished all metallic preparations, especially those containing mercury or arsenic which he considered dangerous poisons (and rightly so). Even as late as the 15th century, the use of mercurial ointments encountered fierce opposition.

Each period in history which witnessed a cultural expansion also witnessed increased progress in the direction of chemotherapy in promoting the preservation of life. China, Egypt, Babylonia, Greece, the Arab world, renaissance Europe, modern civilization. Renaissance Europe witnessed the advent of the alchemists and a new era in man's search for palliatives. It was during this period that the teachings of Paracelsus replaced those of Galen, and compounds of mercury, antimony, and arsenic, although known to be toxic, were found to possess remarkable medicinal properties.

The use of toxic substances in medicine has prevailed from the earliest beginnings of chemotherapy to the present time. Not many years ago, in some parts of Europe it was not uncommon among country folks to eat white arsenic (arsenous oxide). By many it was swallowed daily throughout life,

and the custom was passed down from father to son. It was believed to produce a plump figure (highly prized by young women in 19th century Europe), soft skin, and beautiful complexion. It was also believed to improve breathing. The numbers who died of arsenic "therapy" were not insignificant.

Accounts of science during medieval and pre-renaissance periods suggest that between the 13th and 15th centuries a state of primitive chemotherapy had been reached which was in many ways analogous to our own. The numbers of cures, herbs, potions, witch-doctors, wizards, charms, love philtres, and charlatans had reached an intolerable level.

For more than a thousand years, the grand and ultimate object of all chemical labors had been the search for a "virgin earth," required for the preparation of that mysterious substance which, in the hands of the philosopher or wise man, would yield the Philosopher's Stone, and would change every base metal into gold. In its highest perfection, when used as a remedy, it was believed to cure all diseases, restore youth, and prolong life indefinitely. That such a remarkable substance existed was the firm belief of such learned men as Francis Bacon, Spinoza, Leibniz, and many others. What a powerful concept in stimulating the minds and efforts of man! It is fantastic that the existence of the philosopher's stone should have been regarded for so many centuries as a truth established beyond all doubt, while no one possessed it and each adept only maintained that it was in the posession of another.

But mankind was sorely in need of a rational unifying principle of chemotherapy, and since neither DNA nor serotonin had yet been discovered, perhaps the "philosopher's stone" served as such a principle.

Despite a guiding principle which proved to have been a total fallacy, the concept of the "philosopher's stone" resulted in a remarkable period of discovery and development in the field of chemistry. And we must note in passing that the alchemists' dream of transmutation of metals has, to a great extent, been realized.

Thus we find that throughout history man has attempted, by a variety of chemotherapeutic devices, often involving highly toxic substances, to alleviate "the human condition," to control disease, and thus to promote the preservation of life.

Intrinsic Chemical Mechanisms for the Protection of Living Systems

Life, always a precarious phenomenon, must now more than ever secure all means for its preservation. Obviously, one of the most effective mechanisms for the preservation of life rests in the structure of society; for as man perfects his social organization, the folly of creating ever more efficient means of self-destruction becomes self-evident. The control and ultimate elimina-

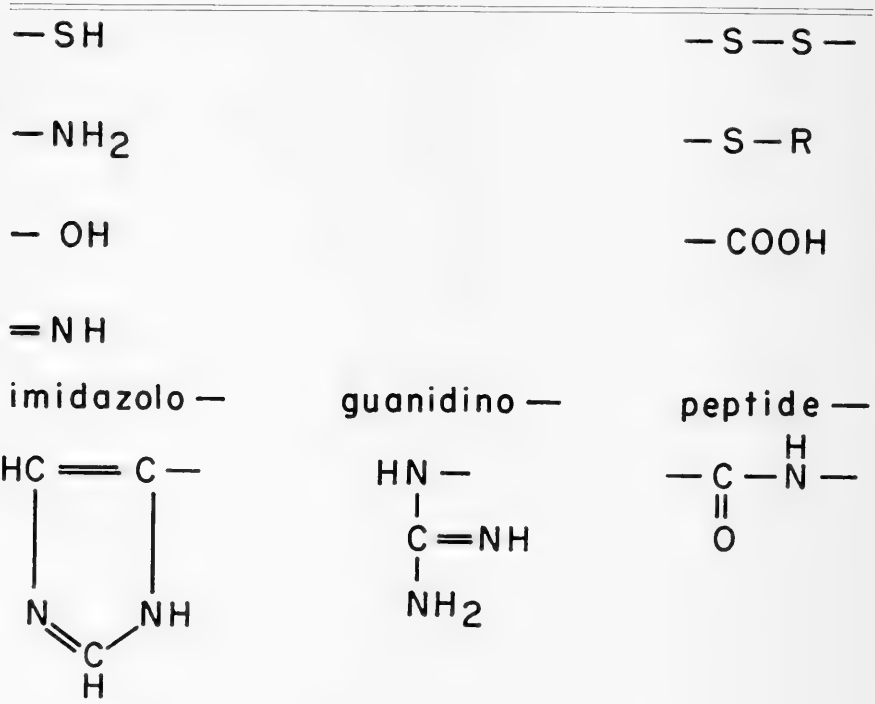
tion of toxic substances in our environment must become the first order of business.

There are other mechanisms of defense, intrinsic chemical ones, which function within the framework and limitations of life and serve to prevent or reduce the damage caused by the chemical substances already described.

Biochemistry has demonstrated the remarkable capacity of living tissues to resist the violation of their chemical and structural integrity by toxic substances. Chemical reactions normally requiring intricate and difficult laboratory procedures are frequently accomplished with great speed by living cells under exceedingly mild conditions. Thus benzene, for example, is detoxified in the dog by oxidation in the liver to phenol, catechol and hydroquinone, products that are conjugated and eliminated in the urine in the form of glucuronides and sulfuric acid esters.[29] In addition to mechanisms of oxidation, living tissues are capable of detoxication reactions involving reduction, hydrolysis and conjugation with carbohydrates, gylcine, glutamine, ornithine, cysteine and others.

These and other mechanisms, to be effective, must preserve the chemical and structural integrity of the vital components of cells and tissues. Of these

TABLE 6. Main functional groups of proteins.



components, I have been concerned for some years with the role of bivalent sulfur compounds, particularly from the standpoint of chemotherapeutic mechanisms. The prevalence of bivalent sulfur compounds in living systems is universal. There is no living matter which is free of such compounds. Of the major functional groups present in proteins (Table 6), the main bivalent sulfur-containing groups are the thioether, disulfide and the free sulfhydryl (-SH) groups. Of these the -SH group is by far the most highly reactive. This property of sulfhydryl groups is the basis for much of the present discussion.

The importance of sulfhydryl-containing proteins and peptides in biochemical systems is well documented;[5, 6, 7] Table 7 lists a few of the many enzymes inactivated by reagents which bind or combine with the SH group.

Table 7. Examples of enzymes inactivated by -SH reagents.

Phosphoglyceraldehyde dehydrogenase	Cholinesterase
Succinic dehydrogenase	Lysozyme
Yeast alcohol dehydrogenase	Glyoxalase
Wheat germ lipase	Pepsin
Adenosinetriphosphatase	Bromelain
Urease	Papain

In a period of a little over 2 years about 135 reports appeared concerning effects of -SH reactants on enzyme systems, and of these about 87% showed definite inhibition by -SH group reagents.[8] So widespread is the requirement of -SH groups for enzyme activity that some workers routinely add one of a number of -SH containing small molecules to virtually every enzyme system as "enzyme activators." These often include compounds such as β-mercapto-ethanol, glutathione, cysteine, thioglycolic acid, and dimercaptopropanol (BAL).

The literature pertaining to the biological importance of sulfhydryl-containing compounds is vast, but a few additional essential relationships are noted briefly:

The sulfhydryl group has been repeatedly implicated in the mechanism of striated muscle contraction, and we have recently demonstrated a similar role in the mechanism of smooth muscle contraction.[17]

The -SH group in proteins is involved in the process of embryonic development as demonstrated by Rapkine and by Brachet.[9] It is involved in the mechanisms of drug-induced diabetes and in the mechanisms of blood clotting.[5]

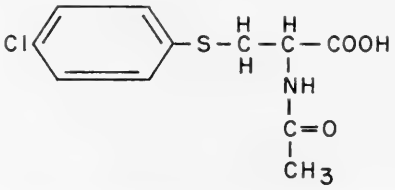
Glutathione, the well-known sulfhydryl-containing tripeptide, has been implicated in a number of enzyme functions. It is widely distributed

throughout the animal kingdom and is particularly abundant in the lens of the eye, in liver, in pancreas, and in spleen.

To underline further the biological importance of the bivalent sulfur compounds, the excretion of mercapturic acids as a means of biological detoxication has long been recognized. A number of compounds are eliminated in this way, and the cysteine appears to originate from dietary sources or from body protein (Table 8).[4]

TABLE 8. Some compounds excreted as mercapturic acids when fed to the dog.

Benzene	Phenethylbromide
Chlorobenzene	Pentachloronitrobenzene
Naphthalene	Nitrobutane
2,4-dinitrobenzene	Bromobutane
p-Fluoronitrobenzene	Benzylchloride
Bromoethane	Anthracene



p-Chlorophenylmercapturic Acid

Bacq[3] used the term "thioloprive" to designate those compounds which act as sulfhydryl scavengers, to deprive the cells of essential -SH groups and thus interfere with cell and tissue function. In recent years the list of "thioloprives" (commonly designated "sulfhydryl group reagents") has grown to enormous proportions.[11, 22] In our studies this list has been extended to a wide variety of chemotherapeutic agents, insecticides, and other chemical substances present in our environment (Table 9).[16]

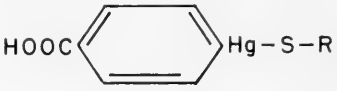
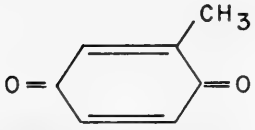
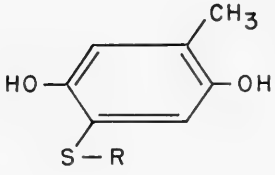
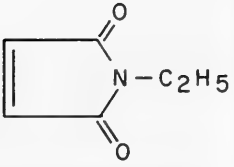
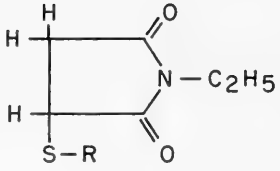
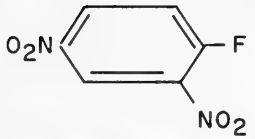
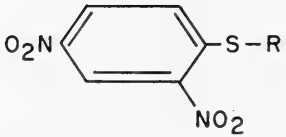
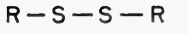
The compounds listed in Table 9 were shown to react readily with the sulfhydryl group of cysteine, glutathione, and of proteins such as ovalbumin and papain under mild conditions comparable to those prevailing in living systems.

TABLE 9. Examples of compounds highly reactive toward the cysteinyl sulfhydryl group.

3-Acetylpyridine	2, 6-dichlorobenzoquinone
p-Aminopropiophenone	Lactonitrile
Patulin	Dihydroxyacetone
Allethrin	Isatin

A few of the many possible types of chemical combinations involving sulfhydryl group reactants and -SH containing molecules are illustrated in Table 10. It is evident that many of the chemical substances (or their metabolic products) mentioned earlier with reference to air pollution, cigarette

TABLE 10. Examples of Sulfhydryl group reactions.

Reagent		Product
		
p-Chloromercuribenzoic acid		
		
Methylbenzoquinone		
	RSH →	
N-ethylmaleimide		
		
2,4-Dinitrofluorobenzene		
H_2O_2 Hydrogen Peroxide		

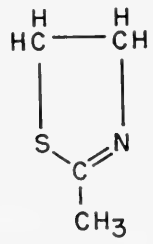
smoking, insecticides, chemotherapeutic agents, etc. may be included in the broad class of compounds known as sulfhydryl group reactants, for these would include quinones, derivatives of heavy metals, active halogen compounds, unsaturated aliphatic derivatives, carbonyl compounds, oxidizing agents, alkylating agents, and others. Thus, by virtue of reactions such as those illustrated in Table 10, toxic substances of the most diverse kind may react with available SH compounds in living systems, resulting in the inhibition of vital enzyme systems and interference with normal physiological processes.

From the available evidence it is reasonable to conclude that the bivalent sulfur compounds, and particularly those possessing or capable of supplying the free sulfhydryl group constitute a vital but highly vulnerable mechanism for the maintenance of living processes.

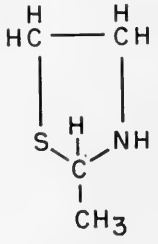
That the integrity of the sulfhydryl group is essential for a great many enzyme reactions has been established. But the exact structural nature of the sulfhydryl group in native proteins has not yet been resolved. The terms "hidden," "sluggish," and "freely active" are still used to describe the observed but poorly understood properties of sulfhydryl-containing proteins.

It would appear likely, *a priori*, that the sulfhydryl group, so vulnerable to chemical assault and yet so vital, would exist in a form of chemical combination which would prevent its undue depletion and yet would allow its rapid release when required. One such form, suggested by Linderstrøm-Lang[19] in 1940, is the thiazoline ring as illustrated by the structure of 2-methyl thiazoline, the compound he studied as a model. The properties of thiazolines and the structurally related thiazolidines (see below) have been studied extensively[25] since the provocative hypothesis of Linderstrøm-Lang, but no evidence for the existence of such structures in native proteins has been produced. However, the polypeptide antibiotic, bacitracin A, has been found to contain a thiazoline ring.[1, 13]

Our own studies on certain aspects of the thiazoline problem[18] continue to suggest that such structures cannot yet be excluded as possible components



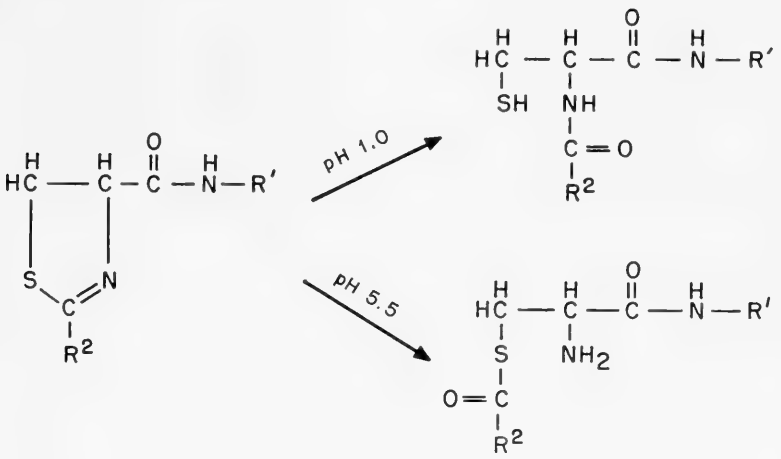
2-Methyl-2-thiazoline



2-Methyl thiazolidine

in native proteins. The thiazoline hypothesis still constitutes a challenging approach to the interpretation of a variety of fundamental biochemical problems.

Our studies on the properties of such cyclic structures permit us to speculate concerning the mechanisms of certain drug-protein interactions. Thiazolines have been shown to undergo rapid ring cleavage in two possible ways to yield a free sulfhydryl group and the corresponding N-acyl derivative, or to yield a thioester and the corresponding free amino group:



This ring cleavage is catalyzed by acids, bases and by ammonium salts under a variety of conditions.[16, 19]

Since all of the sulfhydryl group reactants we have examined also react with model thiazoline compounds, we may further speculate that thiazolines, if present in proteins, are caused to undergo ring cleavage by a variety of sulfhydryl group reactants, and the resulting -SH (or $-NH_2$) groups interact with the appropriate active functional group of the drug or other chemical substance.

Thus in a certain sense the highly reactive bivalent sulfur compounds of low molecular weight, particularly those capable of furnishing free sulfhydryl groups, may constitute one of man's essential mechanisms for defense of vital enzyme systems against foreign chemical agents. For living systems are endowed with an abundant supply of such compounds in the form of glutathione, cysteine, cystine, methionine, thioctic acid, ergothioneine, etc. These substances may act to trap and chemically alter invading foreign chemicals, and may also serve to protect the cells and tissues from the damaging effects of ionizing radiations.

However, similar reactions, involving sulfhydryl-containing molecules of

high molecular weight may account for drug sensitivity reactions (the drug acting as a hapten) and for a variety of diseases induced by chemical agents.

Whether the active sulfhydryl group of proteins, so essential to living processes, resides in the thiazoline, thiazolidine or some other protective structure remains to be established by further research.

I have roamed rather widely in this brief survey in an effort to give a panoramic view of a possible intrinsic chemical mechanism in the defense and maintenance of living systems.

Although science has made spectacular advances since the days of Paracelsus, we find that now, as then, our pharmacopoeias are filled with lists of toxic compounds; now, as then, sulfur occupies a key role in our theories of chemotherapy; and our search for universal panaceas has become perhaps a more sophisticated version of the search for the philosopher's stone. As a by-product of the logarithmic rise in technological achievement we face an ever growing challenge to the preservation of life. For science to wrest from nature more of the secrets of the chemical mechanisms involved in the defense and maintenance of living systems, one of the guidelines which must be pursued ever more intensively is the detailed biochemistry of the intrinsic bivalent sulfur compounds.

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