

AN ABSTRACT OF THE THESIS OF

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Title Use of a Radiation Resistant Organism as a Protectant  
from Lethal Effects of Irradiation in Mice

Abstract approved [REDACTED]  
(Major Professor)

In 1956, workers at Oregon State University discovered a microorganism which survived ten thousand times the gamma radiation dose fatal to man. Research efforts designed to explain this resistance have been underway since that time. As a result of some of these studies it was postulated that the microorganism might contain a compound or compounds which protected it from the toxic effects of radiation. This experimentation consisted of attempts to isolate this compound or compounds and determine if it (they) was capable of protecting mice from the lethal effects of radiation.

Experiments were begun by injecting whole bacterial cells into mice which were subjected to a lethal dose of irradiation. It was observed that cells injected into the peritoneal cavity of mice before or after irradiation protected some of them from a lethal dose of irradiation. The effect of extracts from the cells was then examined.

The cytoplasmic and particulate material was isolated from H1 by sonication of whole cells and these were tested for their

protective efficacy. Though both fractions protected mice from the lethal effects of radiation the particulate material gave better results.

Cellular extracts of pigment material were examined for their protective ability as well as various other cellular extracted material. The sulfuric acid or petroleum ether extracted cellular material gave the best results.

The Miescher's method for isolating nuclear material yielded material which was the most effective radiation protective material of those nuclear materials tested. Even though the nuclear material prepared by Miescher's method was crude, containing associated lipid, pigment and protein material, chemical analysis was performed. The results indicated more sulfur than expected.

The crude nuclear material was purified further by separating the lipid-pigment material from it. The lipid-pigment material of the nucleic acid extracted material appeared to be the protective fraction rather than the nucleic acid material. When purified still further by saponification or when the methyl derivative was prepared, the lipid portion had more protective properties than the pigment material. The pigment-lipid material was also crudely separated on a silicic acid column and the first fractions which were removed (neutral fats and bound lipids) appeared to be the protective portion.

A survey of the literature was performed in an effort to determine whether a similar compound had been analyzed previously for its irradiation protection. A definite need for a study of

this type existed as the protective compounds known to date were not satisfactory. These compounds usually were required to have been administered before the irradiation dose had been received, and effective concentrations were toxic to the animals.

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1963



USE OF A RADIATION RESISTANT ORGANISM AS A  
PROTECTANT FROM LETHAL EFFECTS OF IRRADIATION IN MICE

by

JOAN PRISCILLA PAYNE KILBOURN

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USE OF A RADIATION RESISTANT ORGANISM AS A  
PROTECTANT FROM LETHAL EFFECTS OF IRRADIATION IN MICE

INTRODUCTION

In 1956, workers at Oregon State University isolated a microorganism which survived ten thousand times the gamma radiation dose fatal to man. Research efforts designed to explain the resistance have been in progress since that time. As a result of these studies compounds were indicated which protected them from the toxic effects of radiation. Experiments were begun by injecting whole bacterial cells into mice which had been subjected to a lethal dose of irradiation. It was observed that cells injected into the peritoneal cavity before or after irradiation protected mice from some of the lethal effects of irradiation. The effectiveness of extracts from the cells was then examined, including pigment, cell wall, nuclear material and lipid. The most consistent results were obtained from the nuclear and lipid fractions.

The nature of the nuclear material was examined. These studies included an approximate analysis of the mineral and inorganic elements; the amount of nitrogen, phosphorous, sulfur, etc. Chromatographic techniques were used to identify the amino acid and purine-pyrimidine base composition. Similar work was carried out on the lipid material. Statistical and Mathematical methods were used to substantiate its protective action.

In surveying the literature an effort was made to determine whether similar compounds had been analyzed previously for irradiation protection. From such a survey there was indicated a definite need for a study of this type as the protective compounds known to date were not satisfactory. These compounds usually were required to have been given before the radiation dose had been received, and effective concentrations were toxic to the animals.



## HISTORICAL

Roentgen discovered X-rays in 1895. These rays were found as a result of the fluorescence which they produced on a platinum-barium cyanide screen (703, p. 741-746). The following year Becquerel noticed that potassium uranyl sulfate, produced rays similar to X-rays. Subsequently, many related compounds were found which produced gamma radiation. Gamma rays occur spontaneously as a result of decay, whereas X-rays are produced from the bombardment of a target with electrons (303, p. 1-3).

The St. Louis Post Dispatch speculated on this inconceivable new power of radioactivity sixty years ago, especially on its use in war and as an instrument for the destruction of the world (303, p. 3).

The first person in America to suffer from acute radiation was working on the Manhattan Project for the production of an atomic bomb. On August 21, 1945, he received a dose of radiation when an uncontrollable chain reaction occurred and within 24 hours was dead (286, p. 233; 379).

The biologists found that radiation caused many effects, varying in intensity with the dose applied. These effects ranged from insignificant to instant death with convulsions (294, p. 68-91). It was also noted that microorganisms which did not appear damaged by radiation could produce progeny which were different from their parents or mutants (389; 488, p. 69-99; 523).



The Army Quartermaster Corps became interested in radiation for the sterilization of foods following the last world war. In 1956, workers at Oregon State University discovered a microorganism which was 10 times more resistant to gamma radiation than most others (14). Investigations of the organism were begun and nutritional studies revealed that methionine, thiamine and biotin were the only organic substances required. Other experiments demonstrated its sensitivity to heat, antibiotics and its tolerance for high concentrations of copper (221, p. 14-56; 242; 433; 640; 645). Cultures gave off a disagreeable odor which was traced to a sulfur compound such as mercaptoalkylamine and hydrogen sulfide. Pigments from this organism were studied by Bellamy et al., (433) who believed that they act as an energy trap. Jong Lee (489, p. 9-27) was unable to draw a correlation between its pigmentation and resistance.

A summary of compounds which have been tested for radiation protective properties can be listed under eight main types plus miscellaneous ones as follows:

1. Anoxia producing compounds such as cyanide and nitriles
2. Sulphydryl substances, such as cysteine, cysteamine and cystamine, which contain -SH (covalent group) or a potential -SH group and a strong basic function such as amine or guanidine on the molecule.
3. Amines, amino acids and peptides
4. Hormones

5. Chelating agents
6. Extracts from biological material and hematopoietic tissue
7. Lipids
8. Irritants

The following list of compounds includes the results with regard to radiation protective properties and reference to the experimenter.

COMPOUND	EFFECT	REFERENCE
2 Acetamido 4' Chlorodiphenylsulfide	none	(802, p. 106)
P Acetamido beta Dimethylaminopropio-phenone	none	(802, p. 121)
2 Acetamidophenylthionoacetate	none	(802, p. 107)
2 (Acetamido-tert-butyl)isothiosemi-carbazide	none	(802, p. 77)
Acetate	none	(802, p. 141)
Acetic Acid	none	( 7, p. 400)
Acetoacetate	none	(802, p. 141)
N Acetoacetylcysteamine	none	(615)
S, 2 Acetoaminoethylisothiuronium bromide hydrobromide	none	(232)
Acetone	slight	( 51, p. 400; 568)
Acetonitrile	none	( 7, p. 399)
1 Acetyl 2 (acetamido-tert-butyl)-isothiohydantoin	none	(802, p. 77)
Acetyl aminoacetophenonethiosemi-carbazone	protects	(622)
Acetylamino mercaptothiodiazole	protects	(646)
Alpha Acetylbenzylcyanide	none	(802, p. 138)
Acetylcholine	protects	(705)
Acetylcholine and Adrenaline	protects	(704; 706)
Acetylcholine and SH Adrenaline	none	(704)
Acetylcholine Chloride	protects	(585)
Acetylcholine and indoleamine	protects	(706)
Acetylcholine Iodide	none	(704)
Acetylcholine and Beta Mercapto-ethylamine	protects	(706)
Acetylcholine and serotonin	protects	(706)
Acetylcholine and Tryptamine	protects	(706)

N Acetylcysteamine	protects	(478; 615)
Acetylcysteine	protects	(802, p. 58)
N Acetylhomocysteinethiolacton	protects	(483)
S Acetylmercaptoethylamine	protects	( 7, p. 401)
N Acetyl beta Mercaptoethylamine	protects	(234)
N Acetyl 2 Mercaptoethylamine	protects	(232)
Acetyl beta Methylcholine bromide	protects	(130)
Acetyl beta Methylcholine chloride	protects	(130; 131)
N Acetylmethylcysteamine	none	(615)
2 Acetyl 1 Naphthol	protects	(802, p. 149)
Acetylsalicylic acid	none	(858)
ACTH	protects	(5; 88; 429; 730)
	none	(155)
Actinomycin D	protects	(210)
Adenosine monophosphate	protects	(802, p. 144)
Adenosine triphosphate	protects	(802, p. 144)
Adrenalectomy	protects	(776)
	slight	(696)
	none	(142)
Adrenaline	protects	(340; 682)
	slight	(708)
Adrenaline and acetylcholine	protects	(704)
Adrenochrome	slight	(802, p. 117)
Adrenoxyl	slight	(106)
Aeron	none	(235)
Age (Young more sensitive)	protects	(198; 717)
Agmatine	protects	(802, p. 150)
Alanine	protects	(802, p. 145)
Alpha Alanine	protects	(769)
N beta Alanyl derivative of Cysteamine	slight	(802, p. 68)
Beta Alanylaminoethanethiol	none	(232)
Albumin	protects	( 7, p. 400; 56)
	none	(460)
Aletheine	slight	(615)
Alinamin	protects	(372; 452)
Alpha Alkoxyglycerol	protects	(130)
Alkoxyglycerol	protects	(120)
Alkoxyglycerol esters	protects	(119; 120; 121; 312)
S Alkylhomocysteine	none	(802, p. 59)
Alloxan	none	( 7, p. 400)
S Allyl derivative of Cysteamine	none	(802, p. 69)
S Allyl derivative of Isothiouronium	none	(802, p. 77)
Allyl Alcohol	protects	( 7, p. 399)
	none	( 47, p. 176)
Allylamine	slight	( 7, p. 399)



3 Allyl 4 Aminouracil	protects	(406)
Allylcyanide	protects	( 7, p. 399)
Allylisopropylbarbiturate	slight	(802, p. 111)
S Allylmercaptoethylamine	protects	( 7, p. 401)
Allylthiourea	protects	( 7, p. 400; 47, p. 176)
	none	(802, p. 84)
Aloe Juice	protects	(555)
Altitude	protects	(455)
	none	(747)
Amino Acid Mixture	protects	(387)
P Aminoazobenzene	none	(802, p. 151)
P Aminobenzene disulfide	none	( 25)
P Aminobenzene sulfonic Acid	none	(802, p. 106)
O Aminobenzenethiol	none	(232)
2 Aminobenzimidazole	none	(802, p. 151)
P Aminobenzyl derivative of Isothio- uronium	none	(802, p. 77)
P Aminobenzylisothiuronium bromide	none	(713)
P Aminobenzoic Acid	protects	( 7, p. 398; 499)
	none	(150; 564; 802, p. 151)
2 Aminobenzothiazole	none	(802, p. 101)
2 Aminobutane 1 Thiol	slight	(802, p. 66)
1 Aminobutane 2 Thiol	slight	(802, p. 66)
S, 4 Aminobutylisothiuronium bromide hydrobromide	none	(713)
S, 2 Aminobutylisothiuronium bromide hydrobromide	protects	(802, p. 72)
S, 2 Aminobutylisothiuronium sulfate	none	(802, p. 72)
Alpha Aminobutyric Acid	none	(802, p. 145)
2 Amino 4 Carboxythiazoline	none	(713)
Aminocardinicrotoxin	none	(367)
2 Amino 2' Chlorodiphenyl sulfide	none	(802, p. 102)
2 Amino 1 Cyclohexamethiol	protects	(802, p. 67)
2 Amino 4, 4' Dichlorodiphenyl sulfide	none	(802, p. 105)
2 Amino 3, 4' Dichlorodiphenyl sulfide hydrochloride	none	(802, p. 106)
2 Aminoethanesulfate	slight	(802, p. 104)
2 Aminoethylcyanide	none	(802, p. 138)
S (2 Aminoethyl)cysteine monohydro- chloride	protects	(802, p. 58)
S (2 Aminoethyl)cysteine dihydrochloride	none	(802, p. 58)
2 Aminoethyldisulfide dihydrochloride	protects	(232)
S (2 Aminoethyl)homocysteine monohydro- chloride	none	(802, p. 59)
Aminoethylisothiurea	protects	(150)



Aminoethylisothiuronium bromide hydrobromide	protects	(16; 81; 82; 96; 98; 201; 203; 206; 209; 229; 233; 234; 253; 276; 337; 339; 386; 390; 444; 474; 491; 513; 514; 528; 529; 530; 531; 565; 631; 669; 677; 687; 713; 828; 839; 841)
2(2-Aminoethyl)mercaptothiazolene dihydrobromide	none	(713)
S,2 Aminoethyl N Methylisothiuronium chloride	protects	(615)
Aminoethylthiophosphate	protects	(753)
Aminoethylthiophosphoric Acid	protects	(370)
2 Aminoethylthiosulfuric Acid	protects	(417)
Beta Aminoethylthiosulphuric Acid	protects	(393; 753)
2 Amino 4 Iminothiazoline	slight	(802, p. 93)
2 Amino 5 Isothiuronium methylthiazo- line bromide hydrobromide	protects	(339)
Beta Aminomercaptan	protects	(328)
2 Amino 5 Mercaptothiodiazole	protects	(646)
2 Amino 6 Methylbenzothiazole	none	(802, p. 94)
1 Amino 3 Methylbutane 2 Thiol	slight	(802, p. 101)
5 Amino 4 Methylcytosine	slight	(802, p. 66)
2 Amino 4 Methyl 5 Iodo 6 Mercapto- pyrimidine	protects	(802, p. 143)
2 Amino 4 Methyl 3 Mercaptopropionic Acid	none	(802, p. 143)
2 Amino 2 Methylpropylisothiuronium sulfate	none	(802, p. 59)
5 Amino 4 Methyluracil	protects	(802, p. 72)
1 P Aminophenylbiguanide hydrochloride	slight	(802, p. 143)
Amino 3 Phenylbutane 2 Thiol	none	(802, p. 151)
2 Aminopropane 1 Thiol	none	(802, p. 66)
1 Aminopropane 2 Thiol	protects	(802, p. 66)
3 Aminopropionitrile	protects	(802, p. 66)
	none	(802, p. 138)

P Aminopropiophenone	protects	(23; 98; 239; 342; 611; 615)
P Aminopropiophenone and N,N'-bis (2 hydroxyethyl) dithiocoxamide	protects	(621)
S (3 Aminopropyl)N,N dimethylisothiuronium	protects	(802, p. 74)
Aminopropylisothiuronium	protects	(234)
S 3 Aminopropylisothiuronium bromide hydrobromide	protects	(233; 390)
S (3 Aminopropyl)N' methylisothiuronium	protects	(802, p. 74)
3 Aminopropylthiosulfate	protects	(802, p. 106)
3 Aminopropylthiosulfuric Acid	protects	(417)
P Aminosalicylic Acid	none	(802, p. 151)
Aminothiazol, MethyleneBlue and Dinitrophenol	protects	(505)
2 Aminothiazole	none	(713)
2 Amino 1, 3, 4 thiadiazole	none	(802, p. 88)
2 Aminothiazoline	protects	(713)
O Aminothiophenol	none	(475; 615)
N 2 Aminothiozoly derivative of Cysteamine	none	(802, p. 68)
5 Amino 1, 2, 4 Triazine 3 Thiol	none	(802, p. 88)
3 Amino 1, 2, 4 Triazole	protects	(285)
	none	(301)
Ammonium Chloride	slight	( 7, p. 398)
Ammonium Dithiocarbamate	protects	(615)
Ammonium Nitroso-phenylhydroxylamine	protects	(192)
Ammonium Thiocyanate	none	(802, p. 146)
Ammonium Thiosulfate	none	(802, p. 146)
Amodiaquin	protects	(365)
Amodiaquin 1 Oxide	protects	(365)
Amphetamine	slight	(239)
N Amyl Alcohol	slight	( 7, p. 399)
Analaptics	slight	(802, p. 114)
Anaerobic growth independent of anoxia	protects	(853)
Androgens	none	(542)
Aniline	protects	( 7, p. 398)
N M Anisyl derivative of Cysteamine	none	(802, p. 68)
N O Anisyl derivative of Cysteamine	protects	(802, p. 68)
N P Anisyl derivative of Cysteamine	none	(802, p. 68)
Antibiotics	protects	(185; 287; 536)
Antihistamine	protects	(349; 541)
Antipyrine	none	(367)
Arabinose	none	(802, p. 141)

Arginine	slight	( 7, p. 398)
	none	(802, p. 145)
Arsenic Trioxide	none	(802, p. 146)
Arsenious Oxide	protects	( 7, p. 401)
Arterenol	protects	(615)
Ascorbic Acid	protects	(415; 600; 639)
	none	(802, p. 142)
Asparagine	slight	(802, p. 145)
Aspartic Acid	protects	( 7, p. 398)
Asphyxia	protects	(869; 870)
Atropine	protects	( 25; 682)
	none	(131; 362)
Atropine Sulfate	none	(131)
Aureomycin	protects	(639)
Avertin	none	(846)
Azide	protects	( 51)
Azobenzene	none	(802, p. 151)
Azulon	protects	( 69)
Bacterial Polysaccharides	protects	( 96)
Badach's Serum	none	(106)
Banthine	none	(361)
Baramyl	none	(541)
Barbital	protects	(367)
Barium	none	(211)
Barium Meal	slight	(183)
Batyl Alcohol	protects	(241)
Becaptan	protects	(638)
Beef Embryo Extracts	protects	(358)
Beer Yeast	protects	(587)
Beets	protects	(591)
1 Behenyl 2 naphthol	protects	(802, p. 149)
4 Behenyl pyrocatechol	slight	(802, p. 149)
4 Behenyl pyrogallol	protects	(802, p. 149)
Belenke's Therapeutic Serum	none	(144)
Benactyzine	none	(858)
Benzene	protects	( 7, p. 399)
Benzene azo 4 Thymol	slight	(248)
Benzene azo 2 Methyl 4 Thymol	slight	(248)
4 (Benzeneazo)thymol	protects	(802, p. 149)
Benzestrol	protects	(601)
Benzilimidazoline hydrochloride	protects	(585)
Benzimidazolyl beta Mercaptoethylamine	protects	(796)
Benzimodozolyl derivative of Cysteamine	slight	(802, p. 69)
Benzoic Acid	protects	( 7, p. 400)
2, 4' Benzophenone dicarboxylic Acid	none	(802, p. 121)
Benzoquinone	protects	( 7, p. 400)
Benzothiazole	none	(802, p. 101)



Benzothiazolyl derivative of Cysteamine	slight	(802, p. 69)
2 Benzothiazolyl diethyl dithiocarbamate	none	(802, p. 94)
2 Benzothiazolyl dimethyl dithiocarbamate	slight	(802, p. 94)
3(2-Benzothiazolylthio)propylisothiouronium dichloride	none	(802, p. 77)
Benzotriazole	slight	(446)
1 Benzoyl 2 (Acetamido-tert-butyl)isothiohydantoin	none	(802, p. 77)
N Benzyl derivative of Cysteamine	none	(802, p. 68)
Benzyl derivative of Isothiouronium	slight	(802, p. 77)
S Benzylcysteamine	none	(615)
S Benzylcysteine	none	(802, p. 58)
Benzylmalonic Acid	none	(802, p. 149)
Benzylmercaptan	slight	(802, p. 102)
Benzylphenylhydrazine	none	(802, p. 151)
N(2(3 Benzylthiazolidylidene))Aniline	none	(802, p. 77)
Benzyltryptamine	none	(802, p. 126)
Betaine	none	(131)
Bioflavonoids	protects	(32)
	none	(272)
Bis (2 Acrylamido 4 Chlorophenyl)sulfide	none	(802, p. 106)
Bis (Beta Alanyl aminoethyl)disulfide	none	(232)
Bis (Beta Alanyl aminoethyl)sulfide	none	(802, p. 106)
Bis (Beta Aminoethyl)disulfide	protects	(234)
1, 6 Bis (P Aminomethylphenoxyhexane)	protects	(622)
Bis (2 Aminophenyl)disulfide	slight	(802, p. 104)
4 Bis (Benzeneazo)thymol	protects	(802, p. 149)
2, 2' Bis (Benzothiazolyl)disulfide	none	(802, p. 101)
N, N' Bis (2 Benzothiazolylmercaptomethyl)urea	none	(802, p. 101)
1, 2 Bis (2 Bromoethylthio)ethane	none	(802, p. 105)
Bis (Butylaminoethyl)sulfide	none	(802, p. 105)
1, 2 Bis (S Carboxymethylthiocarbamyl)ethane	slight	(802, p. 94)
N, N' Bis (Carboxypentyl)dithiooxamide	protects	(621)
3 (Bis(2 Chloroethyl)amino)propionitrile	none	(802, p. 138)
Bis (2 Cyanoethyl)amine	none	(802, p. 138)
Bis (2 Cyanoethyl)ether	none	(802, p. 138)
1, 4 Bis (Dimethylamino) 2 Phenylthio 2 Butene	none	(802, p. 105)
4,4' Bis (Dimethylamino)thiobenzophenone	none	(802, p. 121)
Bis (Ethylaminoethyl)sulfide	none	(802, p. 105)
Bis (2 Guanidinoethyl)disulfide	protects	(700; 709)
N, N' Bis (2 Hydroxyethyl)dithiooxamide	protects	(621)
Bis (Isopropylaminoethyl)sulfide	none	(802, p. 105)
1, 2 Bis (S Isothioureido)ethane	none	(802, p. 77)
3, 6 Bis (S Isothioureidoethyl)2, 5 diketopiperazine dihydrochloride	none	(802, p. 77)



Bis (S Isothioureidoethyl)ether dihydrochloride	none	(802, p. 77)
1, 5 Bis (S Isothioureido)pentane	none	(802, p. 77)
Bis (P (S Isothioureido)phenyl)sulfone dihydrochloride	none	(802, p. 77)
2,3 Bis (S Isothioureido)1 propanol	none	(802, p. 77)
Bis (S Isothiouronium 3 Diethyl)formol dichloride	none	(802, p. 77)
1, 4 Bis (4 Morpholinyl)2 Phenylthio 2 Butene	none	(802, p. 105)
1, 3 Bis (P Nitrophenyl)2(P Carbethoxyphenyl)guanidine	none	(802, p. 151)
Bis (Pentamethylenethiuram)disulfide	none	(802, p. 94)
1, 3 Bis (2 Pyridyl)2(2 Dimethylaminoethyl)guanidinium trichloride	none	(802, p. 151)
1, 3 Bis (3 Pyridyl)2(2 Hydroxyethyl)guanidine	none	(802, p. 151)
P, P' Bis (2 Thiolcarbonylacetylamide)di-phenyl	none	(802, p. 107)
S,S' Bis(Thiomorpholine)disulfide	slight	(802, p. 104)
3, 6 Bis (2(4 Thiomorpholinyl)ethyl) piperazine 25 Dione	none	(802, p. 102)
Bis (Tribromomethyl)sulfoxide	slight	(36)
N', N' Bis (9 Xanthylenyl)dithiooxamide	slight	(621)
Blood Transfusion	protects	(287; 639)
Blood Serum Proteins from previously irradiated animals	protects	(460)
Bone Marrow	protects	(11; 12; 15; 151; 164; 170; 184; 202; 287; 340; 404; 577; 643; 814; 821)
	slight	(56)
	none	(83)
Bovine Properdin	none	(545)
Bran, Oats and Ascorbic Acid	none	(163)
Bread	none	(372)
British anti-Lewisite	protects	(565; 656; 740; 863)
	none	(478)
Broccoli	protects	(214; 764)
5 Bromodeoxyuridine	none	(84)
Bromohydrate of S Beta Aminoethylisothio-urea	protects	(669)
2 Bromolysergic Acid	none	(802, p. 125)

Bufolenidine	slight	(649)
Bufotenine	slight	(649)
Bufox	none	(106)
Butanol	slight	( 7, p. 399)
Butter Fat	protects	(273; 327)
	none	(326)
Butylated Hydroxytoluene	protects	(271)
N Butyl derivative of Cysteamine	none	(802, p. 69)
2 Butylaminopropylthiol	none	(232)
Cabbage	protects	(247; 591; 763; 764)
	none	(561)
Cadaverine	none	(802, p. 150)
Cadmium Chloride	slight	(660)
Caffeine	protects	(235; 463)
	none	(367)
Caffeine Citrate	none	(239)
Calcium	none	(211)
Calcium Chloride	protects	(366; 639)
Calcium Cyanamide	none	(802, p. 138)
Calcium Gluconate	protects	(845)
Calcium Flavonate	none	(217; 524)
Caprylate	none	(802, p. 141)
Caprylic Acid	protects	( 7, p. 400)
Carbaminoyl Choline	protects	(802, p. 115)
Carbamylcholine	protects	(131)
Carbamylcholine Chloride	protects	(130)
Carbazole	protects	(802, p. 152)
2 Carbethoxymethylbenzothiazole	none	(802, p. 101)
Carbethoxymethyl P Chlorophenylldithio- carbamate	none	(802, p. 94)
Carbomethoxymethylbenzylmethyldithio- carbamate	slight	(802, p. 94)
Carbomethoxymethylmorpholinylldithio- carbamate	slight	(802, p. 94)
Carbon Dioxide	protects	(157; 340; 571)
Carbon Monoxide	protects	(105; 340; 453)
2 Carboxyethyl derivative of Isothio- uronium	none	(802, p. 77)
2 Carboxyethylisothiuronium bromide	none	(713)
Carboxylic Acid	protects	(387)
Carboxymethyldimethyldithiocarbamate, P Chlorophenyl Ester	none	(802, p. 94)
N Carboxymethylrhodamine	none	(802, p. 94)
O Carboxyphenylmercaptan	none	(802, p. 102)
Carrots	none	(372; 591)
Casein	slight	(224)

Castration	none	(479; 712)
Catalase	protects	(2; 106; 442; 485; 572)
Alpha Chloral	none	(282)
Chloralhydrate	none	(106)
Chloramphenicol	protects	(367)
	protects	(318)
	none	(544)
2 Chloro 4 Aminobenzoic Acid	none	(802, p. 151)
2 Chlorobenzothiazole	slight	(802, p. 99)
4 Chlorobenzyl dimethyldithiocarbamate	none	(802, p. 94)
2 Chloro 10 (3 Dimethylaminopropyl) phenothiazine	protects	(43; 350)
Chloroform	none	(330)
P Chloromercuribenzoate	none	(606)
P Chloromercuribenzoic Acid	none	(802, p. 139)
N (4 Chlorophenyl)rhodanine	slight	(802, p. 94)
Chlorpromazine	protects	(19; 45; 85; 152; 359; 498; 618; 813)
	none	(467; 858)
Chloroquine	protects	(802, p. 115)
Delta 6 Cholesterol 3 Beta O 1, 5 Alpha hydroperoxide	protects	(447)
Choline	slight	(7, p. 398)
	none	(802, p. 115)
Chondroitin Sulfate	protects	(612)
Citrus	protects	(754)
	none	(217; 524)
Citrovorum Factor	none	(126)
Citrulline	none	(802)
Coal Gas	protects	(106)
Cobalt	protects	(229; 593; 594)
	slight	(595)
Cobalt Bromide	protects	(7, p. 401)
Cobalt Iodide	protects	(593)
Cobaltous Chloride	protects	(311; 593)
Coenzyme A	protects	(802, p. 142)
Colcemide	protects	(660; 668)
Colchicine	protects	(802, p. 129)
Complexone III-Procaïne	protects	(150)
Cortineurine	protects	(478)
Corticoids (Cortisone)	protects	(86; 110; 123; 660)
	none	(155; 260; 313; 633; 861)



Corn Oil	protects	(326; 327)
Cotton Seed Oil	protects	(273; 274)
Creatine	slight	(802, p. 152)
Creatinine	none	(802, p. 152)
Cyanamide	none	(802, p. 138)
Cyanide	protects	(51; 499)
Cyanocobalamin	protects	(127)
2 Cyanoethyl derivative of Isothiuronium	none	(802, p. 77)
Cyanoethyl derivative of Cysteamine	none	(802, p. 69)
2 Cyanoethylisothiuronium Chloride	none	(713)
Cyanomethyl derivative of Isothiuronium	none	(802, p. 77)
Cyanomethylisothiuronium Chloride	none	(713)
Cyclohexane	slight	( 7, p. 399)
Cyclohexene	slight	( 7, p. 399)
N Cyclohexyl derivative of Cysteamine	none	(802, p. 68)
S Cyclohexyl derivative of Cysteamine	slight	(802, p. 69)
N Cyclohexyl 2 Benzothiazole sulfamide	none	(802, p. 101)
Cyclohexylethyl derivative of Isothiuronium	none	(802, p. 77)
Cyclohexylethylisothiuronium Bromide	none	(713)
1 Cyclohexylhexahydrobenzoic Acid	none	(362)
Cyclooctatetraene	slight	( 7, p. 399)
Cyclopentylpropionate	none	(542)
Cyclopropane	none	(868)
Cytidylic Acid	none	(802, p. 144)
Cytosine	protects	(802, p. 143)
Cystamine	protects	(73; 87; 143; 241; 339; 376; 492; 534; 671; 822)
Cysteamine	protects	(44; 45; 51; 87; 150; 218; 292; 324; 339; 391; 393; 452; 478; 506; 556; 558; 637; 671; 677; 699; 771; 798; 801; 822)
Cysteinamine	protects	(672; 700)
	none	(152)



Cysteine	protects	(19; 45; 51; 91; 124; 162; 246; 324; 339; 351; 391; 406; 408; 438; 447; 450; 454; 456; 466; 478; 525; 593; 598; 599; 600; 602; 604; 606; 611; 613; 618; 632; 636; 665; 684; 740; 741; 787; 837)
Cysteine ethyl ester hydrochloride	none	(381; 735)
Cysteine hydrochloride	protects	(232)
Cysteine hydrochloride and Aminoethyl- thiouronium	protects	(232; 438)
Cysteine S Alpha Propionic Acid	protects	(530)
Cystinamine	none	(802, p. 58)
Cystine	protects	(51; 478)
Cystine diethyl ester dihydrochloride	slight	(452)
Decamethylenediamine	none	(600)
4 Decanoylprogallol	none	(232)
Defatted Liver Supplement	slight	(802, p. 150)
Desicated Non Fat Liver Supplement	protects	(470)
Desoxycorticosterone	protects	(274)
Desoxycorticosterone acetate	protects	(274)
Desoxycorticosterone emanthate	none	(371)
Desoxyribonucleic Acid	protects	(802, p. 127)
Deuterium Oxide	protects	(786)
Dextran	protects	(421)
N Diacetyl galactosidyl derivative of Cysteamine	none	(592)
N, S Diacetylcysteamine	slight	(802, p. 147)
S, N Diacetylmercaptoethylamine	protects	(95; 96)
N, S Diacetyl 2 Mercaptoethylamine	none	(802, p. 68)
N, S Diacetyl Beta Mercaptoethylamine	slight	(615)
1, 3 Diacetyltriphenylguanidium	slight	(234)
N, N' Diallyl Dithiooxamide	protects	(232)
	protects	(234)
	none	(802, p. 151)
	protects	(621)

2, 6 Diaminobenzothiazole	none	(802, p. 101)
1, 2 Diaminocyclohexane tetracetic Acid	protects	(622)
2, 2' Diamino 5, 5' Dichlorodiphenyl sulfide hydrochloride	none	(802, p. 105)
2, 6 Diamino 5 Nitro 4 Methylpyrimidine	none	(802, p. 143)
4, 5 Diamino 2, 8 Triazinethiol	none	(713)
4, 6 Diamino 2, 8 Triazinethiol	none	(802, p. 98)
4, 6 Diamino 1, 3, 5 Tridazine 2 Thione	none	(802, p. 88)
Dibenamine	none	(368)
Dibenzanthracene	protects	(7, p. 399)
Dibenzene Azo 2, 4 Thymol	protects	(248)
Dibenzothiophene	none	(802, p. 102)
Dibenzoylethylene	none	(802, p. 121)
N, N Dibenzyl derivative of Cysteamine	none	(802, p. 68)
N, N' Dibenzyl N, N' Bis (2 Mercaptoethyl) Ethylene Diamine derivative of Cysteamine	none	(802, p. 68)
Dibenzylsulfoxide	slight	(802, p. 107)
N, N Dibutyl derivative of Cysteamine	none	(802, p. 68)
2 (Dibutylaminoethylmercapto)2 Thiazoline	none	(713)
2 Dibutylaminoethylmercaptothiazoline	none	(802, p. 98)
Di (Butylaminoethyl)sulfide	none	(232)
3 Di N Butylaminopropyl P Aminothionobenzoate	none	(802, p. 107)
3 Dibutylaminopropylthiol	none	(232)
Dibutylcysteamine	protects	(564)
Dibutylsulfoxide	slight	(802, p. 107)
4,6 Dichloro 7 (Diethylaminoethylamino) benzothiazole	none	(802, p. 101)
Didymium nitrate (Rare Earth Mixture)	none	(527)
N, N Diethyl derivative of Cysteamine	none	(802, p. 68)
N Diethylalanine	slight	(7, p. 398)
N, N Diethylalanine	protects	(802, p. 145)
Beta Diethylaminoethyl Ester	none	(362)
S, 2 Diethylaminoethylisothiuronium chloride hydrochloride	none	(713)
2 Diethylaminoethylguanidinium sulfate	none	(802, p. 151)
2 Diethylaminoethylmercaptan	slight	(232)
Di (Ethylaminoethyl) sulfide	none	(232; 615)
Beta Diethylaminoethyl 9 Xanthene Carboxylate Methobromide	none	(802, p. 118)
3 Diethylaminopropylmercaptan	slight	(232)
Diethylammoniumdiethyldithiocarbamate	protects	(802, p. 89)
Diethylcyanamide	none	(802, p. 138)
N Diethylcysteamine	protects	(615)
Diethyldeoxystilbenedipropionate	none	(844)
Diethyldisulfidemercaptoethylamine	none	(25)
Diethyldithiocarbamate	protects	(615)
Diethyldithioloxalate	none	(802, p. 107)

Diethylether	none	(867; 868)
N, N' Diethylethylenediamine	none	(802, p. 151)
Diethylsulfoxide	slight	(802, p. 107)
Diethylstilbestrol	protects	(711)
	slight	(542)
Di (2 Guanidoethyl)Disulfide Dihydro- bromide	protects	(713)
Dihydrooxypropyltheophylline	protects	(585)
Dihydroquercetin	none	(217; 524)
2, 4 Dihydroxybenzophenone	none	(802, p. 121)
Dihydroxydiethylsulfoxide	slight	(802, p. 107)
Dihydroxyphenylalanine	slight	( 7, p. 398)
2 (3', 5' (Diiodo 4 Pyridine) 2 Mercapto) Acetic Acid	none	(802, p. 103)
Diisoamylsulfoxide	slight	(802, p. 107)
N, N Diisobutyl derivative of Cysteamine	none	(802, p. 68)
Diisobutylsulfoxine	slight	(802, p. 107)
Diisopropyl derivative of Cysteamine	none	(802, p. 68)
Di (Isopropylaminoethyl)sulfide	none	(232)
N, N' Diisopropylethylenediamine	none	(802, p. 141)
Diisopropylfluorophosphate	protects	(854)
Diisopropylfluorophosphonate	protects	(855)
Diisopropylsulfoxide	slight	(802, p. 107)
Di (Isothiuronium 3 Diethyl)Formal Di- chloride	none	(713)
S, 2 Di (Isothiuroniummethyl)amine Di- bromide hydrobromide	none	(713)
Di (S, 2 Isothiuroniummethyl)ether Di- chloride	none	(713)
S, 2 Di (Isothiuroniummethyl)ethylamine dibromide hydrobromide	none	(713)
S, 2 Di (Isothiuroniummethyl)methylamine dibromide hydrobromide	none	(713)
2, 3 Di (Isothiuronium)propanol dichlor- ide	none	(713)
Dimedrol	none	(757)
Dimedrol and Streptomycin or Biomecin	none	(757)
Dimercaptocholine iodide	none	(704)
2, 3 Dimercaptopropanol	protects	(232; 572; 863)
Dimercaptopropionic	protects	(338)
2, 3 Dimercaptopropylsulfonic Acid	protects	(802, p. 103)
4, 5 Dimercaptopyridoxine	protects	(115)
2, 5 Dimercapto 1, 3, 4 Thiadiazole	none	(802, p. 94)
Dimerole	protects	(216)
2, 6 Dimethoxyacetophenone	none	(802, p. 121)
1, 4 Dimethoxybenzene	none	(802, p. 149)
N, N Dimethyl derivative of Cysteamine	protects	(802, p. 68)



Dimethylamine	protects	(802, p. 145)
	slight	(7, p. 398)
Dimethylaminoazobenzene	none	(802, p. 151)
P Dimethylaminoazobenzene	none	(426)
4 (2 Dimethylaminoethoxy)N(3, 4, 5 Trimethoxybenzoyl)benzylamine	slight	(622)
Dimethylaminoethyl derivative of Cysteamine	none	(802, p. 69)
2 Dimethylaminoethyl P Aminothionobenzoate	none	(802, p. 107)
S, 2 Dimethylaminoethyl 1', 3' Dimethylisothiuronium bromide hydrobromide	none	(713)
Beta Dimethylaminoethylisothiuronium bromide hydrobromide	protects	(234)
S, 2 Dimethylaminoethylisothiuronium chloride hydrochloride	none	(713)
2 Dimethylaminoethylmercaptan	slight	(232)
2 Dimethylaminoethylthiosulfate	protects	(802, p. 106)
2 Dimethylaminoethylthiosulfuric Acid	protects	(417)
S, 2 Dimethylaminoethyl 1', 3', 3' Trimethylisothiuronium bromide hydrobromide	none	(713)
2 (3 Dimethylaminopropionamido)4', 5 dichlorodiphenyl sulfide monohydrochloride	none	(802, p. 106)
Dimethylamino 1, N Propyl 3 N (2 Chloro) phenothiazine hydrochloride	protects	(152)
2 (N, N Dimethylaminopropyl) 4, 4' Dichlorodiphenyl sulfide monohydrochloride	none	(802, p. 105)
2 (N, N Dimethylaminopropyl) 4', 5 Dichlorodiphenylsulfide monohydrochloride	none	(802, p. 105)
S (1 Dimethylamino)2 propylisothiuronium chloride hydrochloride	none	(713)
Dimethylammoniumdimethyldithiocarbamate	protects	(802, p. 89)
N Dimethylcysteamine	slight	(564)
N, N Dimethyl Gamma (N Dodecylthio)Allylsamine	none	(802, p. 105)
1, 4 Dimethyl 7 Isopropylazulene	slight	(695)
4, 6 Dimethyl 2 Mercaptopyrimidine	none	(475)
4, 5 Dimethyl 2 Mercaptopyrimidine	none	(615)
N, N Dimethyl Gamma (Phenylthio)allylamine	none	(802, p. 105)
(Dimethyl 2,5 pyrolyl)4 Thymol	slight	(248)
4 (2', 5' Dimethylpyrryl)Thymol	slight	(802, p. 149)
Dimethylsulfide	slight	(802, p. 104)



Dimethylsulfone	none	(802, p. 104)
Dimethylsulfoxide	protects	(36)
	none	(35)
2, 2 Dimethylthiazoline	none	(802, p. 98)
N, N Dimethylthiomorpholinium Iodide	none	(802, p. 102)
N, N Dimethyl Gamma (P Tolythio)allyl-amine	none	(802, p. 105)
Di-octinylsulfide	none	(25)
Dinisine	protects	(216)
Dinitra	none	(106)
2, 4 Dinitrophenol	none	(113; 525)
	protects	(304; 630)
2, 4 Dinitrophenyl Dimethyl Dithiocarbamate	slight	(802, p. 89)
N, N' Dioctodecylidithiooxamide	protects	(621)
Diphacil	protects	(807)
N, N Diphenyl derivative of Cysteamine	none	(802, p. 68)
Diphenylamine	none	(802, p. 151)
N, N' Diphenylbenzidine	none	(802, p. 151)
Diphenyldisulfide	none	(802, p. 106)
Diphenylhydantoenate	protects	(19)
4 Diphenylmethane 1, 1 Dimethylpiperidiniummethylsulfate	none	(802, p. 118)
1, 3 Diphenyl N Methyl 2 Propylamine	none	(802, p. 151)
1, 2 Diphenyl 3 Propylamine	none	(802, p. 151)
Diphenylsulfoxide	slight	(36)
Diphenylthiophthalide	none	(802, p. 107)
Dipotestosterone	none	(542)
N, N Dipropyl derivative of Cysteamine	none	(802, p. 68)
Di N Propylsulfoxide	slight	(802, p. 107)
Disodium Phosphate	slight	(366)
2, 5 Di Tert Amylhydroquinone	none	(802, p. 149)
2, 5 Ditertbutylhydroquinone	protects	(271)
1, 6 Dithia 4, 9 Diazaspiro (4, 4') Nonane dihydrobromide	none	(713)
Dithiocarbamate	protects	(565)
6, 8 Dithioctanoic Acid	none	(335)
2, 2' Dithioethanol	none	(232)
Dithioglycerol	slight	(802, p. 103)
D L 6, 8 Dithiolactanoic Acid	slight	(360)
Dithiooxamide	protects	(47, p. 176)
Dithiopentaerythrite	none	(475; 615)
Dithiopentaerythritol	none	(802, p. 91)
1, 3 Dithiopropanol	none	(475)
2, 3 Dithiopropanol	none	(475)
Dithiopropanol	none	(478)
Ditolylsulfoxide	slight	(802, p. 107)
Divinylether	none	(867; 868)

Divinylsulfone	protects	( 7, p. 399)
DOCA	none	(313)
3 Docosyl 2, (2', 4' Dihydroxyphenyl) Indole	none	(802, p. 149)
3 Docosyl 2, (2', 3', 4', Trihydroxy- phenyl)Indole	none	(802, p. 149)
DOPA	protects	(615)
Dopamine	protects	(51)
Dose Fractionation	protects	(207)
Dramamine	none	(723)
Echinopsin - 1% nitrate salt	protects	(352)
Embryo Cells	protects	(859)
Endotoxins ( <u>Pseudomona morganii</u> )	protects	(876)
Ephedrine	none	(111; 708)
Ephedrine sulfate	slight	(239)
Epinephrine	protects	( 72; 341; 660; 774)
Epirenamine hydrochloride	protects	(585)
Epsilon (Antiplasmodic Agent)	protects	(395)
Ergothioneine	none	( 7, p. 401)
Erioglaucine	slight	(249)
Estradiol	none	(802, p. 129)
Estradiol Benzoate	protects	( 51; 542; 601; 663; 674)
Estrogen	protects	(601; 711)
Ethanol	protects	(162; 163; 367; 395; 436; 480; 597; 655)
Ethanolamine	protects	( 7, p. 398)
	slight	(802, p. 141)
Ether	none	(330; 480)
Ether Extract of Seeds	protects	(108)
Ethoxyethylidenemalononitrile	none	(802, p. 137)
Ethoxymethylenemalononitrile	protects	(802, p. 137)
Ethoxypropylidenemalononitrile	slight	(802, p. 137)
S Ethyl derivative of Cysteamine	slight	(802, p. 69)
Ethyl	protects	(387)
Ethylamine	slight	( 7, p. 398)
	none	(706)
Ethyl N (P Chlorophenol)Carbamate	protects	(622)
S Ethylcysteine	none	(802, p. 58)
Ethylidithiuronium	none	(234)
Ethylenediamine	protects	( 7, p. 398)
	slight	(802, p. 150)
Ethylenediaminetetraacetic Acid	protects	(223; 615)

Ethyleneglycol	protects	( 7, p. 399)
Ethylenetetrachloride	none	(367)
Ethyl ester Chlorohydrate of 1 Methyl 4 Phenyl 4 Piperidinecarboxylic Acid	protects	(350)
S Ethylisothiuronium bromide	protects	( 37)
S N Ethylmaleimide	none	(117)
Ethylmercaptan	slight	(802, p. 102)
Ethylmercaptoisobutyramide	protects	(622)
2 Ethylthio 4 Amino 6 Oxypyrimidine	protects	(622)
Ethylurea	slight	( 7, p. 400)
Fat	none	(148)
Fatty Acid	none	(802, p. 141)
Flavonoid	protects	(158)
	none	(197)
Fluoroacetate	protects	(802, p. 140)
Fodder	none	(591)
Folic Acid	protects	(198; 325; 532; 580; 772)
	none	(144; 559)
Folinic Acid	none	(128)
Formal	none	(106)
Formaldehyde	protects	( 7, p. 400)
Formate	none	(769)
Formic Acid	protects	(615)
Fructose	protects	(290; 399)
Fumarate	none	(802, p. 141)
S Furfuryl derivative of Cysteamine	slight	(802, p. 69)
Furfurylamine	none	(802, p. 151)
Furfurylmercaptan	slight	(802, p. 102)
S Furfurylbetamercaptoethylamine	protects	(796)
2 Furfurylmethyl derivative of Cysteamine	slight	(802, p. 69)
2 Furfurylmethyl 2' Cyanoethylthioether	none	(802, p. 138)
Gallic Acid	none	(106)
Glucides	none	(591)
D Glucosamine	slight	(245)
Glucose	protects	(290; 591; 639)
	slight	( 7, p. 399)
	none	(399; 615; 655)
Glucuronic Acid	protects	(452)
Glutamic Acid	protects	(770)
Glutathione	protects	( 51; 146; 200; 246; 339; 351; 386; 412;



Glutathione (continued)	protects	442; 558; 600; 789)
	slight	(452)
	none	(232; 382)
Glycerin	protects	(302; 387; 683)
Glycerol	slight	(802, p. 141)
	none	(615)
Glycidyl dimethyldithiocarbamate	slight	(802, p. 89)
Glycine	slight	(296; 508)
Glycine Ethyl Ester	slight	( 7, p. 398)
Gonadotropic Antiserum	protects	(270)
Growth Hormone	none	( 63)
Guanidine	none	(802, p. 150)
1 Guanidino 4 Aminopentane	protects	(802, p. 150)
Alpha Guanidinocinnamic Acid	none	(802, p. 151)
S, 4 Guanidobutylisothiuronium bromide hydrobromide	protects	(713)
Guanine	protects	(770)
Guronsan	protects	(454)
Halothane	none	(867; 868)
Hedonal	none	(625)
Helium	protects	(678)
	none	(654; 670)
Hematoporphyrin	none	(288)
Heparin	protects	(648)
Hepatrol - Liver Extract	none	(106)
N Heptylmercaptan	none	(802, p. 102)
Heroin	protects	(340; 710)
Hesperidin	none	(217; 272)
Hesperidin Methyl Chalcone	protects	(272)
	none	(217; 524)
2 Hexamethylenethiazolidine	protects	(417)
N Hexane	slight	( 7, p. 399)
1, 6 Hexanedithiol	none	(802, p. 103)
Hexanol	none	(330)
N Hexylmercaptan	none	(802, p. 102)
Hippuric Acid	protects	( 7, p. 400)
Histamine	protects	(871)
	none	(829)
Histidine	none	(802, p. 145)
Homocysteine	protects	(115; 452)
	slight	(802, p. 59)
Alpha Homocysteinethiolactone	protects	(583; 615)
Hordeol	none	(107)
Human Properdin	none	(545)
Hydergine	none	(467)
Hydracyanic Acid	none	(751)

2 Hydrazinobenzothiazole	slight	(802, p. 99)
1 Hydrazinophthalazine	slight	(412)
Hydrocortisone	none	(680)
Hydrogen	none	(40)
Hydrogendisulfide	protects	(105)
Hydrogen peroxide	protects	(436; 692)
	none	(792)
Hydroquinone	slight	(7, p. 399)
	none	(106)
Hydrosulfite	protects	(572)
Hydroxyacetoneitrile	protects	(802, p. 137)
2 Hydroxybutyronitrile	protects	(802, p. 137)
4 Hydroxy 6 Diethoxy 3 Methylpyrimidine	none	(802, p. 143)
1 Hydroxy 1 (2', 5' Dimethoxyphenyl)propyl 2 Amine methoxamine	protects	(802, p. 117)
Hydroxyethylbenzylether	protects	(622)
Hydroxyethylether of Belanaphthol	slight	(622)
Hydroxyethylether of O Phenylphenol	slight	(622)
5 Hydroxyindoleacetic acid	none	(802, p. 141)
3 Hydroxyisopropyl derivative of Cysteamine	none	(802, p. 77)
Hydroxylamine	none	(386)
5 Hydroxy 4 Methyluracil	protects	(802, p. 143)
5 Hydroxy Omega N Monoethyl Tryptamine	protects	(244)
M Hydroxyphenylalanine	protects	(802, p. 145)
M Hydroxyphenylethylamine	protects	(802, p. 119)
Beta Hydroxy Beta Phenylisopropylamine	protects	(713)
P Hydroxyphenylmercaptan	slight	(802, p. 102)
Beta Hydroxy Beta N Methylisopropylamine	none	(713)
3 Hydroxypropylisothiuronium chloride	none	(713)
8 Hydroxyquinoline	protects	(47, p. 176)
5 Hydroxytryptamine	protects	(802, p. 119)
5 Hydroxytryptamine	protects	(45; 51; 476; 481; 482; 491; 649; 788)
	none	(823)
4 Hydroxytryptamine	none	(244)
5 Hydroxytryptophan	slight	(160; 649)
Hydroxytyramine	protects	(7, p. 398)
Hyperglycemia	protects	(504)
Hypnotics	protects	(541)
Hypothermia	protects	(467; 520)
Hypoxia	protects	(137; 453; 455; 642; 678)
	none	(679)
Imidazolyl derivative of Cysteamine	slight	(802, p. 69)

Indole	protects	( 7, p. 400)
	none	(706)
Indole acetic Acid	slight	( 7, p. 400)
	none	(802, p. 141)
Indoleacetonitrile	slight	(802, p. 137)
Indolepropionic Acid	none	( 7, p. 400)
Indolethylamine	protects	(706)
Inert Gases	protects	(251)
Iodine	protects	( 7, p. 401)
Iodoacetic Acid	none	(802, p. 139)
5, 10 Iododeoxyuridine	none	(84)
O Iodosobenzoic Acid	none	(802, p. 139)
Iproniazid	none	(481)
Iron	none	(211)
Ischena	protects	(520)
Isobutyl derivative of Cysteamine	none	(802, p. 69)
Isocinchomeronic Acid	protects	(365)
Isocysteine	none	(802, p. 60)
Isonicotinic Acid 1 Oxide	protects	(365)
1 (1 Isonicotinoyl 2 Isopropyl)hydrazine	protects	(802, p. 142)
Isopropanol	none	( 7, p. 399)
Isopropyl derivative of Cysteamine	slight	(802, p. 69)
S, 5 Isopropylaminoamylisothiuronium chloride hydrochloride	none	(713)
Isopropyl N(M Chlorophenyl)Carbamate	slight	(802, p. 110)
Isopropyl N Phenylcarbamate	slight	(802, p. 110)
S Isothioureido S' Isothiourea Dihydro- chloride	none	(802, p. 77)
Beta Ketobutyric Acid	slight	(615)
Alpha Ketoglutarate	protects	(477)
	slight	(615)
	none	(802, p. 141)
Lactate	slight	( 7, p. 399)
	none	(615)
Lauroyl Pyrogallol	protects	(470)
Lemon Bioflavonoid Complex	none	(217)
Lemon Juice	none	(217; 524)
Lettice	protects	(591)
Leucine	none	(802, p. 145)
Leucopene	protects	(293)
Leukocytes	protects	(26; 185)
Limestone	protects	(743)
Linolenic Acid	none	(115)
Linoleic Acid	none	(115)
Lipopolysaccharide	protects	(96)
Liver Cells	protects	(174; 516)
Liver Fat	protects	(274)
Lycopene	protects	(293)



Lysine	slight	( 7, p. 398)
Magnesium	none	(211)
Magnesium sulfate	protects	(366)
	slight	( 99)
Maleic Acid	protects	( 7, p. 399)
Malonic Acid	none	( 7, p. 400)
Malononitrile	protects	( 51; 106)
	none	(592)
Manganese	none	(211)
Manganous Methyl Dithiocarbamate	none	(802, p. 94)
Mapharsol	none	(367)
Margarine Fat	protects	(273)
Megaphen	protects	(776)
	none	(480)
Megaphen and Dolantin	protects	(480)
Meperidine	none	(813; 868)
Meproamate	none	(858)
Mercamine	protects	(458; 871)
Mercaptoalkylamine	protects	(435; 565)
Mercaptoalkylguanidine	protects	(435)
2 Mercapto 5 Aminobenzimidazole	none	(802, p. 65)
2 Mercapto 6 Aminobenzothiazole	none	(802, p. 101)
1 Mercapto 7 Aminoheptane	none	(802, p. 66)
2 Mercaptobenzimidazole	none	(802, p. 66)
2 Mercaptobenzothiazole	protects	(626)
	slight	(802, p. 98)
2 Mercaptobenzoxazole	protects	(802, p. 66)
4 Mercaptobutylamine	protects	(232)
D L Mercaptobutyl 2 Guanidine hydrobromide	protects	(111)
4 Mercaptobutylguanidine hydrobromide	protects	(713)
1 Mercapto 5 Diethylaminopentane	none	(615)
2 Mercaptoethanol	none	(232)
2 Mercaptoethyl N <sup>+</sup> Allylguanidine hydrobromide	slight	(713)
2 Mercaptoethylamine	protects	(25; 51; 52; 232; 234; 239; 307; 391; 408; 417; 423; 424; 432; 457; 555; 560; 572; 573; 611; 668; 672; 835; 839; 840; 841)

Mercaptoethylamine derivative of O Arisidyl	protects	(795)
Mercaptoethylamine Ascorbate	protects	( 25)
Mercaptoethylamine Nicotinate	protects	( 25)
Mercaptoethylamine Salicylate	protects	( 25)
Mercaptoethylamine derivative of O Toludine	protects	(795)
2 Mercaptoethyl N', N'' Dibenzylguani- dine hydrobromide	none	(713)
2 Mercaptoethyl N', N'' Dibutylguani- dine hydrobromide	none	(713)
2 Mercaptoethyl N', N'' Diethylguani- dine hydrobromide	none	(713)
2 Mercaptoethyl N', N'' Diphenylguani- dine hydrobromide	none	(713)
2, 2' Mercaptoethyl ether	none	(802, p. 105)
2 Mercaptoethyl N', N'' Ethyleneguani- dine hydrobromide	protects	(713)
2 Mercaptoethylgluconamide	protects	(232)
2 Mercaptoethylguanidine	protects	(208)
2 Mercaptoethylguanidine hydrobromide	protects	(111; 275; 390; 564; 565; 700; 713; 746; 815)
2 Mercaptoethylguanidine hydrochloride	protects	(713)
2 Mercaptoethyl N Isopropylguanidine hydrobromide	protects	(713)
2 Mercaptoethyl N Methylguanidine hydro- chloride	protects	(713)
N, 2 Mercaptoethylmorpholine derivative of Cysteamine	protects	(802, p. 68)
2 Mercaptoethyl N' Phenylguanidine hydro- bromide	none	(713)
N, 2 Mercaptoethylpiperidine	protects	(564)
7 Mercapto 1 Heptylamine	none	(802, p. 66)
3 Mercaptomethyl 4 Hydroxymethyl 5 Hydroxy 6 Methylpyrimidine	slight	(802, p. 66)
2 Mercapto 6 Nitrobenzothiazole	none	(802, p. 101)
3 Mercaptopropionic Acid	none	(802, p. 103)
3 Mercaptopropylamine	protects	(232; 406; 677; 873)
Mercaptopropylamine 2 Aminopropane 1 Thiol	protects	(802, p. 66)
Mercaptopropylamine 1 Aminopropane 2 Thiol	protects	(802, p. 66)
3 Mercaptopropylguanidine	protects	(615)
3 Mercaptopropylguanidine hydrobromide	protects	(713)

3 Mercaptopropylguanidine hydrochloride	protects	(713)
5 Mercaptopyridoxine	protects	(115; 565)
2 Mercaptoquinoline	none	(232)
Mercaptosuccinic Acid	slight	( 7, p. 401)
	none	(615)
2 Mercaptothiazoline	none	(234; 615)
Mercury Chloride	none	(106)
Mescaline	none	(802, p. 119)
Methacholine	protects	(802, p. 131)
Methacil	protects	(802, p. 143)
Methacrylamide	protects	( 7, p. 399)
Methacrylic Acid	protects	( 7, p. 399)
Methacrylonitrile	protects	( 7, p. 399)
Methanol	protects	( 7, p. 399)
Methionine	slight	( 48; 452)
	none	(600; 681)
Methionine sulfoxide	slight	( 36)
Methotriate	slight	(800)
Methoxamine	protects	(708; 733)
Methoxsalen	protects	(496)
P Methoxythiophenol	protects	(292)
5 Methoxytryptamine	protects	(457; 788)
	slight	(244; 649)
Methyl	protects	(387)
N Methyl derivative of Cysteamine	protects	(802, p. 68)
S Methyl derivative of Cysteamine	slight	(802, p. 69)
Methyl derivative of Thiouracil	none	(802, p. 85)
Methylamine	protects	( 49)
	none	(706)
2 Methyl 4 (Benzeneazo)Thymol	protects	(802, p. 149)
2 Methylbenzothiazole	slight	(802, p. 99)
5 Methyl 5 Cyano 2 Pyrrolidine	slight	(802, p. 134)
N Methylcyclohexenyl	slight	(802, p. 110)
N Methylcyclohexylisopropylamine	none	(802, p. 141)
S Methylcysteamine	none	(615)
Alpha Methylcysteine	none	(615)
Methyldiisothiuronium bromide hydrobromide	slight	(234)
Methylene Blue	slight	(505)
Methyl Ester of Linoleic and Linolenic Acid and Amino Acid	protects	(235)
Methyl Ethyl Sulfoxide	slight	( 36)
Methyl Linoleate	protects	(226; 274)
2 Methylmercaptobenzothiazole	none	(802, p. 101)
S Methylmercaptoethylamino	protects	(370; 796)
Methyl Methionine	protects	(452)
Methyl Methionine Sulfonium Chloride	protects	(140)
2 Methyl 1, 4 Naphthohydroquinone Diphosphate	none	(802, p. 142)



2 Methyl 1, 4 Naphthoquinone 2, 3 oxide	none	(543)
Methylpentadiene	protects	( 7, p. 399)
Methyl Pantothenate	none	( 33)
N Methylphenylcysteamine	none	(615)
N Methylphenylisopropylamine	slight	(802, p. 119)
N Methyl 1 Phenyl 2 Propylamine	protects	(802, p. 141)
1 Methyl 4 Phenyl 4 Piperidinecarboxylic Acid	protects	(615)
1 Methyl 3 Piperidylmethylphenyl 2 Thienylacetate	none	(362)
N Methylrhodamine	none	(802, p. 94)
4 Methyl 1 Tetralone 3 Carboxylic Acid	none	(802, p. 149)
2 Methylthiazoline	protects	(802, p. 93)
8 Methyl 5, 8 Thioctic Acid	none	(802, p. 103)
Methyl Thiouracil	none	(357)
Alpha Methyltryptamine	none	(802, p. 126)
Methylurea	protects	( 7, p. 400)
Metrazol	slight	(239)
Minadiol Diphosphate	none	(448)
Minophagen C - A Mixture of Amino Acids	protects	(551)
Mitochondria	protects	( 1)
Monoamine Oxidase Inhibitors	protects	(160)
Monoamine Oxidase	protects	(481)
N Monomethylcysteamine	protects	(615)
Monosemicarbazone of Adrenochrome	none	(802, p. 117)
Monothioethylene Glycol	none	(802, p. 103)
1 Monothioglycerol	slight	(802, p. 103)
	protects	(302)
Moriamin	protects	(584)
Morin	none	(217; 524)
Morphine	protects	(340)
	slight	( 20)
Morphine Chloride	protects	(367)
Morphine Sulfate	protects	( 19; 417)
Alpha ( 4 Morpholinyl)P Toluonitrile	none	(802, p. 138)
S, 2 (1 Morpholyl)ethylisothiuronium bromide hydrobromide	none	(615)
S, 4 (1 Morpholyl)butylisothiuronium bromide hydrobromide	none	(713)
S, 3 (1 Morpholyl)propylisothiuronium bromide hydrobromide	none	(713)
Mustard Greens	protects	(214)
Mustard Seed Extracts	protects	(108)
Myelocytotoxic Serum Therapy	slight	(691)
Nagravon	none	(454)
Nalorphine	protects	( 20)
Naphazoline	protects	(682; 708)
Naphthol	slight	(802, p. 148)

Naringen	protects	(272)
	none	(217; 524)
Nembutal	protects	(625)
Neostigmine	none	(802, p. 115)
Nickelthamide	none	(239)
Nicotinamide and Chloride salt of Cobalt	protects	(676)
Nicotine	none	(361)
Nicotinic Acid	slight	(235)
Nitric Oxide	protects	(628)
	none	(344)
Nitrite Salts	protects	(42)
6 Nitroanthranilic Acid	none	(51)
P Nitrobenzylisothiuronium bromide	none	(713)
Nitrogen	protects	(58; 157; 864)
	slight	(739)
	none	(40)
Nitromin	none	(367)
2 Nitrophenyldimethyldithiocarbamate	slight	(802, p. 94)
1 Nitro 1 Propylisothiuronium bromide	none	(713)
Nitrous Oxide	protects	(129; 250; 279; 330)
Noradrenaline	protects	(682; 708)
Norepinephrine	slight	(802, p. 119)
Norvaline	none	(802, p. 145)
Novocain and Cobalt Iodine	protects	(676)
Nuclear Nucleoproteins	protects	(340)
Nucleic Acids	slight	(452)
Nutritional Factors and Antibiotics	protects	(536)
Alpha Octadecylglycerolether	protects	(252)
2 Octahydro 1 Azocinyethylguanidine	protects	(802, p. 113)
2, 2(Octahydro 1 Azocinyl)guanidine	protects	(412)
Oestradiol	none	(479)
Oleic Acid	protects	(7, p. 399)
Oleic Acid - oxidized	none	(310)
Olive Oil	protects	(34; 511)
Orange II (Sodium Salt)	slight	(7, p. 400)
Ornithine	slight	(802, p. 149)
Orotic Acid	slight	(547)
	none	(452)
Oxalic Acid	none	(7, p. 400)
N Oxide of Reserperine	protects	(364)
Oxybutyrate	protects	(477)
Oxygen	protects	(40)
	none	(13; 92; 215; 311; 315; 316;

Oxygen	(continued)	none	376; 392; 401; 442; 500; 525; 593; 654; 678; 679; 768; 799; 824; 856; 857)
Oxymethoxybenzophenone		protects	(646)
Oxytocin		protects	( 53; 615)
Packed Blood Platelets		protects	( 55)
2 Palmityl 1 Naphthol		none	(802, p. 149)
4 Palmityl Resorcinol		none	(802, p. 149)
Pantetheine		none	(232; 615)
Pantothenic Acid		protects	(586)
		slight	(452)
Pantothenate Omega Methyl		none	( 33)
N Pantothenyl derivative of Cysteamine		none	(802, p. 68)
N Pantoyl derivative of Cysteamine		none	(802, p. 68)
Pantoylaminoethylthiol		none	(232)
Pantoyltaurine		protects	( 33)
Parabiosis		protects	(101; 574; 696; 842)
Paraldehyde		protects	( 19)
Parathormone		none	( 57)
Parathyroid Extract		protects	(660)
Particulate Matter (Inorganic)		protects	(743)
PAS		none	(106)
Pendionide dibromide		none	(361)
Penicillamine (3, 3 dimethylcysteine)		none	(802, p. 60)
Penicillin		protects	(618)
Pentachlorophenylmercaptan		none	(802, p. 102)
3, N Pentadecylcatechol		none	(802, p. 149)
Pentamethyldiethyl 3 Azaheptane 1, 5 diazonium dibromide		none	(802, p. 152)
Pentamethylene Bis (Trimethylammonium) dibromide		none	(802, p. 152)
3 Pentamethylene 3 Methylthiazolidine		protects	(417)
Pentobarbital		none	( 19; 279; 868)
Pentobarbital Sodium and Aminoethyliso- thiouonium		protects	(528; 530)
Pentobarbital Sodium		protects	(618)
Pentose		none	(802, p. 141)
Pentoxyl		protects	(236)
		slight	(235)
		none	(420)
Pentoxyl (5 Hydroxymethyl)4 Methyluracil		protects	(802, p. 143)



Pentylene	slight	(802, p. 141)
Pereston N	protects	(454)
Phenacetylacetonitrile	none	(802, p. 138)
Phenanine	protects	(340)
Phenatin	protects	(24)
Phenatine	protects	(25)
Phenergan	none	(106)
Phenobarbital	protects	(19)
	none	(858)
Phenol	protects	(296)
Phenoxybenzamine hydrochloride	protects	(802, p. 148)
4 Phenoxyl N Butylamine	none	(802, p. 151)
Phentolamine hydrochloride	none	(802, p. 148)
S Phenyl derivative of Cysteamine	slight	(802, p. 69)
N Phenyl derivative of Cysteamine	protects	(802, p. 68)
Phenylalanine	protects	(7, p. 398)
Phenylamidine	protects	(804)
N Phenylamidine of Pyromucic Acid	protects	(804)
N Phenylamidine of Thiophene 2 Carboxylic Acid	protects	(661; 804)
N Phenylbenzamidine	protects	(661; 804)
2 Phenyl 1 Butylamine	none	(802, p. 151)
N Phenylcysteamine	none	(615)
Phenylcysteine	none	(704)
1 Phenyl 4, 4 Dimethylimidazolidine	protects	(802, p. 99)
P Phenylenediamine	none	(802, p. 151)
Phenylephrine	protects	(682)
Phenylephrine	protects	(708)
Phenylephrine Chloride	protects	(708)
Phenylester of Hydroxyacetonitrile	protects	(802, p. 136)
Phenylethanolamine	protects	(802, p. 119)
N Phenylethyl derivative of Cysteamine	none	(802, p. 69)
S Phenylethyl derivative of Isothiuronium	none	(802, p. 77)
Beta Phenylethylamine	protects	(47, p. 176)
	slight	(708)
Phenylethylmercaptoethylisothiuronium chloride	none	(713)
N Phenyl 2 Furamidine	protects	(661)
1 Phenyl 1 Hexylamine	none	(802, p. 151)
2 Phenyl 1 Hexylamine	none	(802, p. 151)
Phenylhydrazine treated Mouse Spleen homogenates	protects	(179)
Phenylisothiocyanate	none	(802, p. 138)
Phenylmercaptan	none	(802, p. 102)
Phenylmercaptomethyl derivative of isothiuronium	none	(802, p. 77)
S Phenyl Beta Mercaptoethylamine	protects	(796)

N Phenyl N Methyl derivative of Cysteamine	none	(802, p. 68)
Phenylphenamine	protects	(25)
O Phenylphenol	none	(802, p. 149)
Phenylpropanolamine	none	(802, p. 119)
2 Phenylthiazoline	none	(802, p. 98)
Phenylthiomethylisothiuronium iodide	none	(713)
3 Phenyl 2 (2', 3', 4' Trihydroxyphenyl) indole	none	(802, p. 149)
Philopan	none	(367)
Phosphate Buffer	none	(162)
Phthalimidoacetoneitrile	none	(802, p. 138)
Picrotoxin	slight	(239)
	none	(367)
Piperidine	protects	(7, p. 400)
2 (3 Piperidinopropionamido)4, 4' Dichloro- odiphenylsulfide monohydrochloride	none	(802, p. 106)
2 (3 Piperidinopropionamido)4', 5' Dichloro- odiphenylsulfide monohydrochloride	none	(802, p. 106)
2 (3 Piperidinopropylamino)4', 5' Dichloro- odiphenyl sulfide monohydrochloride	none	(802, p. 106)
2 (3 Piperidinopropyl) 4', 6' Dichlorodi- phenylsulfide monohydrochloride	none	(802, p. 105)
S, 4(1 Piperidyl) Butylisothiuronium bromide hydrobromide	none	(713)
N Piperidylcysteamine	none	(615)
S, 2 (1 Piperidyl)ethylisothiuronium bromide hydrobromide	none	(713)
S, 3 (1 Piperidyl)propylisothiuronium bromide hydrobromide	none	(713)
Alpha (1 Piperidyl)Alpha toluonitrile	slight	(802, p. 137)
Piromen	protects	(96)
Piromen and Dextran mixed	none	(96)
Pitressin	protects	(802, p. 128)
Pitressin Tannate in Peanut Oil	protects	(852)
Plasma Homologous	slight	(781)
Plasma Protein	slight	(781)
Politamin	protects	(454)
Polyglucin	protects	(503)
Polyvinylpyrrolidone-High Molecular Weight	none	(596)
Polyvinylpyrrolidone-Low Molecular Weight	protects	(596)
Potassium	none	(211)
Potassium Bromide	none	(7, p. 401)
Potassium Chloride	none	(7, p. 401)
	protects	(366)
Potassium Cyanide	protects	(106; 115; 740; 825)

Potassium Iodide	protects	( 7, p. 400)
Potassium Permanganate	none	( 7, p. 401)
Potassium Thiocyanate	slight	( 7, p. 401)
Potatoes Supplemented Diet	none	(561)
Prantal	none	(361)
Prednisone and Prednisolone	protects	(300; 237; 371; 617)
Pregnant Females	none	(673; 760)
Pressure	none	(539)
Procaine	protects	(150)
Procaine Chloride	none	(367)
Procaine, Penicillin and Streptomycin	protects	(274)
Proline	protects	(802, p. 145)
Promethazine	slight	(467)
	none	(813)
Promethazine hydrochloride	none	(802, p. 114)
Promethazine and Refrigeration	none	(802, p. 114)
1, 3 Propanediol	slight	(802, p. 112)
N Propanol	protects	( 7, p. 399)
Propardin	protects	(802, p. 185)
Propionate	none	(802, p. 141)
Propyl derivative of Thiouracil	none	(802, p. 89)
N Propyl derivative of Cysteamine	slight	(802, p. 69)
S, S Propyldiisothiuronium	none	(234)
Propyl Disulfide	protects	(374)
Propylene	protects	(387)
Propylenediamine	protects	(802, p. 150)
Propyleneglycol	protects	(683)
Propylenesulfide	none	(232)
Propylgallate	protects	(332; 333)
Propylhexedrine	none	(802, p. 119)
S Propylisothiuronium bromide	slight	( 37)
Propyl 2 Phenamine	protects	( 25)
2 Propyl Thiazolidine	protects	(417)
Propyl Thiouracil	slight	(357)
	none	(106)
Protein Diets	protects	(608; 609; 693)
Psoralen Benzophenone	slight	(446)
Purine Deoxyribonucleotides	none	(758)
Putrescene	none	(802, p. 150)
Pyrex Glass	protects	(743)
Pyridine	protects	( 7, p. 400)
Pyridine 2, S Isothiuronium bromide		
hydrobromide	none	(713)
Pyridoxal 5 Phosphate	protects	(564)
Pyridoxine	none	(670; 723)
Pyridoxine Hydrochloride	protects	(325; 589)
Pyrilamine Maleate	slight	(739)



Pyrimidine Derivatives	protect	(346)
Pyrimidine Deoxyribonucleosides	none	(758)
Pyrimidine Deoxyribonucleotides	protects	(758)
Pyrogallol	none	(802, p. 149)
Pyromellitic Acid	none	(802, p. 149)
2 (3 Pyrrolidinopropionamido)4, 4' Dichlorodiphenyl sulfide monohydrochloride	none	(802, p. 105)
2 (3 Pyrrolidinopropionamido)4', 5 Dichlorodiphenyl sulfide monohydrochloride	none	(802, p. 105)
2 (3 Pyrrolidinopropylamido) 4, 5' Dichlorodiphenyl sulfide monohydrochloride	none	(802, p. 105)
S, 4 (1 Pyrrolidyl)butylisothiuronium bromide hydrobromide	none	(713)
S, 2 (1 Pyrrolidyl)ethylisothiuronium bromide hydrobromide	none	(713)
S, 3 (1 Pyrrolidyl)propylisothiuronium bromide hydrobromide	none	(713)
Pyruvate	protects	( 51; 573; 477)
Pyruvic Acid	slight	(802, p. 141)
Quartz	protects	(296; 615)
Quercetin	protects	(743)
Quercetin Dehydrate	none	(217)
Quercetin Hesperidin	none	(217)
Quercitrin	none	(524)
Quinacine N omega 10 Dioxide	none	(217; 524)
Quinidine	protects	(365)
Quinoline	protects	(802, p. 142)
Quinoline 2, S Isothiuronium bromide hydrobromide	protects	( 7, p. 400)
Quinoline 1 Oxide	none	(713)
Quinoxaline 1, 4 Di N Oxide	protects	(365)
Reserpine	protects	(356)
Resorcinol	protects	( 45; 364; 481)
Rhodamine	none	(858)
Riboflavin Phosphate	protects	( 7, p. 399)
Ribonucleic Acid	none	(802, p. 149)
Ribonucleotide	slight	(802, p. 92)
Ribose	none	(802, p. 142)
Ribothiazoline	protects	(515; 517; 588)
Royal Jelly	none	(758)
	protects	(802, p. 151)
	none	(802, p. 98)
	protects	(319)

Rutin	protects	(114; 272; 347; 648)
	none	( 94; 199; 217)
Sacchariose	none	(399)
Salicyclic Acid	protects	( 88; 615)
Saline	protects	(131; 234; 290; 366; 858)
	none	(506; 791)
Santoquin and Tocophenol and N, N' Di- phenyl P Phenylenediamine	none	(271)
Sarcosine	none	(802, p. 145)
Serine	none	(769)
Serotonin	protects	(160; 239; 275; 687; 706; 839; 840; 841)
Serotonin and N, N" Bis (2 Hydroxy- ethyl) Dithiooxamide	protects	(621)
Serum (Homologous)	protects	(459)
	slight	(781)
Sex	protects	(673)
Shielding Part of the Body	protects	(790)
Shielding Spleen	protects	(675)
Sodium Amytal	protects	(625)
Sodium Arsenate	protects	(660)
Sodium Azide	protects	(283)
Sodium Benzoate	none	(367)
Sodium Bicarbonate	slight	(366)
Sodium Bisulfate	none	(655)
Sodium Bisulfite	none	(655)
Sodium Bromide	none	(131; 463)
Sodium Cyanate	none	(802, p. 134)
Sodium Cyanide	protects	( 46; 218; 384; 615)
Sodium Diethyldithiocarbamate	slight	( 71)
	protects	(192)
Sodium Diphenylhydantoin	protects	(471)
Sodium Dithionate	none	(550)
Sodium Ethanedithionophosphate	slight	(802, p. 108)
Sodium Ethanedithiophosphonate	protects	(550)
Sodium Ethanemonothiothiophosphonate	slight	(802, p. 108)
Sodium Ethylenediaminetetra Acetate	protects	(192; 658)
Sodium Fluoride	none	(106)
Sodium Fluoroacetate	protects	(45; 50; 54)
Sodium Formate	none	(436)
Sodium Gallate	protects	(333)

Sodium Hydrosulfate	protects	(655)
Sodium Hydrosulfite	protects	(296; 436; 634; 656)
Sodium Menadiol Diphosphate	none	(448)
Sodium Nembutal	protects	(625)
Sodium Nitrate	none	( 7, p. 401)
Sodium Nitrilotriacetate	protects	(192)
Sodium Nitrite	protects	( 41; 162; 173; 324; 342)
Sodium Peroxydisulfate	none	(655)
Sodium Phosphate	protects	(366)
	none	( 7, p. 401)
Sodium Pyrosulfate	protects	(655)
Sodium Salicylate	protects	( 7, p. 399)
Sodium Sulfate	none	( 7, p. 401)
Sodium Sulphydrate	protects	(655)
Sodium Sulfide	none	(600; 615)
Sodium Tetrathionate	none	(615)
Sodium Thiocyanate	none	(802, p. 138)
Sodium Thiophosphate	none	(802, p. 140)
Sodium Thiosulphate	protects	(550; 655)
Somatotrophic Hormones	protects	(348; 468; 469)
Spermidine	none	(802, p. 150)
Spherophysin	none	( 25)
Spinach	none	(591)
Spleen Homogenates	protects	(164; 171; 174; 179; 259; 261; 262; 263; 264; 265; 266; 268; 718; 731)
	none	(546)
Spleen Transplants	none	(106)
Splenectomy	protects	(696)
	none	(302)
Splenocrisis	slight	(106)
2 Stearyl 1 Naphthol	protects	(802, p. 149)
Streptomycin	none	(367)
Strychnine	none	(802, p. 114)
Strychnine Nitrite	none	(367)
Succinate	none	(769)
Succinic Acid	slight	(615)
Succinonitrile	protects	(802, p. 136)
Sucrose	protects	(802, p. 141)



Sulfadine	none	(367)
Sulfonium Chloride	protects	(452)
P Sulfamidophenylmercaptan	none	(802, p. 102)
Beta Sulfanilamidocapronitrile	none	(802, p. 138)
Sulfasuxidine	protects	(766)
Sulfocarbamylguanidine	none	(802, p. 151)
Sulfur	slight	(873)
Sulphocyanide	none	(106)
Sulfoxide	protects	(565)
Sunflower Oil	protects	(566)
Suprarenal Cortex Extract	none	(5)
Syn Diphenylguanidine	slight	(802, p. 149)
Synestrol	protects	(711)
Synkavit	none	(448; 543)
Synthetic Polymer - Polyvinyl Pyrrolidone	slight	(802, p. 141)
Syntocinon	protects	(53)
Taurine	protects	(802, p. 145)
Temperature	protects	(62; 100; 106; 316; 437; 453; 553; 563; 569; 571; 629)
	none	(215)
Testosterone	protects	(479; 614)
	none	(601)
Testosterone Cyclopentylpropionate	protects	(765)
Testosterone Propionate	none	(267; 542; 765)
Tetrabenzene	protects	(244)
3 Tetradecyl 2(2', 3', 4' Trihydroxy-phenyl)indole	none	(802, p. 149)
Tetraethylammonium chloride	none	(361)
Tetraethylammonium chromide	protects	(585)
N, N, N', N', Tetraethyl 4, 4' Diamino-benzophenone	none	(802, p. 121)
Tetraethylthiuram Disulfide	none	(802, p. 94)
Tetraethylthiuram Monosulfide	none	(802, p. 94)
Tetrahydrazoline chloride	protects	(802, p. 151)
Tetrahydrofurfurylamine	none	(802, p. 151)
Tetramethylcystamine	protects	(615)
Tetramethylene Bis Guanidine Sulfate	none	(802, p. 151)
2 Tetramethylene 3 Methyl Thiazolidine	protects	(417)
Tetramethylene Sulfoxide	slight	(36)
2, 2, 4, 5 Tetramethylthiazolidine	protects	(417)
Tetramethylthiuram Disulfide	slight	(802, p. 89)
Tetrasodium 2 methyl 1, 4 Naphthohydroquinone	none	(448; 543)

Tetrasodium 2, 3 Dimethyl 1, 4 naphtho- hydroquinone diphosphate	none	(448; 543; 613)
Tezan	protects	(235)
Thesane Emulsion	none	(554)
Thiacyl	none	(106)
Thialdine	none	(802, p. 77)
Thiamine Diphosphate	none	(802, p. 143)
Thiamine Monophosphate	none	(802, p. 143)
1 Thia 4, 6, 9 Triazaspiro (4, 4') nonane dihydrobromide	slight	(713)
1, 3 Thiazane 4 Carboxylic Acid	none	(802, p. 102)
Thiazolidine	protects	(565)
Thiazolidine 4 Carboxylic Acid	none	(653)
S Thiazolyl 3 Mercaptoethylamine	protects	(796)
S, 4', 2', Thiazolylmethylisothiuronium bromide	none	(713)
N Thienyl derivative of Cysteamine	protects	(802, p. 68)
Thioacetamide	protects	( 7, p. 401)
Thioacetic Acid	none	( 7, p. 401)
Thioammeline	none	(802, p. 55)
O Thiobenzoic Acid	none	( 7, p. 401)
2, 2' Thio Bis (4, 6 Dichlorophenol)	none	(802, p. 106)
4, 4' Thio Bis (6 Tert Butyl M Cresol)	none	(802, p. 106)
Thioctic Acid	protects	(452)
5, 8 Thioctic Acid	none	(802, p. 141)
6, 8 Thioctic Acid	none	(802, p. 141)
Thiocyanide	none	(615)
Thiodiethyleneglycol	none	(802, p. 105)
Thiodiglycolic Acid	none	(802, p. 112)
Thioethanol	none	( 7, p. 401)
Thioethers	none	(802, p. 58)
Thioglycolic Acid	none	(615)
1 Thiohistidine	none	(802, p. 145)
Thiolactic Acid	none	(452)
Thiolacton	protects	(452)
Alpha (Thiolcarbonyl)acetamidilidene	none	(802, p. 107)
1 (-) Thiolhistidine	none	(615)
1 (-) 2 Thiolhistidine	none	(475)
Thioloacetic Acid	none	(802, p. 107)
Thiomalic Acid	none	(106)
Thionoacetanilide	none	(802, p. 107)
Thionobenzanilide	none	(802, p. 107)
Thiopental	none	(129; 868)
Thiophene	none	(802, p. 102)
1 Thiosorbitol	protects	(302)
Thiosulfate	protects	(873)

Thiotaurine	none	(143)
1 Thio 2 Thioureidoethane hydrochloride	none	(232)
Thiouracil	none	(357)
Thiourea	protects	(51; 89; 478; 501; 550; 615; 634; 837)
	none	(106)
Thiuronium Barbiturate	none	(713)
Threonine	none	(769)
Thymidic Acid	protects	(802, p. 143)
Thyroid	none	(100)
	protects	(627)
Thyroidine	protects	( 22)
Time of Day	protects	(619)
Tigan	protects	(132)
Tocophenols	none	(271)
	protects	(385)
Toluedine Blue	protects	(648)
Tolyl derivative of Cysteamine	none	(802, p. 68)
Tranquillizers	protects	(452)
Traumatic Shock	none	(405)
Tri (Beta Diethylamine)ethylamine hydro- chloride	none	(361)
Tribromoethane	none	(846)
Trichloroethylene	none	(868)
Triethanolamine	none	(802, p. 151)
	protects	( 7, p. 398)
S, 3 Triethylaminopropylisothiuronium bromide hydrobromide	none	(713)
Triethyleneglycol	protects	(387)
Triethylenethiophosphoramidate	none	( 83)
Trihydroxy N Methylindole	protects	( 7, p. 400)
2 Tri iodothyronine	none	(319)
1 Tri iodothyronine Sodium	none	(775)
S, 2 Tri (Isothiuroniummethyl)amine tri- bromide hydrobromide	none	(713)
Trimethobenzamide	protects	(132)
Trimethsulfoxonium iodide	none	(802, p. 106)
Trimethylamine	protects	(802, p. 148)
S, 2 Trimethylaminoethylisothiuronium bromide hydrobromide	none	(713)
S, 3 Trimethylaminopropylisothiuronium bromide hydrobromide	none	(713)
Trimethylcolchicinic Acid Methyl Ether D Tartrate	protects	(748)
Trimethylcolchicinic Acid D Tartrate	protects	(802, p. 129)
Trimethyleneglycol	protects	( 7, p. 399)



Triphenylethylamine	none	(802, p. 151)
2, 2, 2, Tris (Diethylamino)ethylamine	none	(802, p. 151)
N, N', N'' Tris (P Nitrophenyl)guanidine	none	(802, p. 151)
Tropinebenzhydrylethermethane sulfate	none	(362)
Tropinebenzhydrylethermethane sulfonate	none	(802, p. 106)
Trypaflavin Fluorescein	none	(336)
Tryptamine	protects	( 51; 71; 706; 871)
Tryptophan	slight	( 7, p. 398)
	none	(655)
Tsien - Polysaccharide of Tea Fungus	protects	( 39)
Turnips	none	(591)
Typhoid - Para Typhoid Vaccine	protects	(660)
Tyramine	protects	( 51)
	none	(615)
Tyrosine	protects	( 7, p. 398)
	none	(802, p. 141)
Ultraviolet Light	protects	(410; 872)
Unithiol	protects	(338)
Uracil	protects	(770)
	none	(655)
Urea	none	( 7, p. 400)
Urethane	protects	(166; 167; 668)
	none	( 83; 367; 383; 467; 480; 568; 625)
Urotropine	protects	(235)
Vitamin B	protects	( 75; 144; 374; 452; 499; 532; 536; 609)
Vitamin C	protects	(532; 609)
Vitamin E	none	(363)
Vitamin K <sub>5</sub>	none	(715)
Vitamin P <sup>5</sup>	protects	( 32; 532; 609; 754; 755)
	none	(197; 217; 524; 773)
Water	none	(401)
	protects	(768)
Xanthorhamin	none	(217; 524)
2 Xyly 2 Mercaptoacetic Acid	none	(802, p. 67)
Yeast Concentrate	protects	(225; 517; 587)

Zinc Dimethyldithiocarbamate	none	(802, p. 94)
Zymosan	protects	(862; 876)

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Interaction between gamma radiation and matter has been demonstrated by photoelectric effect, Compton effect or pair production. Photoelectric effect was reported when gamma rays gave all their energy to an electron removing it from its orbit. Compton effect was reported when gamma rays gave only part of their energy to the electron and kept the rest, being propelled in the opposite direction. Pair production was reported when gamma rays disintegrated forming electrons and positrons (21, p. 86--153; 136; 147, p. 34-42; 182, p. 583-671; 314; 431, p. 22-44).

There are certain characteristics that have been associated with these interactions, such as; The activation of large fractions of molecules receiving energy (623); Transference of energy not occurring singly but in linear groups (74; 702; 874); Excitation energy has been transferred over a distance of 50-100 Angstroms (297); The transfer of electrons occur from an excited molecule to an unexcited molecule (575); Collisions occur between excited and unexcited molecules (805); Excited molecules have either emitted radiation or released electronic excitation (522; 777; 805) and some carotenoid synthesis has been found to be induced by radiation (329; 657).

Photodynamic phenomena have been associated with polycyclic hydrocarbons and photosynthetic processes (31; 688). In photosynthesis, incoming rays have caused the release of an electron which traveled through a path whose ultimate goal was the fixation of carbon dioxide and the release of oxygen (30; 31). The radiation in photosynthetic processes have been found to be of much longer wave length than gamma rays, 4000 to 8000 Angstroms, as opposed to 1 to 0.00001 Angstroms, but similar patterns were obtained as a result of their interaction with matter as determined by electron spin resonance spectra (181). Chlorophyll interacted with radiation to produce an electron and essentially a free radical (10; 305). Carotenoids have been implicated in the procedure also (17; 154; 298; 413; 620). After interaction with the radiation, characteristics of semiconductors were noted (29; 180). A single electron transfer reaction appeared to occur between chlorophyll and quinones (490, p. 136-169; 805; 806).

The primary quantum conversion was from chlorophyll to a compound with two SH bonds according to one proposal (138). A suggested model for this conversion compound was 1, 2 dithiol- and (trimethylene disulfide) which was able to handle large amounts of energy (64). Sulfur containing aromatic compounds showed electron spin resonances and sometimes contained free radicals in substantial amounts (281). Other sulfolipids have been isolated from photosynthetic plants (80; 212).



The radiochemical effects of free radical formation has been studied through electron spin resonance spectra (440). The results included information that most amino acids lost hydrogen atoms from the amino group to form free radicals while cysteine and cystine associated their unpaired electron with the sulfur atom. Peptides lost hydrogen atoms from the alpha carbons of the amino acid residues, while glutathione had an unpaired electron localized on the sulfur atom. Proteins gave characteristic electron spin resonance spectra similar to cysteine, peptides or a superimposition of both (652; 735). Enzymes gave either a pattern similar to cysteine or to polyglycine (38).

Deoxyribonucleic acid did not display radiation induced radicals, possibly due to the rapid recombination of unpaired electrons or to some inherently high radiation resistance of the nucleic acids (716). Carbohydrates lost hydrogen or hydroxyl groups while lipids and carotenoids lost only hydrogen atoms (650; 735; 817).

The end products obtained from radiation and the possible mode of radiation damage have been studied. Alpha amino acids were deaminated with the release of ammonia (9, p. 117; 308), while sulfur amino acids yielded disulfide bonds (9, p. 117). Proteins, such as bovine serum albumin, changed solubility, average molecular weight, sedimentation rate and accessibility of disulfide groups (9, p. 117).

Deoxyribonucleic acid was affected in several ways by radiation. The individual purines and pyrimidines reacted differently though all may deaminate and their rings open (589; 697). Thymine dimers were produced (222; 707; 865); the double bond of cytosine broke in the presence of water (222); guanine degraded to guanidine, while uracil decomposed to urea and oxalic acid (698). The thymonucleohistone depolymerized (761) possibly by the rupture of cross linking hydrogen bonds (193; 194; 635). Internucleotide phosphate ester bonds were either randomly cleft (827) or had specific ester bonds broken (213; 697; 698). Glycosidic linkages were broken with the liberation of bases (697). The depolymerization caused a change in viscosity (133; 134; 135; 188; 187; 194; 213; 494; 495), and lowered the molecular weight (186; 495). Ultraviolet light absorption was decreased (66) and also observed was the swelling of spleen deoxyribonucleic acid in the presence of water (172). An insoluble gel was produced (494) and titratable acid groups were increased (698). It was suggested that the radicals produced in the medium caused the damage and not the direct action of the radiation (135; 734). Oxygen tended to increase the damage caused by radiation (134; 188; 225).

Irradiated carbohydrates gave many and varied results, the aldohexoses did not have one particular point of attack while ketohexoses and hexitols had susceptible primary alcohol groups (614). Polysaccharides depolymerized (614) and mucopolysaccharides degraded,

depolymerized and caused destruction of hexosamine and hexuronic acid moieties (59, p. 149-164; 118).

Irradiated carboxylic and hydroxy acids were converted to keto acids (614). An example is acetic acid which was oxidized to yield glycolic, glyoxylic and oxalic acids, formaldehyde and carbon dioxide (9, p. 117).

Lipids, when irradiated, formed organic peroxides (149, p. 1-25; 396; 397; 817), while fatty acids formed peroxides, hydroperoxides or were completely broken down (369). Carotenoids were bleached by irradiation and peroxides formed (369). Colloids reacted according to their charge, positively charged colloids coagulated and negatively charged colloids had their stability increased (204). Significant changes in epsilon potential of a colloid particle were noted (205). Liquid and solid state polymers were either crosslinked or degraded (147, p. 426-440).

The radiobiological effects of radiation have been studied in various animals and compared with the radiochemical effects (874). Proteins were altered in chemical properties and changed tissue-protein levels resulted from destroyed lymphoid tissue (582). Protein synthesis was insensitive to radiation and the rate of incorporation of glycine into liver slices was not inhibited (426; 582; 605). The decrease in plasma proteins of irradiated animals was not due to decreases in the rate of protein synthesis of the liver but to the increased rate of removal of protein by the tissues (426). Cellular metabolism was required for the



healing of chromosome breaks (430) and protein catabolism was slowed (507). Methionine and glutathione levels were decreased (465).

There was no conclusive evidence that enzyme inactivation occurred as a primary biochemical event though it may have resulted directly from primary biochemical changes (38, p. 359-413; 228; 402; 582; 605; 623; 624). Sulfhydryl enzymes were inhibited due to oxidation of their SH groups (67; 68; 599), while pepsin and non-sulfhydryl enzyme inactivation was due to a modification of the protein (18; 67; 623). Glyoxalase activity decreased in red blood cells as a result of a fall in reduced glutathione, a similar effect was produced by addition of hydrogen peroxide (343; 443). The method of purification of the enzymes lead to different radiation sensitivities (109). DNAase was one of the few enzymes which was activated by radiation (54; 784).

Deoxyribonucleic acid was specifically affected and the reduced content in the cells was accompanied by a decreased rate of incorporation of isotopically labeled precursors into DNA and RNA (9; 526; 543; 578; 582). Spleen DNA was probably released as soluble polynucleotide from an insoluble protein bound form (175; 177) and liver DNA synthesis was resumed within 48 hours (76) though some felt it was never completely inhibited (334). Deoxynucleoside increased and there was a delayed appearance of deoxynucleotides (409) although there was an increase of free deoxypolynucleotides (512). The cell multiplication or some independent process

(intracellular cation concentration) may have been sensitive to radiation and not DNA or RNA synthesis (429; 797; 838; 847; 848), though DNA synthesis was inhibited while RNA and protein synthesis were not (189). Loss of viability of cells bore little relationship to DNA breakdown (782), however the transforming activity of DNA irradiated in saline was destroyed at low levels (240; 398; 783; 785). Chromosome breaks caused by radiation were of two types, those which restituted rapidly and those which were completely restituted after a longer time (533). Cellular metabolism, protein synthesis and oxidatively derived energy (ATP) appeared to be required for some of the repair processes (430).

The absorption of glucose was reduced due to a delay in gastric processes. Tissue breakdown was responsible for accumulation of glycogen as well as depression of glycolysis and respiration (582) though the liver glycogen content fell temporarily (394). A lipemia followed radiation with an increase in neutral fats, phospholipids and cholesterol (582) and a decreased incorporation of lipid into the intestinal wall (394). Peripheral lipid mobilization occurred which was measured by a decrease in lipid concentration of characteristic fat depots and the plasma free fatty acid responses. The plasma phospholipid, free and ester cholesterol and triglycerides were significantly increased after irradiation, while the plasma free fatty acids were unaltered according to another worker (258). Bone marrow lipids showed no

significant alteration (257) and no changes in lipids of rabbit skin (701) or in lipid balance were reported (605).

Cancer was one of the most characteristic long term effects on mammals (165; 549). The leukemia was of three main types, generalized lymphoid, myeloid and reticular neoplasms (112; 196; 243; 441; 533; 576; 816).

The immune response to subcutaneous injections of tetanous toxoids was inhibited (722) and other similar delayed immune responses have been noted (230; 195; 355; 721; 793; 794). A secondary disease developed when tissue transplants were used as therapy treatment for radiation injury. They were caused by reactions of graft against the host (65; 93; 169; 176; 178; 689; 690; 843). Preformed hemolysin levels dropped (449) and there was an impairment of antimicrobial defenses (421). Mitochondrial extracts prepared from leucocytes of irradiated rats showed a lowered bactericidal activity than those prepared from non-irradiated animals (289; 290; 291).

The rate of spread of disease was increased and otherwise latent infections were "activated" (102), e.g. Pseudotuberculosis was activated (714). Bacteremiae in irradiated animals were caused by normal flora (540; 718; 719). Increased susceptibility of mice to plague endotoxin was also noted (720).

Polydipsia was primary and polyduria secondary following irradiation (103; 322). Significant increases in urinary potassium



occurred (103; 322) while serum potassium levels were elevated (103; 322). Similarly, potassium efflux from Escherichia coli was increased (373). Irradiation of the pituitary gland was required for diuresis to occur (427). Intestinal disturbances were attributed to permeability changes with destruction of intestinal mucosal lining, ulceration and hemorrhages (77). The permeability changes have been attributed to the fact that transport mechanism of cells is an energetic transformation which is blocked by irradiation (153).

Oxidative phosphorylation was altered in that the Phosphorous oxygen ratio was reduced (819). Protein phosphorous decreased in all tissues (833) and phosphoprotein phosphatase activity in liver and bone marrow was reduced (833). Hyperactivity of anterior pituitary caused an increase in the blood level of hormones. The excessive amounts of thyroxine and cortical steroids were thought to be responsible for the observed depression of Phosphorous oxygen ratio (79). The correlation of hormones with glycolysis has been proposed (461). The depression of oxidative phosphorylation of mitochondria isolated from spleen, thymus and pea seedlings occurred even following low doses of irradiation (725; 820). The decrease of phosphorylation was not secondary to ATPase activity and development prior to depression of anaerobic glycolysis was observed in spleen homogenates (820). Inhibition of compensatory hypertrophy in the kidney was correlated with suppression of mitotic activity (664).

The blood picture was affected in that the number of erythroid cells, thrombocytes and leucocytes was reduced, while the reticulum and plasma cells were not changed (77; 269; 309; 419; 666). The maturation of myeloid cells was accelerated (309; 566), while reticulum cell transformation was stopped (309; 605). Red blood cells entered the lymph system (667) and there was an increase in venous pressure, a decrease in heart potassium concentration, heart deoxyribonucleic acid and an alteration in length and amplitude of heart sounds (28). Anemia due to decreased hematopoiesis and lowered capillary resistance occurred (70; 77). There was an aggregation of red blood cells (510) and a depression of plasma radioactive iron turnover rate (320; 834). Hypotension was also a symptom (122; 552).

The existence of twelve general metabolic schemes in cells has been recognized: glycolysis, pentose phosphate oxidation cycle, the tricarboxylic acid cycle, electron transport and oxidative phosphorylation, metabolism of glycogen, lipids, cholesterol, steroids, amino acids and proteins, the urea cycle, sulfur metabolism, metabolism of nucleic acids and their derivatives and haem compounds. Of these, the only energy requiring activities of the cell were biosynthesis, mechanical work, active transport, transmission and bioluminescence (220).

Since radiation has been postulated as a random event not all the free radicals indicated will be formed nor will these give the end products and alteration in biological function indicated.

No radiotoxic substance has been found to occur as was once postulated (139) and now workers feel that there are three main points of attack in the cell: Enzymes, Nucleic Acids and Miscellaneous Macromolecules (nutrients, cofactors, hormones etc.) (377).

An enzyme hit was equivalent to a point hit and caused the formation of a free radical in the alpha carbon of the amino acid residue or in the sulfur atom. Most cellular enzymes had as their reactive sites sulfur atoms.

Protection by paramagnetic gases may be due to the fact that they altered electron spin resonance signals of charcoal and may act similar in animals, i.e. donating electrons and neutralizing free radicals (400). Specific binding of the reactive (sulfur) sites by protective compounds or the substrate, are another method of preservation of the activity of the enzyme (101; 109; 227; 254; 255; 331; 464; 472; 730; 866). Metal ions associated with the enzymes may be released by radiation and compounds which will remove these metals have protective ability (136; 233; 255; 256). Oxygen increased the sensitivity of enzymes and compounds which reduced the oxygen tension offer some protection (345; 401; 572; 603; 616; 818) or competitively removing perhydroxyl radicals formed in the presence of oxygen (6).

Inactivation of enzymes by radiation was a repairable type of damage as the nuclear material of the cell was able to synthesize more enzymes (145). A certain amount of time was required for this repair and as has been noted, animals do recover from



low doses of irradiation though mutagenic effects may be noted in their progeny (389; 523).

Nucleic acid hits were neutralized immediately. There were no electron spin resonance signals from DNA after irradiation. In general a univalent free radical was disposed of through reactions with surrounding molecules or with another free radical (652). The close proximity of nuclear material would facilitate this neutralization. Oxygen increases the damage to nucleic acid and potentiated the free radicals by acting as an oxidizing agent.(484).

Deoxyribonucleic Acid was believed to be the template for the synthesis of all the enzymes of the cells. Its destruction or alteration has been considered enough to kill the cell (493; 583). This type of damage was not thought to be repairable in view of the present theories on DNA synthesis (493). Dose fractionation caused an increased survival to a total supralethal radiation dose (61; 141; 168; 191; 207; 354; 462; 562; 641; 759; 811). Some extension of life span occurred with continuous low doses of radiation, and this was attributed to the death of the most susceptible cells leaving the stronger ones (47, p. 441-442). The death of animals after irradiation was not immediate but delayed and it was reported that an attempt at DNA replication was required for death (60). Metabolism was carried on normally until the enzymes needed to be replaced. The nucleic acid was not able to do this and death followed.

Protection from this type of death was by multiplication of nuclear matter or supplying new nuclear material to make up for that which was damaged (229, p. 211; 331). In experiments on amoebae, transplantation of nuclei from unirradiated to irradiated amoebae caused the latter to survive (358). Transplantation therapy of bone marrow cells was considered to replace damaged cells. Though animals lived longer, the altered DNA was considered capable of producing cancer in later years, accelerating normal aging and decreasing the life span (112; 779). Multinucleated cells have been found to be more resistant in some cases, which agrees with the hypothesis that all templates must be destroyed in order to kill the cell (278; 353; 557; 651; 659; 702; 849; 850).

The miscellaneous macromolecules in the cell are usually affected by the loss of hydrogen atoms. The resulting molecules were able to continue in the metabolic scheme unless the processes needed the absent hydrogen atom.

Protection was afforded by supplying unaltered molecules soon after irradiation, thus therapeutic diets have been found to be successful. Microorganisms were better off in this respect since they were grown on a medium which consisted of their nutritional requirements.

Some authors feel that humoral substances in shielded portions of the body or implanted or injected tissues are capable of instituting recovery (407). Cellular extracts of spleens have been

found to be protective in other animals and were thought to protect by supplying humoral substances. Metals which have been ionized in the course of irradiation can be removed by chelating agents (415). Oxygen enhances the effect of radiation and substances which remove it will protect. With heavy suspensions of microorganisms, protection may be afforded by the reduction of oxygen due to the respiration of the dense suspension (116).

Cofactors, inactivated by radiation and not supplied by the medium caused a delay in growth of the microorganisms until they have been synthesized. Since DPNH, FMNH, FADH and TPNH lost hydrogen atoms and were the sources of energy for the cell, energy requiring processes are slowed.

Hormones have been implicated in radiation damage, because of their control of living cells - oxidative phosphorylation and lipid metabolism (334; 461; 473; 509; 627). Radiation was found to cause a direct activation of hormone forming sites or indirectly by its action on cellular materials. Death ultimately was caused by absence in cells of sufficient material to respond to physiological factors of growth and differentiation.

The main action which radiation caused was a lowering of the metabolic activity in the organism. Irritants were able to effect hormonal activity and thus caused increased metabolic activity thereby giving protection. Carotenoid pigments have been implicated as protectants to situations of stress (293; 502). Lipids



and chelating agents tend to remove free radicals or metals and act as energy traps (415).

## MATERIALS AND METHODS

The organism used throughout these tests was a radiation resistant species tentatively named Micrococcus radiodurans (Strain RL).

The basal medium employed for growing the organism was tryptone-glucose-yeast extract supplemented with 0.3% methionine (TGYM). This medium was prepared as follows:

Tryptone - - - - -	5.0 g
Yeast Extract - - - -	1.0 g
Dextrose - - - - -	1.0 g
D L Methionine - - - -	0.3 g
Water - - - - -	1000.0 ml

The solid medium used for slant cultures and plate counts was prepared as follows:

Tryptone - - - - -	5.0 g
Yeast Extract - - - -	2.5 g
Dextrose - - - - -	1.0 g
D L Methionine - - - -	0.3 g
Agar - - - - -	15.0 g
Water - - - - -	1000.0 ml

Physiological saline (0.85% NaCl) was the suspension fluid of choice for bacterial cells or extracts of the cells. Phosphate buffer and Ringer's solution were not used because they were toxic. Glycerol or 95% ethanol was used when they were the only means of suspending the extracts.

The intraperitoneal (IP) route of injection was used in all the experiments unless otherwise indicated. The amount injected was either 0.5 ml or 1 ml, using a 1 ml tuberculin syringe fitted

with a 25 or 26 needle. Injections were made approximately four hours before irradiation or immediately after irradiation unless otherwise indicated. Multiple injections were given at 24 hour intervals before or after irradiation.

Only Swiss white mice, of the Webster strain were used throughout these studies. These were raised and maintained in the Microbiology Department, about four weeks old and weighed between 10 and 20 grams. Litter mates were desired, however, since each mother was limited to a maximum of eight mice, it was necessary in all cases to employ more than one litter. The mice were fed a diet of Purina Laboratory Chow having the following composition:

#### Guaranteed Analysis

Crude Protein not less than - - - -	23.0%
Crude Fat not less than - - - - -	4.5%
Crude Fiber not more than - - - - -	6.0%
Ash not more than - - - - -	9.0%

#### Ingredients

Meat and bone meal, dried skimmed milk, wheat germ meal, fish meal, animal liver meal, dried beet pulp, corn grits, oat middlings, soybean meal, dehydrated alfalfa meal, cane molasses, vitamin B 12 supplement, calcium pantothenate, choline chloride, folic acid, riboflavin supplement, brewers' dried yeast, thiamin, niacin, vitamin A oil, D activated plant sterol, vitamin E supplement, .5% defluorinated phosphate, .5% iodized salt, .075% ferric ammonium citrate, .02% manganese sulphate and a trace of zinc oxide.

A mixture of streptomycin, penicillin and terramycin was occasionally added to the drinking water in order to eliminate gastrointestinal tract infections, however, no significant difference was noted so the procedure was discontinued.





diameter of the field at the base of the machine was 6 cm and at the position of the cage it was 14 cm.

Variation of dose with height was determined using a Victoreen R Meter (767, p. 192) and the results are shown on Table I.

Per cent survivals at 650, 800 and 1000 R are shown on Table II. The survival rates obtained agree with values obtained by other workers (104). A dose of approximately 800 R, requiring 14 minutes exposure at a distance of 17 cm was used for all experiments unless otherwise indicated.

The radiation chamber consisted of a circular plastic fishing fly tackle box, 12 cm in diameter and 3 cm high, with eight compartments separated by glass slides. Holes were bored in the plastic cage to allow air to circulate. The cage was held stationary for the survey work, however, it was mounted on a rotating platform for work statistically controlled in order to assure that each mouse received an identical dose.

#### Method of preparation of whole cells

Broth and slants were inoculated with approximately 1 ml of culture. The broth cultures were shaken to distribute the cells and incubated at 35 - 37 °C. The cultures were then left undisturbed for varying lengths of time. The cells tended to settle to the bottom and within 2 to 3 days a small pale pink sediment of cells could be observed. As growth continued the supernatant turned brown and the sediment a darker pink. The supernatant fluid

Table I

Determination of Lethal Dose of X-Radiation  
for Webster Strain of Swiss White Mice

Exposure time in minutes	Approximate Roentgens given	% Survival after 30 days
11	650	83
14	800	6
18	1000	0

Table II

Variation of Dose with Distance  
from the X-ray Source

Height in cm	R/minute at center of the field	R/minute at 6 cm from center of field
3	199	*(1)
7	135	97
10	102	80
15	64	60
17	59	55
20	49	48
23	43	*

(1) measurement not performed



was poured off and the sedimented cells which remained were resuspended in 5 ml saline. These cells were used for IP injections, one half to one ml of the cell suspension in physiological saline was inoculated per mouse before or after irradiation exposure. A plate count determination of the saline suspension of cells was made for each injection.

Slant cultures were similarly incubated at 35 - 37 °C. The cultures were left for varying lengths of time and 5 ml of saline then added to each slant culture. The cells were scraped loose from the slant aseptically and suspended in sterile saline. One half ml of the saline suspension was injected IP into each mouse and a portion was plated to determine the number of cells present per ml.

#### Method of preparation of Cell Fractions

Approximately 12 grams of a 48 hour culture of wet packed R1 cells were suspended in 50 ml of physiological saline and disrupted by sonication (Model DF 101, 10 Kilocycle, 250 Watt). The preparation was then centrifuged at high speed and the resulting supernatant fluid injected into mice. The sediment was resuspended in 50 ml saline before injections, or further dilutions were made. One ml of either fraction or of the dilutions, was injected IP into mice before they were irradiated.

Five distinct layers were observed after centrifugation:

- I. Cloudy white top layer
- II. Clear pink cytoplasmic layer
- III. Darker pink top layer of sediment
- IV. Lighter pink middle layer of sediment
- V. Black lower layer of sediment

Efforts were made to separate these fractions mechanically and toxicity tests were performed on the crudely separated fractions.

The length of time that the cells were sonicated did not affect the quantity observed in each layer but it did affect the toxic properties of the fractions.

#### Method of preparation of Cellular Extracts

The pigment extraction method of Reimann and Eklund (647) was used with slight modification and is as follows. The R1 cells were grown for a minimum of 48 hours in the desired quantities. They were then removed by centrifugation in a Sharples Continuous flow centrifuge and resuspended in 5 ml absolute methanol per 700 ml original culture. The suspension was allowed to stand about 15 hours at 37 °C. Then 5 ml carbon disulfide (per 700 ml original culture) was added and the mixture allowed to stand at room temperature for 2 to 4 hours. The fractions were separated and the methanol portion filtered or centrifuged to remove cellular debris. The methanol was either removed by evaporation and dissolved in 5 ml of 95% ethanol ( per 700 ml original culture) or 95% ethanol was added without evaporating the methanol. The ethanol was diluted with saline and injected into mice before or after the mice were irradiated.

In an effort to determine whether the pigment was contained in the intracellular material or in the cell wall, the cells were first suspended in 0.15 M Phosphate buffer and varying amounts of

lysozyme added (4; 125). After the cells had stood overnight, they were centrifuged and pigments extracted from both supernatant and sediment.

Cellular extracts were also prepared and tried according to the following method. Approximately 3 grams of wet packed cells were divided into 0.5 g portions. The portions were suspended in 1 ml of different solvents and allowed to stand overnight. After 14 hours the suspensions were centrifuged and the supernatant fluid diluted with saline and injected into mice which had been irradiated. The solvents used and the dilutions made for injections were as follows:

- |                            |         |      |
|----------------------------|---------|------|
| 1. 2% Sodium Bicarbonate   | - - - - | 1:20 |
| 2. 3 N Hydrochloric Acid   | - - - - | 1:20 |
| 3. 1 N Sulfuric Acid       | - - - - | 1:20 |
| 4. Acetone                 | - - - - | 1:20 |
| 5. Petroleum Ether         | - - - - | 1:40 |
| 6. 1 N Potassium Hydroxide | - - - - | 1:20 |
| 7. Methanol                | - - - - | 1:20 |
| 8. N Butanol               | - - - - | 1:20 |
| 9. Carbon Tetrachloride    | - - - - | 1:40 |
| 10. Chloroform             | - - - - | 1:20 |

Method of Schmidt and Thannhauser (694) for the extraction of Deoxyribonucleic Acid.

1. Three grams of wet packed R1 cells were suspended in 50 ml of 5-10% Trichloroacetic Acid.

2. The suspension was shaken for 12 hours and either centrifuged and the supernatant fluid used for subsequent work or the cell suspension was used as such.

3. Fifty ml of 95% ethanol was added to either the suspension or the supernatant fluid and shaken for six hours.



4. The mixture was then centrifuged and the supernatant fluid removed.

5. The sediment was suspended in 50 ml saline and diluted 1:20 before injecting into mice.

Method of Kirby (439) for the extraction of Deoxyribonucleic Acid.

1. Fourteen grams of wet packed cells were suspended in 50 ml of 6% ammonium salicylate.

2. The mixture was shaken for 17 hours and then 50 ml of 5% phenol was added and the mixture centrifuged at a low speed.

3. The supernatant fluid was removed and 50 ml (1:1) petroleum ether - ethanol added.

4. The solution was shaken for 5 hours and centrifuged and the supernatant fluid removed.

5. The sediment was suspended in 50 ml saline and diluted 1:20 before injecting into mice.

Method of Marmur (519) for the extraction of Deoxyribonucleic Acid.

1. Fourteen grams of wet packed cells were suspended in 50 ml (1:1) sodium chloride - ethylenediaminetetra-acetate.

2. The suspension was centrifuged and the sediment resuspended in 25 ml of the sodium chloride - ethylenediaminetetra-acetate.

3. To this suspension 0.1 g of lysozyme was added and the mixture incubated at 37 °C for 11 hours.

4. The amount of preparation was then measured and enough 5 M perchlorate added to make a 1 M solution.

5. An equal volume of (1:1) chloroform - isoamyl alcohol was added and the mixture shaken for 30 minutes.

6. The mixture was then centrifuged at a low speed for 5 minutes to obtain three layers.

7. The top layer was removed and 2 volumes of 95% ethanol layered over it.

8. A glass stirring rod was introduced into the solution and stirred slowly.

9. A stringy coagulate, which attached itself to the stirring rod, was removed and placed in 25 ml saline. The suspension was diluted 1:20 before injecting into mice.

Method of Miescher (537; 538) for the extraction of Nuclear Material.

1. Fourteen grams of wet packed cells were suspended in 50 ml 1 N Sulfuric acid and left to stand 12 to 14 hours at room temperature.

2. Fifty ml of petroleum ether were then added and the mixture shaken for 30 minutes.

3. The ether phase was separated and left at room temperature for 24 to 48 hours, during which time most of the ether evaporated.

4. To the amount which remained 50 ml of saline was added and diluted 1:20 before injecting into mice.

Mouse spleen and liver were treated by the above method. The mice were anesthetized with chloroform before the organs were removed. The organs were minced with a pair of scissors and 10 ml

1 N Sulfuric acid per organ was added. The suspension was left on a shaker for 12 to 14 hours, in an effort to make an unicellular suspension. Petroleum ether and saline were used in the same manner as with the microorganisms above, using 10 ml of each per organ.

Solvents used to extract lipid-pigment complex from deoxyribonuclear - protein complex prepared by Miescher's method were acetone, glycerol, methanol, ethanol, carbon tetrachloride, chloroform, benzene, secondary butyl alcohol, isoamyl alcohol and normal propyl alcohol.

Method of Vernon (832) for the extraction of cytochromes

1. Fourteen grams of wet packed cells were suspended in 50 ml water and heated.
2. Twenty ml of 0.5 N Trichloroacetic acid was added and the solution was kept at 50 °C for 5 minutes.
3. The suspension was left at room temperature 5 minutes and then neutralized with saturated sodium hydroxide using a pH meter to determine the neutral point.
4. Five ml of ammonium hydroxide were added and the suspension centrifuged for 20 minutes.
5. Fifteen ml of 0.5 N trichloroacetic acid was added to the suspension of supernatant fluid and allowed to stand 28 hours at room temperature, then centrifuged and the supernatant fluid discarded.
6. The sediment presumed to be cytochrome was suspended in saline and injected into mice.



#### Method of extraction of Pigment-lipid complex

1. Nine liters of TGYM culture were centrifuged and approximately 14 grams of packed cells obtained.
2. These cells were suspended in 50 ml 1 N Sulfuric acid.
3. The suspension was allowed to stand overnight at room temperature.
4. The suspension was dialyzed against distilled water to remove excess hydrogen ions.
5. The suspension was removed when it reached a pH of 6 to 7.
6. One hundred mls of secondary butyl alcohol were added with stirring.
7. The mixture was filtered through one thickness of Whatman # 1 filter paper.
8. The water phase was removed in a separatory funnel.
9. The alcohol was evaporated to dryness with swirling motion on a hot plate in a hood.
10. The dried material was used per se or separated further on a Silicic Acid column.
11. The dried material was suspended in 2 ml Glycerol and diluted with physiological saline to 10 ml.
12. Injections of 0.5 ml were made intraperitoneally into mice.

#### Method of separation of Pigment-lipid complex on Silicic Acid Column (107; 607)

The sample prepared as indicated above was resuspended in 5 ml

of chloroform and then absorbed on a Silicic Acid Column. A 50 ml graduated biuret, used as a column, was packed with a suspension of anhydrous silicic acid in chloroform. Approximately 20 grams of silicic acid (dried in an oven a minimum of 24 hours) was used per 50 ml biuret. The fractions obtained were as follows:

- I. Colorless - which was removed with chloroform only
- II. Colored - removed slowly with chloroform only
- III. Colorless - which was removed with chloroform-methanol
- IV. Colored - removed with methanol only

Fraction II was never present in large amounts and was pooled with fraction I for much of the work.

Method of saponification of Pigment-lipid complex (375, p. 1122-1123)

The sample prepared as indicated above was dried and resuspended in 150 ml 10% methanolic potassium hydroxide and allowed to stand at 32 °C overnight. After this treatment the non-saponifiable material was extracted with petroleum ether. The remaining fluid was neutralized with 6 N hydrochloric acid and the saponified material extracted with petroleum ether. The petroleum ether extract was evaporated to dryness and the residue resuspended in 2 ml glycerol. The glycerol suspension was diluted with saline to 10 ml volume and 0.5 ml injected before or after irradiation.

Method of methylation of Pigment-lipid complex (90)

The sample prepared as indicated above was dried and suspended in 20 ml diethyl ether and 20 ml of sodium methoxide solution (0.5g sodium per 200 ml absolute methyl alcohol). This was left at room

temperature overnight and the methyl esters recovered by diluting the reaction mixture with 100 ml of distilled water, acidifying with dilute hydrochloric acid and extracting the esters with three 50 ml portions of diethyl ether. The ether extract was washed with two 50 ml portions of distilled water and dehydrated over anhydrous sodium sulfate. The solvent was removed under vacuum before suspending in 2 ml glycerol and 8 ml saline and injecting into mice before or after irradiation. The same procedure was used for making the methyl esters which were separated into their fatty acid ester components on the gas chromatograph.



## RESULTS

## Results of Whole Cell injections

One half to one ml of a R1 cell suspension containing  $1 \times 10^6$  to  $1 \times 10^8$  cells was not toxic when injected IP into mice. Similarly 0.25 to 0.50 ml of equivalent concentrations were not harmful when administered by the intravenous route. Sarcina lutea, which was used as a control, in concentrations comparable to those of R1 was also non-toxic by IP injection, however, Escherichia coli was highly pathogenic when injected into mice under conditions similar to the above and its use as a control was abandoned.

Increased survival to a lethal irradiation dose was observed when R1 cells ( $1 \times 10^6$  to  $1 \times 10^8$  cells per ml) were injected IP into mice approximately four hours before or immediately following irradiation. Results in Table III show that cells obtained from slant or broth cultures were most effective if they were between three and eight days old, although the results are not conclusive.

There appeared to be little difference between cells prepared from broth or slant cultures.

As shown on Table III, whole cells obtained from broth or slant cultures appeared to be more protective than therapeutic when injected into mice. It was also demonstrated that multiple injections of whole cells from either broth or slant cultures did not increase the survival of mice to lethal doses of irradiation. Multiple injections were tried both before or after irradiation and given at 24 hour intervals.

Table III  
Effect of Age of Culture on protection and therapy of lethally irradiated mice

% Survival after 21 days (1)								
Age of Culture in days	IP Injection 4 hours before irradiation (2)				IP Injection immediately after irradiation			
	Slant Culture		Broth Culture		Slant Culture		Broth Culture	
	RI (3)	S. L. (4)	RI	S. L.	RI	S. L.	RI	S. L.
7	25	0	* (5)	0	50	0	25	0
6	67	0	*	0	75	0	25	0
5	15	0	0	0	0	0	0	0
4	26	0	0	0	0	0	25	0
3	42	0	8	0	50	0	25	0
2	23	0	17	0	0	0	0	0
1	0	0	45	0	0	0	50	0
Control (Saline)	6				0			

- (1) A minimum of eight mice were used per group  
 (2) IP - Intraperitoneal  
 (3) RI - Radiation Resistant Micrococcus  
 (4) S. L. - Sarcina lutea  
 (5) \* - Test was not performed

As shown on Table III, Sarcina lutea cells when injected into mice under conditions similar to those used for R1 did not protect or show any therapeutic effect.

#### Results of Cellular Fraction Injections

Cells were fractionated as indicated in the method section, page 62. All sonicated fractions, when injected IP into mice were non-toxic except III, IV and V. These fractions were non-toxic when a 1:6 or greater dilution of the stock solution was made. An increase in the length of sonication time decreased the lethal effect of the pooled sediment fractions, III, IV and V.

Table IV shows that the best results were obtained using the pooled sediment fractions, with a lesser effect in the pooled supernatant fractions, I and II. Various dilutions of the sediment fractions were tested and the highest concentration which was not toxic gave the best results.

Sonication of the bacterial cells causes the release of cytoplasmic material into the suspending media. The supernatant fluid was considered cytoplasmic in origin, while the sediment was composed of the cell wall, nuclear material and other particulate material. Pigmentation was found in both supernatant and sediment fractions. It would appear that the agent responsible for protection predominates in the sediment fraction.

#### Results of Cellular Extraction Injections

No increase in survival of mice to a lethal dose of irradiation was obtained when pigments were injected, which had been extracted



Table IV

Protective effect of R1 Cell Fractions on Mice  
exposed to lethal effects of irradiation

Preparation	% survival 21 days (1)
Supernatant fluid (I and II) (2)	33
Sediment Fraction (III, IV and V) diluted 1:50	0
Sediment Fraction (III, IV and V) diluted 1:300	67
Sediment Fraction (III, IV and V) diluted 1:400	50
Sediment Fraction (III, IV and V) diluted 1:500	17
Saline Controls	11

(1) a minimum of eight mice were used per group

(2) I. Cloudy white top layer

II. Clear pink cytoplasmic layer

III. Darker pink top layer of sediment

IV. Lighter pink middle layer of sediment

V. Black lower layer of sediment

Fractions were prepared as indicated on page 62

from the whole bacterial cell with ethanol and heat. A slight therapeutic effect of the pigment was noted when cells were first sonicated and then the pigment extracted (Table V).

The pigments extracted with carbon disulfide by the method of Reimann and Eklund (647) were toxic. The methanol soluble pigments were not toxic, however, omitting the carbon disulfide extraction step of the method did not increase survival significantly. Results are shown on Table V.

Mice injected IP with pigment extracted from sonicated or lysozyme treated cells showed survival not significantly different from that of pigment extracted from whole cells. Multiple injections of the methanol soluble pigments was no better than a single injection.

Pigments extracted from Sarcina lutea according to the above procedure of Reimann and Eklund gave approximately the same results as pigments extracted from R1, as shown on Table V. It would appear that the therapeutic effect shown by injection of carotenoid pigments is non-specific.

It was observed that the pigments diffused into the medium as the cultures aged. As shown on Table VI increased survival was observed when mice were injected with 1 ml of such medium. However, injection of control broth under similar conditions was not effective.

Table V

Effect of R1 Cellular Extracts on Mice  
exposed to lethal effects of irradiation

Extractant used	When Injected	% Survival after 21 days (1) R1 <u>Sarcina lutea</u>
Ethanol (Sonicated cells)	B (2)	0 * (4)
	A (3)	25 *
Methanol (whole cells)	B	0 0
	A	28 25
(Cytoplasm)	B	13 17
	A	8 0
(Cell Wall)	B	11 0
	A	7 0
Saline	A	0 *
Water	A	0 *
Sodium Bicarbonate	A	0 *
Hydrochloric Acid	A	0 *
Sulfuric Acid	A	50 *
Acetone	A	0 *
Petroleum Ether	A	50 *
Potassium Hydroxide	A	0 *
Chloroform	A	0 *

(1) A minimum of eight mice were used per group

(2) Injections were performed 4 hours before irradiation

(3) Injections were performed immediately after irradiation

(4) Test was not performed



Table VI

Protective effect of pigments in medium  
on Mice exposed to lethal effects of irradiation

Age of Culture (1) in days	% survival after 21 days (2)
0 (Control)	0
1	33
2	67
3	33
4	50
5	17

(1) Injections were made four hours before irradiation

(2) A minimum of eight mice were used per group

As indicated under methods (page 64) several other cellular extracts were obtained. These were tested for their radiation protection properties. Sulfuric acid and petroleum ether extracts were the most effective protectants (Table V).

#### Results of Nuclear Material Injections

Sulfuric acid and petroleum ether extractable materials were investigated further. The original method of Miescher (537; 538) for extracting the nuclear portion of cells was by acid treatment and ether extraction. After extraction the nuclear material was injected into mice immediately after being exposed to 800 R. A survival of approximately 25% was obtained, which was equivalent to the extract from approximately 0.1 g of cells. Doubling the concentration increased the survival, however, higher concentrations were toxic.

It was felt that the residual ether was toxic since it was possible that it had not been completely removed from the nuclear material before dilution with saline. The protective action, however, was destroyed when the material was evaporated to dryness before suspending in saline. It was possible that the material imparting protection was destroyed in the drying step.

Another reason for the toxicity could be the low pH of the nuclear portion. Apparently some of the sulfuric acid was soluble in the ether giving the final preparation a pH of about 2. A suspension of the nuclear portion of R1 was dialyzed to remove the excess hydrogen ions and thereby decrease the acidity. The

maximum survival noted with this preparation was slightly better than without dialysis.

Two fractions were obtained when whole cells were treated with lysozyme (4; 125); the cell wall debris and the cytoplasmic material. It appeared that the nucleic acid - protein complex isolated from the cell wall debris (27; 685) was a more effective protectant than that from the cytoplasmic material.

The effect of the time of injection of nuclear material with respect to irradiation was investigated ( Table VII). The most effective injections were made five hours before irradiation or within a 24 hour period following irradiation.

Multiple injections in a series of four, three and two, 24 hours apart, using material prepared by Miescher's method did not show a marked advantage over a single injection ( Table VIII).

Nuclear material extracted from Sarcina lutea and mouse spleen and liver gave results similar to R1, when tested under comparable conditions. Deoxyribonucleic acid prepared by other methods ( pages 64-66 ) was studied and survival compared with that of Miescher's method (Table IX). The protection observed was probably due to nucleic acids generally and not specifically to R1 nucleic acids.

Amino acid composition of the nuclear protein was determined as shown on page 113. A mixture of the various amino acids was tested for its effect on mice before lethal irradiation. Results



Table VII

Effect of time of injection of Nuclear Material from R1 on  
Mice exposed to lethal dose of irradiation

Time in hours	When injected	% survival after 21 days(1)
96	B (2)	0
72	B	0
48	B	0
24	B	25
5	B	50
3	B	0
1	B	0
0.25	B	0
1	A (3)	0
2	A	0
4	A	0
6	A	25
24	A	25
48	A	0
72	A	0
96	A	0

- (1) A minimum of eight mice were used per group  
 (2) Injections were made before mice were irradiated  
 (3) Injections were made after mice were irradiated

Table VIII

Effect of Multiple Injections of Nuclear Material from  
R1 on Mice exposed to lethal dose of irradiation

Number of Injections (1)	When injected	% Survival after 21 days (2)
4	B (3)	0
3	B	20
2	B	20
1	B	46
1	A (4)	23
2	A	46
3	A	20
4	A	0

- (1) Injections performed at 24 hour intervals  
 (2) A minimum of eight mice were used per group  
 (3) The final injection was 24 hours before irradiation  
 (4) The first injection was immediately after irradiation

Table IX

Therapeutic effect of injections of nuclear material from  
R1, Sarcina lutea, Mouse spleen and liver on  
mice exposed to a lethal dose of irradiation

Method of Preparation	% Survival after 21 days (1)			
	R1	S. L. (2)	Mouse	
			Liver	Spleen
Miescher	29	12	25	12
Kirby	16	0	* (3)	*
Schmidt and Thannhauser	0	25	*	*
Marmar	8	0	*	*

(1) A minimum of eight mice were used per group

(2) Sarcina lutea

(3) Test was not performed



indicated that combinations of serine or lysine plus alanine and phenylalanine provided a slight but significant protection.

The protection shown by the nuclear material was destroyed completely when exposed to gamma radiation or by heating for five minutes in a steamer.

#### Results of Cytochrome injections

The method of isolation of the nuclear protein is similar to that for the isolation of cytochromes. Cytochromes were isolated and found to be less effective than nuclear protein. When the nuclear protein isolation method was varied so as to duplicate the cytochrome extraction a slight protection was obtained.

#### Results of Lipid-Pigment Complex injections

The nuclear protein complex was mixed with warm ethanol to precipitate the protein and nucleic acid and release the pigment - lipid complex. The nuclear protein so precipitated was then injected into mice in various concentrations either before or after they had received 800 R. The precipitates were ineffective, however, the ethanol extracts retained some activity when administered before irradiation.

Toxicity of the precipitated nuclear protein and supernatant material was reduced if it was dialyzed before extraction with ethanol. All subsequent extractions were made with dialyzed nuclear protein complexes.

The warm ethanol appeared to denature the protein causing inactivation. Efforts were made to find another solvent for

extracting the pigment lipid complex from the nuclear protein without heat. The effective solvents used were acetone, ethanol, secondary butyl alcohol and normal propyl alcohol. The complexes so extracted, as well as the decolorized sediment were injected into mice before or after irradiation. The secondary butyl alcohol extracts, as shown on Table X, were by far the best protectants.

In order to remove the secondary butyl alcohol from the preparation before dilution with saline the extract was evaporated carefully on a hot plate. It was suggested that this procedure might inactivate some of the protective factor, therefore, evaporations were performed under a vacuum over a hot water bath. As shown on Table XI similar results were obtained by either method.

Sulfuric acid was used for initial suspension of the bacterial cells. Replacing the sulfuric acid with hydrochloric acid did not change the protective nature of the preparations as shown on Table XI.

The time of injection with regard to irradiation was investigated by varying time before and after X-ray exposure. Table XII indicates that the closer injections are made to the time of irradiation, either before or after, the greater the effect.

Five, four, three and two injections of the lipid - pigment complex were used to increase the survival of mice to lethal doses of irradiation. Secondary butyl alcohol extracts and a pool of

Table X

Effect of Various Extracts of R1 Nuclear Material  
in mice exposed to lethal dose of irradiation

Solvent	Extract	When injected	% Survival 21 days (1)
Ethanol	Pigment-lipid	B (2)	25
		A (3)	0
	Nuclear Protein	B	0
		A	0
Acetone	Pigment-lipid	B	0
		A	0
	Nuclear Protein	B	0
		A	0
N Propyl Alcohol	Pigment-lipid	B	25
		A	0
	Nuclear Protein	B	0
		A	0
Sec. Butyl Alcohol	Pigment-lipid	B	25
		A	25
	Nuclear Protein	B	0
		A	0
Controls - Glycerol:Saline		B	12
		A	6

- (1) A minimum of eight mice were used per group  
 (2) Injections were performed 4 hours before irradiation  
 (3) Injections were performed immediately after irradiation



Table XI

Effect of heat or acid on protective and therapeutic properties of Lipid-Pigment Material of R1

Acid	Method of removal of alcohol	When injected	% Survival after 21 days (1)
Sulfuric Acid	Vacuum	B (2)	50
		A (3)	12
Hydrochloric Acid	Atmospheric Pressure and heat	B	12
		A	25
Hydrochloric Acid	Vacuum	B	25
		A	25

- (1) A minimum of eight mice were used per group  
 (2) Injections were performed 4 hours before irradiation  
 (3) Injections were performed immediately after irradiation

Table XII

Effect of time of injection of Pigment-Lipid material from R1  
on Mice exposed to a lethal dose of irradiation

Time in hours	When injected	% Survival after 21 days (1)
96	B (2)	0
72	B	12
48	B	0
24	B	0
6	B	25
6	A (3)	62
24	A	50
48	A	25
72	A	25
96	A	12

- (1) A minimum of eight mice were used per group  
 (2) Injections were made before mice were irradiated  
 (3) Injections were made after mice were irradiated

extracts which had been separated by means of a Silicic Acid Column (see below) were used. Survival obtained with various multiple injections are shown on Table XIII. It would appear that the optimum protection is obtained with two injections. Table XIV shows the results obtained using 40 mice per group in order to insure statistically valid results.

Lipid material from Sarcina lutea, mouse spleen and liver was tested for protective effect. Per cent survivals obtained from these preparations when injected into mice before or after irradiation are shown on Table XV. Mouse lipids isolated from spleen and liver gave more protection than that from microorganisms. These results might give an explanation for the lipid mobilization which is observed in animals soon after irradiation (258). The lipid mobilization might be the way animals protect themselves and by injecting lipid material the protection is increased.

The secondary butyl alcohol extract was crudely separated into its constituent parts by plating on a Silicic Acid Column with chloroform and eluting with chloroform-methanol solutions. The protection obtained by the various fractions are shown on Table XV. The first material eluted from the Silicic Acid Column was presumed to be neutral fats. The second was bound lipids and the last slower portion was pigments.

The fatty acids, isolated by saponification, were injected into mice and the protection compared with that obtained with the



Table XIII

Effect of Multiple injections of Lipid-Pigment material  
on Mice exposed to a lethal dose of irradiation

Number of Injections (4)	When Injected	% Survival after 21 days (1)	
		B.S. (2)	Fractions S.A.C.(3)
5	B (5)	25	37
4	B	0	25
3	B	25	25
2	B	50	0
1	B	50	12
1	A (6)	0	25
2	A	75	25
3	A	25	50
4	A	37	25
5	A	37	25

- (1) A minimum of eight mice were used per group  
 (2) Secondary Butyl Alcohol soluble Lipid-Pigment Material before separation on Silicic Acid Column  
 (3) Secondary Butyl Alcohol soluble Lipid-Pigment Material after separation on Silicic Acid Column - a pool of fractions I, II and III - see page 69  
 (4) Injections performed at 24 hour intervals  
 (5) The final injection was 24 hours before irradiation  
 (6) The first injection was immediately after irradiation

Table XIV

Effect of two injections of Lipid-Pigment Material on Mice  
exposed to a lethal dose of irradiation using a  
Statistical valid population of mice

Fraction	When injected	% Survival After 21 days (1)
Before Separation (2)	B (3)	42
	A (4)	55
Silicic Acid Column I and II (5)	B	35
	A	32
Silicic Acid Column III (6)	B	17
	A	22

- (1) A minimum of forty mice were used per group
- (2) Secondary Butyl Alcohol soluble Lipid-Pigment Material before separation on Silicic Acid Column
- (3) Injections performed 48 and 24 hours before irradiation
- (4) Injections performed immediately and 24 hours after irradiation
- (5) Secondary Butyl Alcohol soluble Lipid-Pigment Material after separation on Silicic Acid Column - a pool of Fractions I and II - see page 69
- (6) Secondary Butyl Alcohol soluble Lipid-Pigment Material after separation on Silicic Acid Column - Fraction III - see page 69

Table XV

Effect of various separated Lipid-Pigment Material on mice  
exposed to a lethal dose of irradiation

Fraction	When Injected	% Survival after 21 days	
		(1) R1	(2) S.L.
Lipid-Pigment Material	B (3)	20	0
	A (4)	25	25
Silicic Acid Column Fraction I - page 69	B	30	0
	A	20	0
Silicic Acid Column Fraction II - page 69	B	12	* (5)
	A	0	*
Silicic Acid Column Fraction III - page 69	B	17	25
	A	8	0
Silicic Acid Column Fraction IV - page 69	B	8	*
	A	0	*
Saponified Lipid-Pigment Material	B	20	*
	A	15	*
Methyl Ester Derivatives of Lipid-Pigment Material	B	20	*
	A	15	*
Mouse Liver Pigment- Lipid Material	B	13	
	A	37	



Table XV, continued

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Mouse Spleen Pigment-		
Lipid Material	B	37
	A	56
Controls - Glycerol:Saline	B	12
	A	6

---

- (1) A minimum of eight mice were used per group
- (2) Sarcina lutea
- (3) Injections performed 4 hours before irradiation
- (4) Injections performed immediately after irradiation
- (5) Test was not performed

first fraction eluted from the Silicic Acid Column. A small but significant effect was noted (Table XV). Carotenoid pigments are non-saponifiable material, thus the material injected was presumed to be the fatty acids (saponifiable material).

The methyl esters of the fatty acids were injected into mice before subjecting them to a lethal dose of irradiation and a slight but significant survival was obtained (Table XV). Therapeutic treatment was less effective than protective treatment.

#### Controls

The materials which were used for suspending the fractions of R1 in order to inject them into mice, were tested for their ability to protect mice from a lethal dose of irradiation. Results of the various solvents tested are found in Table XVI.

Blood slides were taken from at least one animal of most of the groups. These were stained with Wright's stain and observed. No protection of leukocytes was obtained with any of the fractions tested (750).

Table XVI

Controls - Solvents used to suspend fractions  
for injection into mice

Treatment	When Injected	% Survival after 21 days (1)
No injections		6
Males		3
Females		3
Saline	B (2)	6
	A (3)	0
TGYM medium	B	0
95% Ethanol and saline	B	0
	A	6
Glycerol and saline	B	12
	A	6

- 
- (1) A minimum of eight mice were used per group  
 (2) Injections performed 4 hours before irradiation  
 (3) Injections performed immediately after irradiation



## METHODS OF CHEMICAL ASSAY

## Spectral Analysis

A Beckman Model #IR5 Infrared Spectrophotometer with a wavelength range of 2 to 16 microns was used for analysis of the infrared spectrum of the various extracts studied. The moisture in the extracted sample was first partially removed by evaporation at room temperature and then any remaining moisture removed by vacuum desiccator with Drierite ( $\text{CaSO}_4 \cdot \frac{1}{2} \text{H}_2\text{O}$ ). A minimum of 24 hours later the sample was removed and suspended in one to two drops of Nujol. After a uniform emulsion was made it was placed between two sodium chloride crystals and clamped in an adapter which was held stationary in the instrument. A sample of nujol was run as control.

A Cary Recording Spectrophotometer, Model #11 was used to determine the wave-length in the range of 3000 angstroms to 8000 angstroms. Samples were suspended in saline and diluted so that a suitable reading could be made. The instrument was blanked against saline for all determinations. Quartz glass cells were used throughout the measurements.

A Cary Recording Spectrophotometer, Model #11 was used in the range 2000 to 3000 angstroms. Samples were suspended as above for visible spectra and the instrument blanked against saline for all determinations.

### Preparation of sample for Inorganic element analysis

The wet oxidation was performed by mixing the sample of nuclear or lipid-pigment material obtained from a nine liter culture with 5 ml of concentrated nitric acid and 2 ml of concentrated sulfuric acid. The mixture was heated gently and evaporated to white fumes of sulfuric acid with a yellow or colorless fluid remaining. The mixture was cooled and 2 to 3 ml of water added, and the solution again evaporated to white fumes. The mixture was cooled and 10 ml of water added and heated to boiling. This mixture was diluted to 25 ml and used for element analysis. (686, p. 384-385).

For sodium fusion a pyrex tube, 6 by 3/4 inches, was supported vertically in a clamp. A small cube of sodium was placed in the tube and heated until the sodium vapors rose 4 to 5 cm in the tube. A small amount of the substance was dropped onto the sodium and the tube heated to redness for about 1 minute. The tube was allowed to cool and then 3 to 4 ml of methyl alcohol added to decompose the unreacted sodium. Then 8 ml of water was added and the solution boiled gently for a few minutes. The solution was filtered and the clear, colorless filtrate was used for element analysis (836, p. 1040-1041).

### Element Analysis

One ml of sample in which the phosphorous was to be determined, was diluted with saline or water in a tube. A control tube was run containing one ml of saline or water, depending on which was

used. To the 1 ml sample or control 4 ml 10% trichloroacetic acid was added, followed by 1 ml molybdate II reagent, and 0.5 ml amidol reagent and 3.5 ml distilled water. The per cent transmittance was then read on a Leitz-Photoelectric Colorimeter with red filter. Molybdate II reagent was made by dissolving 2.5 gms ammonium molybdate in 20 ml distilled water; adding 30 ml 1 N sulfuric acid and diluting to 100 ml with distilled water. Amidol reagent is prepared by dissolving 10 grams sodium bisulfite in 50 ml of water and adding 0.5 gms amidol (2,4 diaminophenol dihydrochloride) (375, p. 631).

One ml of sample in which the nitrogen was to be determined was diluted with saline or water in a tube, a control tube containing 1 ml of saline or water depending on which was used. To the 1 ml sample or 1 ml control, 1 ml Nessler's reagent and 5 ml distilled water were added. The per cent transmission of the resulting solution was determined on a Leitz Photo-electric Colorimeter with a blue green filter. Nessler's reagent was prepared by dissolving 6.8 gms of Nessler's compound in 10 ml distilled water; adding 85 ml 10% sodium hydroxide and diluting to 100 ml with water (375, p. 878-879).

One ml of sample, in which the sulfur was to be determined was diluted with saline or water in a tube. The control tube contained 1 ml of saline or water depending on which was used. To the 1 ml sample or control was added 1 ml of potassium hydroxide solution, 1 ml N-ethyl-maleimide solution and 4 ml water. The per



cent transmittance was then read on a Leitz Photoelectric Colorimeter with a blue filter. The potassium hydroxide solution was prepared by dissolving 1.4 grams potassium hydroxide in 100 ml isopropanol and shaking for approximately 30 minutes. The N-ethylmaleimide solution was made by dissolving 0.625 gms N-ethylmaleimide in 100 ml isopropanol (78).

One ml of sample in which the amount of protein was to be determined, was diluted with saline or water in a tube. A control tube contained 1 ml of saline or water depending on which was used. To the 1 ml sample or control was added 1 ml Biuret solution, 1 ml 23% sodium sulfate and 4 ml water. The per cent transmittance was then read on a Leitz Photoelectric Colorimeter with a red filter. Biuret solution was prepared by dissolving 1 gm sodium potassium tartrate,  $1\frac{1}{2}$  ml 20% copper sulfate solution and 0.5 gms potassium iodide in 44 ml of 0.2 N sodium hydroxide and diluting to 111 ml with 0.2 N sodium hydroxide. (375, p. 927).

Two ml of sample in which the iron was to be determined, was diluted with saline or water in a tube. A control tube contained 2 ml of saline or water, depending on which was used. To the 2 ml sample or control was added 2 ml of 20% potassium thiocyanate and 2 ml (1:1) dilution of hydrochloric acid and water. The per cent transmittance was then read on a Leitz Photoelectric Colorimeter with a blue filter (685, p. 363-375).

Two ml of oxidized preparation in which calcium was to be determined, was stirred with 1 ml 4% ammonium oxalate until a

precipitate occurred. The precipitate was separated by sedimentation and dissolved in 2 ml of 1 N sulfuric acid. The resulting solution was titrated against potassium permanganate. The amount of potassium permanganate used was an indication of calcium present (375, p. 644-645).

To 5 ml of oxidized preparation in which molybdenum was to be determined, was added 1 ml of ferrous ammonium sulfate solution, 3 ml of stannous chloride solution, 3 ml of 10% potassium thiocyanate and 10 ml amyl alcohol. The solution was blue-black when molybdenum was present. Ferrous ammonium sulfate solution was made by dissolving 0.5 g in 50 ml of 0.2 N sulfuric acid. The stannous chloride solution was prepared by dissolving 1 gm in 10 ml of 1 N HCl (686, p. 468).

One ml of oxidized sample in which copper was to be determined, was mixed with one ml of dithizone and 5 ml of water. The carbon tetrachloride phase turned from green to brown in the presence of copper. Dithizone solution was a 1:100 dilution of dithizone in carbon tetrachloride (686, p. 317). Since dithizone also gives a color with other oxidizing agents an alternate method was used: 2 ml of sample which had been oxidized with sulfuric acid and nitric acid in the wet state was added to 5 ml of 20% ammonium citrate solution. To this mixture was added 1.0 ml of 0.1% sodium diethyldithiocarbamate and 5 ml of carbon tetrachloride. The carbon tetrachloride phase became brown if copper was present (686, p. 300).

Two ml of oxidized sample in which aluminum was to be determined was diluted with sulfuric acid to 5 or 6 N, approximately 2.5 ml of concentrated sulfuric acid, to make 20-25 ml. This solution was then treated with 2.5 ml of 6% cupferron solution and shaken vigorously for 40 seconds with 10 ml of chloroform. The chloroform layer was removed and the aqueous phase was washed with 5 ml of chloroform to displace the drop of cupferrate solution on the surface of the aqueous layer. The aqueous solution was next shaken for 30 seconds with 5 ml of chloroform which was drawn off. Then 0.5 ml of 6% cupferron solution was added and the extraction made successively with a 10 ml portion and two 5 ml portions of chloroform. The solution was transferred to a 100 ml erlenmeyer flask and made alkaline to litmus paper with 10 N ammonia. A 5 N hydrochloric acid was added dropwise until the solution was acid and then 5.0 ml in excess (686, p. 160).

The sample, once it had been extracted, was then diluted to 100 ml and 20 ml of the solution transferred to a 100 ml flask and 30 ml of water, 1.0 ml of 5% gum sandarac solution, 5.0 ml of ammonium acetate buffer and 2.0 ml of 0.2% ammonium aurintricarboxylate added. The mixture was then heated to boiling in a water bath and boiled for 5 minutes, then cooled to room temperature and 4.0 ml of ammonium borate solution added. A red color was produced if aluminum was present. Reagents included ammonium acetate buffer, made by dissolving 156 gms ammonium acetate and 108 gms ammonium chloride in 500 ml water, and ammonium borate solution, made by



dissolving 93 gms boric acid in 66.7 ml ammonium hydroxide and 933.8 ml water (686, p. 146-149).

Two ml of oxidized sample in which magnesium was to be determined, was mixed with 1 ml of molybdate I reagent and diluted with 5 ml water. One-half ml aminonaphtholsulfonic acid reagent and 2 ml water were added and the solution examined for a blue color. Molybdate I reagent was made by dissolving 25 gms of reagent grade ammonium molybdate in about 200 ml water. Five hundred ml of 10 N sulfuric acid was placed in a 1 liter volumetric flask and the molybdate solution was added, and diluted with washings to 1 liter with water. Aminonaphtholsulfonic acid reagent was prepared by placing 195 ml of 15% sodium bisulfite solution in a glass stoppered cylinder, adding 0.5 gms of 1,2,4 aminonaphtholsulfonic acid and 5 ml of 20% sodium sulfite. The cylinder was then stoppered and shaken until the powder was dissolved (375, p. 648-649).

Two ml of oxidized sample in which vanadium was to be determined, was diluted with 7.5 ml water and 2 to 3 drops of 2.5% 8 hydroxyquinoline solution. A blackish color would appear if vanadium was present (686, p. 615).

Two ml of oxidized sample in which cobalt was to be determined, was mixed with 1.0 ml of 0.2M citric acid solution and 1.2 ml of phosphate boric acid buffer. Then exactly 0.5 ml of Nitroso R salt solution was added with stirring and the solution was boiled for 1 minute in a water bath. One ml of concentrated nitric acid was then added and the solution boiled for 1 minute in a water bath.

A red color denoted the presence of cobalt. The phosphate boric acid buffer was made by dissolving 6.2 gms of boric acid and 35.6 gms of disodium phosphate dehydrate in 500 ml 1.0 N sodium hydroxide and diluting to 1 liter. The nitroso R salt solution was a 0.2% aqueous solution (686, p. 287).

Two ml of oxidized sample in which antimony was to be determined, was mixed with 8 ml of 1 M phosphoric acid and 5.0 ml of 0.02% aqueous Rhodamine B solution. Ten ml of benzene was added and the mixture shaken. If the pink color went into the benzene layer antimony was present (686, p. 165).

Two ml of oxidized sample in which arsenic was to be determined was diluted to 15 ml with water. To this solution was added 3.0 ml ammonium molybdate solution and 2.0 ml of hydrazine sulfate (0.5%). This was mixed thoroughly and heated in a water bath at 90-100°C for 10 minutes. A blue color indicated the presence of arsenic. Ammonium molybdate solution was prepared by dissolving 1.0 gms of ammonium molybdate in 100 ml of 1:9 sulfuric acid and adding 50 ml of 1 N hydrochloric acid (686, p. 190).

Two ml of oxidized sample in which nickel was to be determined, was diluted to 12 ml with water and 10% sodium citrate in an amount of 5 ml was added. A few drops (2 to 3) of concentrated ammonium hydroxide were then added and 2 ml of dimethylglyoxime. A wine red color at this point indicated the presence of nickel. The dimethylglyoxime was a 1% solution in 95% ethanol (686, p. 470).

Two ml of oxidized sample in which manganese was to be determined, was mixed with 5 ml nitric acid - phosphoric acid solution and placed in a screw capped culture tube. This mixture was then mixed with 50 mg of potassium periodate and heated for  $1\frac{1}{2}$  hours on a boiling water bath. Upon removing the tubes 3 drops of reagent solution were added and the solution examined for an orange color. The reagent solution was 0.1 gms of 4,4 tetramethyldiamino triphenylmethane in 5 ml phosphoric acid and 95 ml water. The nitric acid-phosphoric acid solution consists of 300 ml 1 N nitric acid, 375 ml phosphoric acid and 162.5 ml water (686, p. 430).

Two ml of oxidized sample in which tungsten was to be determined, was made alkaline to litmus with 1 ml 40% sodium hydroxide. The solution was then boiled vigorously and diluted to 10 ml. One ml of 15% potassium thiocyanate, 4 ml of concentrated hydrochloric acid and 1 ml of 10% stannous chloride were added and the solution examined for a yellow color (686, p. 584).

Two ml of oxidized sample in which zinc was to be determined, was mixed with 5 ml of 10% sodium citrate and ammonium hydroxide added to make an alkaline solution to litmus followed by an excess of 0.2 to 0.3 ml. The solution was extracted in a separatory funnel with a few ml of 0.01% dithiozone. The carbon tetrachloride extract was then shaken with 10 ml 0.02 N hydrochloric acid and the latter transferred to a 25 ml flask and 1 ml of 25% sodium thiosulfate, 5 ml of acetate buffer were added. The solution was then mixed vigorously with 5.0 ml of 0.001% dithizone in carbon tetra-



chloride. The acetate buffer consisted of 16.4 grams of sodium acetate, 11.3 ml acetic acid and 88.2 ml water (686, p. 616).

#### Other Determinations

Test for catalase activity of whole bacterial cells, sonicated fractions and nuclear material was performed as follows. One ml of the suspension to be tested, one ml of phosphate buffer (pH 7), 1.1 ml of saline and 1 ml of 1 N sodium hydroxide were mixed in a Thunberg flask. When the mixture had equilibrated in the closed system, after 10 minutes,  $\frac{1}{2}$  ml of hydrogen peroxide was added, and the change in height of the manometric fluid was noted. Hydrogen peroxide was used in a 3% solution and the amount added was estimated to be approximately equivalent to 500 microliters of oxygen (306).

Viscosity measurements were performed with a Viscometer - 80-100 sec., Scientific Supply Company. The length of time required for the liquid to fall between two lines was determined as the viscosity (295, p. 220-221).

The amino acid constituents of the deoxyribonuclear protein were analyzed by first decomposing the sample with acid or alkali and then plated against a suitable solvent on a paper chromatograph.

The hydrolyzing solutions used were as follows:

1. Hydrochloric acid - - - - - 1:1
2. Hydrochloric acid - - - - - Concentrated
3. Sulfuric acid - - - - - Concentrated
4. Perchloric Acid - - - - - 70%
5. Nitric acid - - - - - Concentrated
6. Sodium hydroxide - - - - - Saturated

The solvent systems used were as follows:

1. N Butanol 9, Acetic Acid 1, Water 2.5
2. Sec. Butanol 15, Formic Acid 3, Water 2
3. Sec. Butanol 13, Ammonia Hydroxide 36
4. Propanol 7, Water 3
5. N Butanol 4, Acetic Acid 1, Water 5
6. Acetone 10, N Butanol 4, Hydrochloric Acid 2  
(744, p. 82-103)

The developing agents used included ninhydrin for amino acids, and N-ethyl-maleimide for SH groups.

The purine pyrimidine composition of the deoxyribonuclear protein was analyzed by first hydrolyzing the sample with acid and then plating against a suitable solvent on a Whatman # 1 Paper chromatograph.

The hydrolyzing solutions included hydrochloric acid (1:1) (518; 745) and perchloric acid 0.5 to 0.7 N (416, p. 358). The solvent systems used were a mixture of 700 ml tertiary butanol, 132 ml concentrated hydrochloric acid and 168 ml water, or a mixture of 172 normal butanol and 28 ml water. With the former solvent system the spots formed by the migrating compounds were determined under ultraviolet light by their fluorescence (518; 745), in the latter solvent system a developer of (1:1) 0.4% thymol blue in acetone and 2% silver nitrate in water was used (744, p. 240).

The purine and pyrimidine bases were in the acid form after hydrolysis and thus the  $R_f$  values obtained corresponded to adenylic, cytidylic, guanylic and thymidylic acids.

Methyl esters were prepared from the lipid-pigment extract as indicated on pages 69-70. These were analyzed on an Aerograph Hy-Fi Model 600 Hydrogen Flame detector. Hydrogen flow rate was 20 ml per minute from a Model 550 Hydrogen Generator. The temperature was held between 178°C and 198°C and chart speed was set at 30 inches per hour. A 9 foot column packed with 20% diethylene glycol succinate on Clite 546 was used. The methyl esters were suspended in diethyl ether and 5 microliters plated on the column. Diethyl ether was used as a reference point for all measurements. Known samples were run under similar conditions and the peaks obtained compared with the unknown lipid-pigment material. Estimates were made on the basis of this comparison as to the length of the carbon chains in the lipid-pigment material which could be isolated as methyl esters (280).

Solubility tests were carried out in order to determine the organic classification of the lipid-pigment material. The arbitrary ratio of 0.10 gms of solid or 0.20 ml of liquid per 3 ml of solvent was used. The solvents and concentrations used were as follows:

1. Water
2. Ether
3. Sodium hydroxide - - - 5%
4. Hydrochloric acid - - 5%
5. Sodium bicarbonate - - 5%
6. Sulfuric acid - - - - concentrated
7. Phosphoric acid - - - 85%

(836, p. 1052-1056)



Tests for various organic groups, which might be present in the lipid-pigment material, were performed. In a small dry test tube 0.5 grams of the material, in which alcohol and ether groups were to be determined, was treated with 0.3 to 0.4 ml of acetyl chloride and examined for a reaction. (836, p. 1065-1068).

Two drops of the material, in which aldehyde and ketone groups were to be determined, were added to 3 ml of 2:4 dinitrophenylhydrazine reagent and shaken. If no precipitate formed immediately it was allowed to stand for 5 to 10 minutes. A crystalline precipitate indicated the presence of carbonyl compounds. The 2:4 dinitrophenylhydrazine reagent was prepared by suspending 2.0 grams of 2:4 dinitrophenylhydrazine in 100 ml of methanol and adding cautiously and slowly 4 ml of concentrated sulfuric acid. The mixture became warm and the solid dissolved completely (836, p. 1060-1061).

One drop or 0.05 grams of the material, in which carboxylic acid and phenol groups were to be determined, was dissolved in 5 ml of water and 1 drop of ferric chloride solution (5%) was added. The mixture was observed for color production (836, p. 1071-1072).

A preliminary test for ester and anhydride groups was performed by dissolving a drop of material or a few crystals in 1 ml of 1 N hydrochloric acid. The color produced when 1 drop of 5% ferric chloride solution was added was examined. If a pronounced

violet, blue, red or orange color was produced, the hydroxamic acid test described below was not applicable.

The hydroxamic acid test consisted of mixing 1 drop or several crystals of the compound with 1 ml of 0.5 N hydroxylamine hydrochloride in 95% ethanol and adding 0.2 ml of a 6 N aqueous solution of sodium hydroxide. The mixture was heated to boiling and after the solution had cooled slightly, 2 ml of 1 N hydrochloric acid were added. Since the solution was cloudy 2 ml of 95% ethanol were added. The color produced when 1 drop of 5% ferric chloride solution was added was observed and compared with that obtained in the preliminary test. A positive test was a distinct burgundy or magenta color as compared with the yellow color observed when the original compound was tested with ferric chloride solution in the presence of acid (836, p. 1062-1065).

A small amount (0.2 grams or 0.2 ml) of the lipid-pigment material, in which the unsaturated hydrocarbons were to be determined, was dissolved in 2 ml of water or in 2 ml of acetone and 2% potassium permanganate solution added dropwise. The test was negative if no more than 3 drops of the reagent was decolorized (836, p. 1057-1058).

One ml of the lipid-pigment material, in which the presence of reducing sugars was to be determined, was added to 5 ml of either Benedict's solution or Barfoed's solution and heated for five minutes. A positive test occurred if the solution turned

green, yellow or brown. Benedict's solution was prepared by mixing the following ingredients: 18.0 grams Copper sulfate, 200 grams sodium carbonate, 200 grams sodium citrate, 125 grams potassium thiocyanate, 5 ml of a 1% solution of potassium ferrocyanide and 1000 ml of water. Barfoed's solution was prepared by mixing 13.3 grams of copper acetate in 200 ml of water and adding 1.8 ml of glacial acetic acid (375, p. 1322).



## RESULTS OF CHEMICAL ASSAY

## Spectral Analysis

Pigments isolated from R1 were suspended in Nujol and examined on the Infrared Spectrophotometer (299; 487, p. 14-76; 579; p. 379-498; 644; 701; 742; 851). Absorption bands for N-H or O-H, C=O (acids), C-C, C-O and S-H were observed. The preparation was quite crude and no conclusions were drawn.

The nuclear material isolated from R1 appeared to have the following bonds: N-H or O-H, C=O (acids), C-S, P=O, C-O (esters), C-C, C-O and S-H. The presence of sulfur bands was not expected. Their presence could be explained when amino acid analysis was run and cysteine and cystine were found to occur in the protein associated with the nuclear material. The other bonds were as expected for nuclear material and the spectra obtained were similar to those obtained with commercially prepared deoxyribonucleic acid.

The lipid-pigment material isolated from R1 appeared to have the following bonds: N-H or O-H, C=O, C-S, C-O (esters), C-C, C-O and S-H. Sulfo-lipids were believed to cause these sulfur absorption bands.

The nuclear material isolated from R1 gave a peak between 2000 and 2500 Angstroms and between 2500 and 3000 Angstroms when assayed in the Ultraviolet light region on a Cary Recording

Spectrophotometer. Commercially prepared deoxyribonucleic acid gave similar results, as did nuclear material isolated from Sarcina lutea and Mouse liver and spleen.

The lipid-pigment material isolated from R1 gave an absorption peak between 2000 and 2500 Angstroms, when assayed in the Ultraviolet light region on a Cary Recording Spectrophotometer. Similar results were obtained from lipid-pigment material after saponification and with the fractions isolated from the Silicic Acid Column.

Nuclear Material isolated from R1 and Sarcina lutea gave absorption peaks between 6000 and 8000 Angstroms, when assayed in the Visible Light region on a Cary Recording Spectrophotometer. No peaks were obtained with the other nuclear material tested. The nuclear material isolated from R1 and Sarcina lutea was pink and yellow respectively, while the mouse liver and spleen nuclear material was colorless.

Lipid-pigment material isolated from R1 did not give any peaks in the visible region of the Cary Recording Spectrophotometer. Saponified lipids or material which had been separated by means of the Silicic Acid Column did not give any peaks either.

#### Element Analysis

The presence or absence of various inorganic elements was determined in the nuclear material isolated from R1, Sarcina lutea and mouse spleen and liver. Table XVII shows the results obtained from this analysis.

Table XVII

## Approximate Inorganic Chemical Analysis of Nuclear Material

Element	Rl	<u>Sarcina</u> <u>lutea</u>	Mouse	
			Spleen	Liver
Phosphorous	+ (1)	+	- (2)	+
Nitrogen	+	+	+	+
Sulfur	+	+	-	+
Iron	+	+	+	+
Calcium	+	+	-	+
Molybdate	+	-	-	+
Copper	-	-	-	-
Aluminum	+	tr (3)	tr	+
Magnesium	+	+	-	+
Vanadium	-	-	-	-
Cobalt	-	-	-	-
Antimony	+	+	+	+
Arsenic	-	-	-	-
Nickel	-	-	-	-
Manganense	+	+	-	tr
Tungsten	+	+	-	+
Zinc	+	+	* (4)	*

(1) The element was present according to the test

(2) The element was absent according to the test

(3) The element was present in trace amounts according to the test

(4) The test was not performed



The presence or absence of various inorganic elements was determined in the lipid-pigment material isolated from R1 and Sarcina lutea. Table XVIII shows the results obtained from this analysis.

#### Other Determinations

The viscosity of nuclear material isolated from R1 and Sarcina lutea was determined before and after gamma radiation. Results obtained indicated that the viscosity did not increase as much with the nuclear material isolated from R1 as with the nuclear material isolated from Sarcina lutea or deoxyribonucleic acid obtained commercially.

Since hydrogen peroxide was one of the harmful end products of irradiation and catalase was able to destroy hydrogen peroxide, the presence of catalase in a preparation may explain part of its protective ability. Catalase activity was determined in whole R1 bacterial cells, sonicated cells and nuclear material. Results are as follows:

Whole cells - - - -	343 ul oxygen evolved
Sonicated cells - -	245 ul oxygen evolved
Nuclear Material -	0 ul oxygen evolved

The oxygen evolved was from an equivalent of 0.03 grams of wet packed cells.

The amino acids associated with the nuclear material isolated from R1 were determined and the following were found: Cysteine, cystine, serine, threonine, glycine, alanine and phenylalanine. No effort was made to quantitate these amino acids, though the

Table XVIII

## Approximate Inorganic Chemical Analysis of Lipid-Pigment Material

Element	RI	<u>Sarcina lutea</u>
Phosphorous	+ (1)	- (2)
Nitrogen	tr (3)	tr
Sulfur	tr	-
Iron	-	-
Calcium	tr	-
Molybdate	-	-
Copper	-	-
Aluminum	+	+
Magnesium	+	-
Vanadium	-	-
Cobalt	-	-
Antimony	+	+
Arsenic	tr	-
Nickel	-	-
Manganense	-	-
Tungsten	-	-
Zinc	+	-

(1) The element was present according to the test

(2) The element was absent according to the test

(3) The element was present in trace amounts according to the test

intensity of the ninhydrin spots on the paper chromatograph seemed to indicate that cysteine, cystine, serine, threonine and glycine were present in large amounts.

The purine pyrimidine composition of the nuclear material isolated from R1 was examined. No effort was made to quantitate the amount, however  $R_f$  values for adenylic, cytidylic, guanylic and thymidylic acids were obtained.

Methyl esters of the lipid-pigment material were prepared and the lengths of the carbon chains in the preparation assayed by means of Column Gas Chromatography. Results obtained are shown on Table XIX. Methyl esters were also prepared from the various fractions separated on a Silicic Acid Column and results are presented in Table XIX.

The solubility of the lipid-pigment material was examined for the purpose of classification. It was found to be in the group containing the lower members of the homologous series of alcohols, aldehydes, ketones, acids, esters, phenols, anhydrides, amines, nitriles and polyhydroxy phenols. The lipid material was soluble in water and ether, but was not soluble in sodium hydroxide, sodium bicarbonate, hydrochloric acid, sulfuric acid and phosphoric acid. The lipid-pigment material isolated from Sarcina lutea was similar to R1 in solubility.

Because of the solubility of the lipid-pigment material, various tests were performed to determine which organic groups were



Table XIX

Carbon Chain length of Methyl Esters isolated  
from Lipid-Pigment Material of R1

Number of Carbons	Double Bonds	B.S. (1)	Silicic Acid Column (2)		
			I & II	III	IV
8	0 (3)	+ (4)	+	+	- (5)
13	0	+	-	-	-
14	0	+	+	+	-
14	1 (6)	+	-	-	-
15	0	+	+	-	-
16	0	+	+	-	-
16	1	+	+	-	-
17	0	+	-	+	-
18	0	+	-	-	-
18 iso.	0	+	+	-	-
18	1	+	-	-	-

- (1) Secondary Butyl Alcohol soluble Lipid-Pigment Material before separation on Silicic Acid Column  
 (2) Fractions obtained from Secondary Butyl Alcohol soluble Lipid-Pigment Material after separation on Silicic Acid Column - see page 69  
 (3) A peak was obtained on the Gas Chromatograph which corresponded to a known methyl ester which did not have any double bonds  
 (4) A peak was obtained on the Gas Chromatograph which corresponded to a known methyl ester of this carbon length  
 (5) No peak was obtained on the Gas Chromatograph which corresponded to a known methyl ester of this carbon length  
 (6) A peak was obtained on the Gas Chromatograph which corresponded to a known methyl ester which had one double bond

present. A summary of the results are in Table XX.

Since the pigment does impart protection to mice, it was felt that it might be the protective agent in R1. In order to test this suggestion, cells were grown with and without diphenylamine added to the media. Diphenylamine has been implicated as a specific inhibitor of pigment synthesis (161; 812; 830). The R1 cells after being grown in the presence of diphenylamine (0.01 grms per 1 liter TGYM ) were irradiated in 0.15 M phosphate buffer and the per cent drop in number of cells surviving recorded. Without diphenylamine ( $5 \times 10^8$  to  $1.6 \times 10^8$ ) or a 32% survival was noted, while with diphenylamine in the medium ( $4.9 \times 10^8$  to  $0.015 \times 10^8$ ) or 0.3% survival was noted. Therefore it was felt that the pigment or related lipids played a part in protection.

There was some question concerning the role of diphenylamine as a metabolic inhibitor. Therefore varying amounts of diphenylamine were added to 50 ml flasks of TGYM. The cells were harvested by centrifugation, dried in a vacuum and weighed. With 0.005 and 0.001 grams of diphenylamine added, a marked reduction in weight of cells was noted, also a decrease in the amount of pink pigmentation. With 0.005 to 0.00001 grams, no reduction in weight of cells occurred, but the intensity of the pigmentation did vary, being darker with less diphenylamine.

It was felt that the diphenylamine was a specific pigment or associated compound inhibitor in limited concentrations but as the

Table XI

Tests for various organic groups in Lipid-Pigment  
material isolated from R1 and Sarcina lutea

Organic Group	R1	<u>Sarcina lutea</u>
Alcohol or ether	- (1)	-
Aldehyde or ketone	+ (2)	+
Carboxylic acid or Phenol	-	-
Ester or anhydride	-	-
Unsaturated hydrocarbons	-	-
Reducing sugar	-	-

(1) The organic group was absent according to the test

(2) The organic group was present according to the test



concentration was increased it inhibited the other metabolic processes.

It was noted that aeration with a 1:1 mixture of oxygen and nitrogen or no aeration caused a slight decrease in growth and pigment production. Oxygen alone increased the amount of growth and pigmentation.

## DISCUSSION

The main objective of this experimentation was to determine if a radiation resistant microorganism produced a compound or compounds which protected it from the lethal effects of radiation. A further objective was to isolate this compound or compounds and determine if it (they) was capable of protecting mice from the lethal effects of radiation.

The concept that an organism or extracts of an organism might protect another organism from radiation was not new. As shown in The Historical Section of this thesis, several workers had observed that factors from seeds, yeast concentrates, bacterial polysaccharides and endotoxins had protective properties (39; 96; 108). The exact nature of the protective property is not known though many suggestions have been made and some have been presented earlier.

Therefore, suspensions of whole R1 cells were injected into mice before or after the mice were irradiated. As indicated on Table IV, mice receiving R1 cells had a better chance of survival than those receiving Sarcina lutea cells (a radiation sensitive bacteria used as a control organism). On the basis of the observed differences in survival it was felt that the radiation protection was due to a specific compound in R1 and not just to the presence of particulate material in the peritoneal cavity. This point needed to be clarified as Smith et al had found that IP injections of inorganic particulate material protected animals from radiation (743). The protection afforded by inorganic particulate material

was therapeutic in nature and afforded little effect when injections were made before irradiation. The R1 cells were effective if injected either before or after irradiation.

In view of the survival obtained from injections of whole bacterial cells to lethally irradiated mice, cell fractionation was undertaken. Sonication of bacterial cells yielded two distinct fractions: cytoplasmic and particulate (cell wall, nuclear material and cellular debris). The protection afforded by the particulate material was better than that from the cytoplasmic, though both were effective (Table IV).

It had been hoped that the "protective property" could be localized in just one particular portion of the bacterial cell. Since this was not the case, as indicated on Table V, extraction methods were next examined.

The most noticeable characteristic of R1 colonies was their flesh pink pigmentation. The pigments of R1 have been studied by Lee (489, p. 9-27) and their production compared with the development of radiation resistance. It was suggested that the pigments might be the desired "compound", especially as sonication of whole cells left the pigmentation in both cytoplasmic and particulate material. It had been observed by others that some types of pigments interacted with visible radiation in photosynthetic processes and that some pigment synthesis was light induced. Carotenoid pigments were also believed by some authors to act as radiation protectants for bacteria, as pigment-less mutants were more sensitive to visible light (161).



Diphenylamine was a specific pigment inhibitor (161; 812; 830) and when RL cells were grown in its presence they showed a reduced amount of pigment. These pigment-less cells were more sensitive to gamma radiation than the pigmented cells.

Pigment formation or the formation of colored (oxidized) forms of pigment also appeared to be induced by ultraviolet light and oxygen tension.

Pigments were isolated from RL by hot ethanol and this crude pigment extract was found to be therapeutic in nature. Using a more elaborate technique of Reiman and Eklund a slightly better result was obtained (Table V.). Comparison of survival obtained with pigments isolated from Sarcina lutea indicates that this was not the compound in which we were interested. The source of the pigment (cytoplasm or cell wall) did not greatly effect its protective ability. Lycopene, a carotenoid pigment has been found to have radiation protective properties (293). These authors felt that the lycopene acted as a producer of non-specific responses to stresses (502). They found that injection of lycopene extracts provided protection against a variety of bacterial infections against development of ascites tumors as well as the consequences of total body irradiation (502).

Ellinger et al had found that saline extracts of splenic tissue had protective ability (262;264). Extracting solutions used in these experiments included acids, bases, salts, organic solvents (752) and water. The best results were obtained with

extracts of R1 using sulfuric acid or petroleum ether. The original method of isolating nuclear material was by acid digestion and ether extraction (537, 538).

When nuclear material was extracted from R1 by the above method, protection was obtained. Other workers had found similar results, i.e. nuclear material had protective ability. The protection obtained with nuclear material from R1 was slightly better than that with nuclear material isolated from Sarcina lutea, mouse spleen or liver (Table IX).

The nuclear material needs to be injected either 24 hours before or after irradiation in order to be effective (Table VII). The reason for this time requirement is not known. It is suggested that when injected before irradiation it would reduce oxygen tension or combine with the sensitive sites. When injected after irradiation it may act to supply precursors for DNA synthesis.

Multiple injections of nuclear material increased the survival, although when four injections were made no survival was noted (Table VIII). There may be a toxic effect associated with this material which, when accumulated to a certain level, removes the beneficial effect. If the compound acted as an anoxia producing compound, it could suggest that the animals have a lower limit of oxygen with which they can live. If the compound protects by binding with sensitive sites, it could suggest that the animals need some of these free sites (unbound) in order for normal metabolism to be carried on by the animals.

Even though the nuclear material was crude, containing associated lipid, pigment and protein it was felt that a chemical analysis would be beneficial. Infrared, ultraviolet and visible spectra of the material were compared with spectra of commercially prepared DNA and found to be similar. Phosphorus and nitrogen were present in the nuclear material, which would be expected, as well as sulfur. Amino acid analysis of the nuclear material showed that cysteine and cystine were present as well as several others. The presence of these two amino acids would explain the positive test for sulfur. Cysteine may be the protective portion of the nuclear material. It is a known protectant whose method of protection was postulated to be the result of anoxia.

Metal ions were good electron donors and supposedly could have supplied electrons to damaged material of the cell (136).

An attempt was made to separate the nucleoprotein material from the pigment-lipid complex. The nuclear material was pigmented and the protective portion might be the pigment-lipid complex associated with the nuclear material, especially as purified nuclear preparations gave less protection.

The precipitation of nucleoprotein was done with warm ethanol. The resulting precipitated nucleoprotein was unable to protect mice from the lethal effects of irradiation, but the soluble pigment-lipid complex was protectant. Results obtained from the various fractions of the pigment-lipid complex separated by Silicic Acid Column are shown on Table XV. This fractionation was



crude because of the quantities plated on the Silicic Acid Column, but the results would indicate that the major protection was found in the neutral fats and bound lipids. Saponification of the lipid-pigment material gave survival comparable to that obtained from the first fraction collected on the Silicic Acid Column. Similar results were obtained with methyl ester derivatives of the pigment-lipid material. This would eliminate the carotenoid pigment as sole protective agent, but the possibility that the pigment and lipid together are the effective fraction was considered.

The lipid-pigment complex was most effective if injected after irradiation. The possibility that this therapeutic effect was due to its ability to remove free radicals has been presented by other workers.

Less than 4 multiple injections were effective, but when 5 injections were tried, toxic effects were noted. As indicated in discussion of nuclear material, toxic effects of compound, while not apparent with one injection, may be additive.

Various lipids have been found to protect animals from the lethal effects of radiation. Donating electrons or acting as free radical traps have been suggested as some of the mechanism by which lipids repaired radiation damage. Chelating agents (lipids) may also act to remove free metal ions which are released by irradiation.

One of the noticeable symptoms of radiation damage in animals

was an alteration in lipid metabolism. Release of lipids may be one of the animals means of defense against radiation. The lipid-pigment material may have acted to supplement the animals defense. This suggestion was supported by the fact that lipid material isolated from mouse liver and spleen gave better results than that isolated from R1 or Sarcina lutea.

The lipid material of R1 has been postulated to occur as the outer coat of the cell on the basis of work with other bacteria (521, 749). Lysozyme action is not quite typical with this organism, though the gram reaction of the organism is changed indicating that cell permeability is broken down. The suspending fluid does not become viscous as is the case with typical lysozyme sensitive organisms. The observation that the cell wall of this organism is different and that it has a lipid coat which may moderate radiation seems to be the best one considered to date. Many of the results presented here can best be explained on the basis of pigment-lipid complex being the protective compound for R1 cells. Noticeably, the effect of diphenylamine on pigment production and the corresponding decrease in resistance of the organism and the formation of pigmentation induced by ultra-violet light correlated the pigment-lipid material with radiation resistance. The radiation must act upon the nuclear material of the microorganism in order to kill it. Thus some method of reducing the effect of free radicals produced by radiation before they reached the nuclear material would protect the organism.

The lipid-pigment material which was isolated from R1 may act to remove free radicals formed by radiation.

It has been shown in electron micrographs that this microorganism grew in tetrads with each individual cell having more than one nucleus at all times. If these nuclei were each an independent entity and could carry on the function of the cell without the others the radiation dose would need to be greater in order to kill the organism. If the protection afforded by a protective lipid-pigment coat were added to that afforded by the multinucleated state of the microorganism the total resistance of the organism would undoubtedly be increased.

The presence of sulfur in the lipid-pigment material, though in only trace amounts, might suggest that the lipid may be a sulfo-lipid and thus the lipid material could act as a free radical acceptor. Sulfo-lipids have been found in photosynthetic plants and their role in photosynthetic processes has been investigated. All the well known protective agents known to date, cysteine, aminoethylisothiuronium bromide hydrogen bromide and the mercaptoalkylamines have sulfur atoms. Perhaps one of these is associated with the lipid-pigment coat thus giving the protection noted. This has been suggested as mercaptoalkylamine gases are produced by the microorganism. The argument contrary to this would be the fact that these compounds act before radiation whereas what has been used in these experiments appeared to be affect if given before or after radiation.



The additive nature of the lipid-pigment coat and the multi nuclei is the most attractive hypothesis and the one which is here presented as the mode of protection of radiation resistant micrococcus species designated R1. Mathematical analysis of the survival curve of R1 carried on by Puria (in progress) gives additional information which agrees essentially with the hypothesis presented here.

## SUMMARY

The main objective of this experimentation was to determine if a radiation resistant microorganism had a compound or compounds which protected it from the lethal effects of radiation. A further objective was to isolate this compound or compounds and determine if protection could be conferred to higher animals.

The whole R1 bacterial cells were injected into mice before and after they received a lethal dose of irradiation. Protection of mice to lethal effects of irradiation was obtained with whole cell preparation of R1 whereas similar preparations of Sarcina lutea did not protect.

The cytoplasmic and particulate material was isolated from R1 by sonication of whole cells and these were tested for their protective efficacy. Though both fractions protected mice from the lethal effects of radiation the particulate material gave better results.

Cellular extracts of pigment material were examined for their protective ability as well as various other cellular extracted material. The sulfuric acid or petroleum ether extracted cellular material gave the best results.

The Miescher's method for isolating nuclear material yielded material which was the most effective radiation protective material of those nuclear materials tested. Even though the nuclear material prepared by Miescher's method was crude, containing associated lipid, pigment and protein material, chemical analysis was

performed. The results indicated more sulfur than expected.

The crude nuclear material was purified further by separating the lipid-pigment material from it. The lipid-pigment material of the nucleic acid extracted material appeared to be the protective fraction rather than the nucleic acid material. When purified still further by saponification or when the methyl derivative was prepared, the lipid portion had more protective properties than the pigment material. The lipid-pigment material was also crudely separated on a silicic acid column and the first fractions which were removed (neutral fats and bound lipids) appeared to be the protective portion.

The protection afforded by the pigment-lipid complex was additive and relatively consistent and good protection was obtained. It was felt that the compound which protects R1 has indeed been found and that it is this lipid-pigment complex.



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