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ARMY RESEARCH TASK REPORT | REPORTS CONTROL SYMBOL CSCRD-6(R2)

ACCESSION NUMBER 36164 **CONFIDENTIAL** | PROJECT, TASK, OR SUBTASK NO. 3A012501A805Q101

1. REQUESTING AGENCY
The Army Medical Service
Office of The Surgeon General
Washington, D. C., 20315

2. FUNDING AGENCY
Army Medical R&D Command
Office of The Surgeon General
Washington, D. C., 20315

3. CONTRACTING AGENCY
NA

4. CONTRACTOR AND/OR GOV'T LABORATORY
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Walter Reed Army Medical Center
Washington, D. C., 20012
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5. PRINCIPAL & ASSOC. INVESTIGATORS/PROJECT OR ACTION OFFICER
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576-2280 or Interdepartmental Code 198, Ext 2280 See Continuation Sheet 49

6. TITLE OF: PROJECT ☐ TASK ☐ SUBTASK ☒ Chemical protection against irradiation (U)

7. DATE OF REPORT DAY 30 MONTH June YEAR 1964

8. RESUME' (C) The Antiradiation Drug Development Program continues to make progress in the development of agents useful for protecting animals against ionizing radiation injury. The toxicity associated with the lipid soluble agents has been reduced and their effectiveness against radiation has been increased. The water soluble agents have been expanded to include new effective functional groups, thereby increasing the flexibility in this part of the program. For the first time in this laboratory, monkeys have been protected against fully lethal doses of radiation. The chronic studies preliminary to administering these compounds to man have been initiated. The progress of the last year is such that the staff is confident that with sufficient effort, these agents can be made effective without appreciable side effects.

9. KEY WORDS
Radiation injury, antiradiation drugs, medicinal chemistry, pharmacology, organic chemistry, toxicity, mice, dogs, monkeys, bacteria, man, contracts, patents.

10. SUPPORTING PROJECTS
Not Applicable

11. COORDINATION WITH OTHER MILIT. DEPARTMENTS & GOV'T AGENCIES
☒ NO ☐ YES See Continuation Sheet

12. PARTICIPATION BY OTHER MILIT. DEPTS. & GOV'T. AGENCIES CONFIDENTIAL
☒ NO ☐ YES See Continuation Sheet

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ACCESSION NO. 36164

ARMY RESEARCH TASK REPORT CONFIDENTIAL

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13. PROJECT, TASK OR SUBTASK NUMBER: 3A012501A805 | 01 | 01 |

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15. SECURITY OF WORK (34): 3

16. TYPE OF REPORT: 3 | 1263

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19. R&D CATEGORY (67): 1

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20. CONTRACT NUMBER: DA

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22. ESTIMATED COMPLET. DATES: 1CONT | 2 | 3 | 4 | 5

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23. PRIORITY (11-14): 1

24. PROGRAM ELEMENT (15-26): 6.11.25.01.1

25. CMR&D CODES: N/A

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a. Est. Total Cost (11-15):
b. % Spent Intern. (16-18): 1
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c. Total Obligation (22-26):
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e. " " " +1 (34-40)
f. " " " +2 (41-47)
g. " " " +3 (48-54)
h. " " " +4 (55-61)
i. " " " +5 (62-68)
j. " " " +6 (69-75)
k. Total Man Years of Effort (76-78):

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CONFIDENTIAL

ANNUAL PROGRESS REPORT

Project No. 3AO12501A805 Title: Ionizing Radiation Injury - Prevention and Treatment
Task No. 01 Chemical protection against irradiation
Subtask No. 01

Description: The development of an antiradiation agent for man.

Progress:

I. General

Progress in the development of antiradiation agents for man continues to be made. During the last year the two major classes of agents previously discovered were further developed and have yielded additional variations which appear promising.

The lipid-soluble antiradiation class has been improved by the addition of rings isolated by 3 to 4 methylene groups from the nitrogen function of MEA. By this maneuver the cardiac toxicity, vasoconstriction, and beta adrenergic blockade produced by WR-1607 has been eliminated while still retaining the high degree of efficiency, the lack of convulsions, and the lack of histamine release which constituted the advantage for WR 1607. The new agents still produce emesis in the dogs, but do not produce emesis in monkeys. They are still not effective by mouth. The elimination of cardiac toxicity and peripheral vasoconstriction means that these agents lack the two major side effects known to mitigate against testing these agents for tolerance in man.

The water soluble class of antiradiation agents is still the most effective class of agents known in terms of reduction of apparent radiation dose. In the hydroxyl series, WR 2347 is the best agent but has the disadvantage of being unstable and requires relatively large amounts for protection. Two new functional groups have been discovered which extend the number of variations possible in the water soluble class of antiradiation agents. Excellent protection has been obtained by use of an alkyl amide substituant on MEA. Excellent protection has also been obtained using amino alkyl derivatives if the mercaptan is converted to a thiophosphate. This latter class of compounds

as mercaptans had been dropped two years ago but as thiophosphates are among our promising water soluble agents. All members of the water soluble class are capable of producing emesis in the dog but not in the monkey.

A number of agents not belonging to the amino thiol family continue to be tested for their ability to protect mice against radiation injury. We estimated that about 3,500 random structures have been examined to date. The amino thiol derivatives appear to be superior to any and all such random structures.

The chemical typewriter and the data processing procedures for the handling of structural information is being completed with the idea of handling a million structures per year. The possibility to manipulate large amounts of structural information will provide the Army Medical Service with a system designed to make investigators more effective.

A. Chemical Synthesis Program

1. Contract

The chemical synthesis program during FY 64 was operating on a $700,000 budget. There were 23 contracts active during the year, three of which were of the nonsynthetic type. The twenty synthesis contracts are broken down as follows: Industrial - 5, 25%; academic - 11, 55%; and nonprofit research house - 4, 20%. Three synthesis type contracts were terminated during the year; one in each category. In addition, one nonsynthetic contract was terminated and one industrial contract was started. A breakdown of the money spent on synthesis type contracts for the year is as follows: industrial - 65%; academic - 15%; and nonprofit research house - 20%. Eliminating the still active but nonproducing contracts, that is, those that have been extended in time to allow proposals to be submitted or reports to be written, we will enter FY 65 with 12 synthetic type contracts and two nonsynthetic type.

The following figures are given on a projected basis, an estimate being made for the period between the writing of this report and the end of the fiscal year. There were about 255 compounds submitted from the contract synthetic laboratories at an average cost of about $2800 per compounds. This relatively higher cost per compound during FY 64 compared to FY 63 is attributed to several factors. One is that the full impact of the reduction in the budget and number of contracts two years

ago is now being felt; the pipeline is now fairly empty. Another important reason is that the chemistry is becoming more difficult because of the increased sophistication of the compounds requested by WRAIR. Finally, the readily available starting materials for compounds of interest has now been exhausted so that it is necessary to synthesize intermediates, analogues of which were at one time commercially available. The total number of accessioned compounds from all sources as of the end of FY 64 is about 3,075. During the year a total of about 445 compounds from all sources was submitted. The total cumulative dollar obligation is approximately $4,000,000.

The major change in approach during the year has been the decision to exploit Bunte salts and de-emphasize mercaptans. Because of encouraging results with some phosphorothioic acids during the year, this class also is assuming increasing importance, and may, before the end of FY 65 receive as much attention as the Bunte salts. The switch in emphasis from the mercaptans to the Bunte salts has resulted in some extremely difficult purification problems in the important area of polyhydroxyalkylaminoethanethiosulfuric acids. These problems have slowed the acquisition of compounds markedly during the year. At this writing this situation is expected to improve immediately. This is no problem in the case of the insoluble Bunte salts. The Bunte salts of interest are now being made through three main routes: (1) the opening of epoxides with aminoethylthiosulfuric acid, sodium salt; (2) conversion of disulfides using ammonium sulfite; and (3) the alkylation of aminoethylthiosulfuric acid using appropriate halides. The high activity obtained with compound 3-(2-mercaptoethyl)propionamide prompted synthetic efforts toward obtaining compounds with acidic or weakly basic groups attached to the nitrogen atom of MEA through a chain of a varying number of methylene groups. Methods have been fairly well worked out for these synthesis when the number of methylenes is two; when the number is greater than two, special syntheses have to be devised. This has proved to be a very difficult problem and is not yet solved. This is a fine example of productivity slowing down because of various chemical difficulties.

What appears to be significant breakthrough has developed in the cyclohexylalkyl and phenylakyl aminoethylthiosulfuric acids. This has lead to a concentration of effort on the synthesis of compounds containing rings (aryl, carbocyclic, and heterocyclic) attached to the nitrogen atom of aminoethylthiosulfuric acid through methylene chains of various lengths. Variations

on this structure, for example, separation of the ring from the methylene chain by means of an oxygen or sulfur atom is also being looked at.

Interesting activity has also been observed when the nitrogen is of the amidine type. From the work this year, it is apparent that many interesting compounds may be available through ethylene imine chemistry. One example is the propionamide mentioned above. It is planned to exploit this area in the coming year. The high activity of the compound 2-amino-2-mercaptomethyl-1,3-propanediol led to much effort both for the modification of this compound and for the introduction of this compound as a moiety in other compounds of proven activity. This year also saw the first concerted activity through synthesized fluorinated derivatives of active or related compounds. This effort was just beginning to be productive from the chemical synthesis standpoint. The contractor, unfortunately, has voluntarily withdrawn from the program. The number of fluorinated compounds that we have obtained is, at this time, perhaps too small to make a definite judgement on the value of introducing fluorine into essential agents. At the present time there are no plans to place this work elsewhere.

Submission of chelates for testing during the year has been slow. The area of mixed disulfides, thiosulfonates and related compounds has continued to receive attention. Compounds related to o-aminoethyl-dithiobenzoic acid, an unexpedtedly active compound, have been pursued. A number of miscellaneous compounds have been examined. There are two propane dithiol derivatives which show interesting activity (WR 2694 and 2712).

There are now in operation two contract-supported laboratories for the synthesis of larger amounts of promising compounds. These two laboratories should be sufficient for the time being to support the large animal studies and chronic feeding tests although for some types of compounds expanded facilities will be needed.

The no-dollar agreement which facilitates the recepit of unpatented compounds for testing continues to be of real value to the program and it is anticipated that increasing use will be made of this method of obtaining compounds in the coming year.

2. WRAIR Medicinal Chemistry Laboratory

A total of 22 compounds were submitted for testing as potential antiradiation agents. Nearly all of these compounds are new to the chemical literature.

A new procedure was developed for the preparation of mixed (unsymmetrical) disulfides containing the cysteamine moiety. By combining the sodium salt of 2-aminoethanethiosulfuric acid and a sodium mercaptide in methanol solution at 0°, the by-product, sodium sulfite, precipitated from solution immediately leaving the mixed disulfide in the methanol solution. Yields of product were limited by the extensive disproportionation of the mixed disulfides in alkaline solution. Six mixed disulfides ($RSSCH_2CH_2NH_2$) were submitted for testing as hydrochlorides. The reaction mentioned above has been carried out successfully using the selenium Bunte salt, 2-aminoethaneselenosulfuric acid, to yield the selenosulfide, 1-amino-3-selena-4-thiatetradecane.

The reactions of 1-amino-2-bromopropane hydrobromide and 2-amino-1-bromopropane hydrobromide with sodium thiosulfate have been investigated in collaboration with Dr. J. W. Low, Department of Biophysics, WRAIR. Using infra-red and nuclear magnetic resonance spectorscopy it was found that in the reaction of the former amino-halide with sodium thiosulfate, two isomeric Bunte salts are formed, i.e. 1-aminopropane-2-thiosulfuric acid and 2-aminopropane-1-thiosulfuric acid; the latter amino-halide, under identical conditions, yields one Bunte salt, 2-aminopropane-1-thiosulfuric acid. Mechanisms for the two reactions have been postulated.

The reactions of the selenium Bunte salt, 2-aminoethaneseleno-sulfuric acid, have been studied further. From this compound there has been synthesized the selenium heterocyclic compound, 2-aminoselenazoline; seleno-cystamine; and a selenosulfide, mentioned above. The selenocystamine has been oxidized to 2-aminoethyl 2-aminoethaneselenolselenonate (analogous to a thiosulfonate) and selenohypotaurine (2-aminoethaneseleninic acid). Attempts to prepare selenotaurine by various methods have failed due to the instability of the compound. Selenocystamine has been reduced to selenocysteamine (2-aminoethaneselenol).

The ring opening of ethyleneimines with thiosulfate and seleno-sulfate to yield 2-aminoalkylthiosulfuric acids

and 2-aminoalkyl-selenosulfuric acids is being investigated. A study is underway to determine the pH dependence of these reactions and their applicability to the preparation of potential antiradiation drugs.

Additional work is being directed toward synthesizing some heterocyclic alkyl aminoethanethiosulfuric acids.

B. Evaluation of the Antiradiation Action of Chemicals

1. The Mouse Program

The mouse evaluation program continues to be the standard reference program by which chemicals are examined for effectiveness against radiation injury, see Figure 1. A total of 2727 compounds have been examined to date of which 426 were examined during the last year and 653 were examined on contracts with the Woodward Research Corporation. The total examined this year between the two facilities is 1089. The numbers of compounds that fall into different categories of activity is shown in Figure 2. The compounds tested for oral administration 1/4 and 1/8 doses, duration, dose reduction, diet, etc., represent further testing on agents originally found to be effective. As can be seen from the activity of new compounds 10% of the compounds tested had good activity and 340 of all the compounds tested had some activity. This group of compounds includes some off the shelf items. The percentage of activity in synthesized compounds would have been higher if we had confined ourselves specifically to the compounds which were selectively synthesized.

The presumptive screening test to discover new classes of antiradiation compounds has been developed and is working fairly satisfactorily. Pseudomonas in the test system has given rise to some difficulties but chlorination of the water may solve the difficulties. This work is being conducted on contract.

Agents which were found to be effective in mice were tested in combinations at the maximum tolerated drug levels and also in combinations at drug levels which were ineffective if the agents were given alone. Enhanced protection was seen in a number of instances. The ability of MEA to detoxify 1607 previously reported was found to include the hydroxyl derivatives

of MEA and also affected other lipid soluble Bunte salts. The pharmacologically active agent S-ethylisothiourea, WR 539, was very effective when combined with lipid soluble derivatives.

Dose reduction factors were determined for a number of antiradiation compounds. The lipid soluble agents are in general not as potent as the non-lipid derivatives. One combination had a dose reduction factor of 2.

The administration of candidate antiradiation drugs at high levels in a diet has resulted in the development of osteolathyrism. While we do not expect to administer agents at these high dietary levels, we feel that lathyrism represents a problem. However, the lack of lathyrogenic activity in the simple alkyl derivatives is another point in favor of the single alkyl derivatives.

2. Bacterial Testing Program

Bacterial testing was converted entirely to a contract operation during the past year. As a result of difficulties attendant with the switch-over (failure to deliver necessary equipment), there is no specific progress to report in spite of the fact that bacterial testing and rating of compounds with respect to their protective factor has been in the past an important part of this program. Bacterial testing is now functioning again at approximately 3,000 agents per year. Automatic data processing procedures have been initiated which we hope will reduce the time lag between test data and results.

3. Large Animal Testing

The Large Animal Testing Section has extended work to include monkeys as well as dogs. In addition, chronic clinical administration of agents has been initiated on monkeys on contract. An appreciable part of the Large Animal Testing Section has been devoted to the toxicity of candidate antiradiation agents including examination of a series of structures for their ability to produce ganglionic blockade.

C. Pharmacology of Antiradiation Chemicals

1. Animals

The pharmacologic side effects of candidate antiradiation chemicals continues to be one of the major stumbling blocks in the development of a candidate antiradiation agent for man. As previously reported, we do not any longer have difficulty with convulsant or histamine release of candidate agents. A special series of tests were established within the past year to evaluate in vitro cardiac toxicity, beta-adrenergic blockade, peripheral vasoconstriction, neuro-muscular blockade and parasympathetic activity. These tests have been effective in providing us with information on agents lacking the adverse pharmacologic side effects of 1607.

2. Man

A number of agents have been furnished to a contractor for evaluation of the ability to depress in vitro serum rheumatoic factor. These studies will be carried out in vivo as soon as clearance is available. Agents have also been examined for their ability to protect against nitrogen mustard lethality. The water soluble agents appear to be highly effective against nitrogen mustard. The best agent is WR 2347. This agent may well have potential clinical utility in nitrogen mustard therapy and therefore may well provide us with pharmacologic information.

II. Detailed Reports

A. Structure vs Function (also see Section M)

The lipid soluble derivatives reported last year have been extended. The most promising agents are reported on Figure 3. This chart contains the first indication that it is possible to use rings in the development of effective antiradiation compounds. WR 2691 has the advantage that it is effective at relatively low drug levels. Figure 4 contains a report supplementing last year's intensive report on the hydroxyl derivatives. These compounds are becoming increasingly difficult to make. WR 2753 represents the most promising agent to be added to the hydroxyl series. Figure 5 represents a summary of the amino alkyl amino series which were previously considered to have limited potential usefulness.

This series was extended by converting the mercaptans to thiophosphates with an attendant marked improvement in activity. This series has not been examined for histamine release but the zwitterionic nature of the compounds should mean that histamine release will be less than with the corresponding thiols. In this series we have one comparison with a corresponding Bunte derivative; the Bunte is not active in spite of the fact that both the thiol and thiophosphate are active.

Figures 6 thru 9 constitute a detailed review of amino alkyl amino ethyl mercaptans. Although there is consistent activity in this class of agents there is no compound as a mercaptan which appears promising enough to merit further development. As can be seen by inspection of the WR numbers, most of the compounds reported here were developed early in the program. On Figure 6 WR 2220 and related structures represented an attempt to develop agents effective at low drug levels. There was some activity with this compound so that the thiosulfate might be more auspicious. These long chain compounds cannot be tested for anticipated cardiotoxicity since the present screen can handle only thiosulfates. There is some activity in corresponding hydroxyl group substitutions corresponding to favorable substitutions in the single hydroxyl series on the terminal nitrogen but the activity of 1079 is less than 729. For example, WR 1751 in Figure 6 is active but 2416 in Figure 7 is lacking in activity. These hydroxyl derivatives were made to see if the histamine release properties of these diamines can be altered. Figure 8 summarizes an attempt made over the last two years to develop bis agents because of an early finding that bis compounds are effective at lower millimolar levels then corresponding non-bis compounds. The activity of the compounds on Figure 8 exceeds the activity in the corresponding amino alkyl thiols shown on Figure 5 where x=3 thru 6. These compounds, therefore, constitute candidates for development as corresponding thiosulfates or thiophosphates. Figure 9 represents an attempt to combine the bis and hydroxyl series with the expectation of reducing histamine release. This series designed originally to develop WR 342,, one of the best agents in 1960, has been relatively unsuccessful.

Figures 10 thru 18 represent a detailed review of substitutions on the carbon chains between the nitrogen and the sulfur. The most promising compound in this series is WR 2389 on Figure

11. On Figure 10 WR 621 was one of the best ten compounds in 1960. As can be seen from a comparison of the indices, this compound no longer has the same interest, since the rest of the program has been relatively successful. These substitutions were originally made in order to develop compounds having asymmetric centers with the expection that optical resolution would result in compounds having an improved therapeutic ratio. In Figure 11, WR 2389 is not effective at drug levels lower than 500 mg/kg but it does offer protection at the two top tolerated drug levels. This compound represents the only structure which retains activity at interesting levels when a substitution is on the carbon chain between the nitrogen and the sulfur. It has the disadvantage of requiring relatively large amounts for protection but attempts are being made to modify it in order to see if a greater efficiency can be obtained. All the compounds in Figure 12 contain double substitutions on the carbon carrying the amine. WR 2649 has definitely interesting activity and since it is an amino Bunte derivative presumably could be modified by the addition of the lipid soluble group to give even more interesting compounds. The carbon to which the nitrogen is attached is quaternary so that ganglionic blockade might be expected if water solubilizing groups were used, but should not be a problem if lipid soluble groups were used. In this series it can be seen from a comparison of 2648 and 2579 that the Bunte is again superior to the mercaptan. In both cases the cyclo pentane derivative is very much aperior to the cyclo hexane.

Figures 13 thru 17 represent a review of amino thiols having substitutions on the carbon carrying the mercaptan. In Figure 13 WR 166 having an index of 4.0 was on the best 20 list in 1960. Because it has an asymmetric carbon, the compound was resolved into the d and l isomers. The l isomer had an approximate LD_{50} appreciably greater than the d form but had no greater ability to protect against radiation injury. It is conceivable, therefore, that the l isomer may have a greater margin of safety. These compounds have not been converted to the corresponding thiosulfate. Resolution of these compounds was difficult. We had previously made an intensive effort to develop alkyl variations on the nitrogen of this series but without success. We therefore feel after this experience that this group of compounds is not suitable for further development in spite of the fact that there is an appreciable difference in the approximate LD_{50} of the two optical isomers. Figure 14 lists compounds

having poly functional groups on the carbon carrying the sulfur. WR 2623 is also related to one of the unithiol series developed by Tank, although all of his derivatives had substitutions containing alkyl sulfonic groups on the nitrogen. Neither Tank's compounds nor these examples were effective against radiation injury. WR 2622 has an unexpected toxicity. Figure 15 represents an attempt to combine the hydroxyl series of compounds with derivatives having a methyl group on the carbon carrying the sulfur function. WR 2516 is the parent compound in this series. It is not as effective as the MEA Bunte. WR 2277 is an analogue of a compound in the hydroxyl series which has an index of 4.2. WR 2557 is an analogue of 1901 having an index of 3.8. It has the advantage that it goes at .4 millimoles per kilogram as compared to .7 for 1901. WR 2455 is an analogue of 843 and has approximately the same index. WR 2703 is an analogue of 1898 and has essentially the same characteristics.

In Figure 16, WR 2226 is an analogue of 728 but does not offer any protection in spite of the good protection obtained with 728. The tolerated level for .9 mM/kg as compared to .7 mM/kg for 728. The other compounds do not exceed in effectiveness analogous MEA derivatives where comparisons are possible.

Figure 17 contains a summary of all compounds having double substitutions on the carbons carrying the sulfur function. In general we do not have the analogous thiosulfates. WR 1553 has essentially identical information as that shown for 339 on Figure 11. 1987 may be compared to 2648 in Figure 12. It is tolerated at considerably lower levels than 2648. Other exact comparisons are not possible since it is obvious from Figure 12 that the thiosulfates are superior to the corresponding mercaptans. We do not have available thiosulfates corresponding to the mercaptan shown on Figure 17. Figure 18 contains compounds which have substitutions on both carbons between the nitrogen and the sulfur. In general it can be seen that these compounds are not active. WR 2872 and 2871 were made with the hope that these compounds would offer protection at low drug levels without producing side effects associated with chelation. The compounds were administered at drug levels comparable to 1607 but did not offer protection.

On Figure 19 a series of amidine derivatives is reviewed. As can be seen from the chart there is consistent activity in this series with good protection at low drug levels with 2743. It is interesting that the phenbutyl side chain is active, but the decyl side chain is not. This series of compounds represents a different nitrogen function and therefore merits exploitation. Figure 20 represents amidine derivatives in which there is also a substitution on the carbon carrying the sulfur function. There are three compounds for direct comparison with Figure 19. WR 1552 is comparable to 1551 except that it exhibits better protection. WR 2187 is comparable to 1756 but does not offer any protection in spite of the fact that it is administered at a higher dose. The same may be said about 2050 as compared to 1868. We therefore feel on the basis of this present experience that methyl groups on the carbon carrying the sulfur function do not represent an auspicious series for further development.

In Figure 21 a series of hydroxyl amines are reported which demonstrate good activity. In view of the prominence given to the AET series of drugs, this class is indeed an interesting one since the hydroxyl amino guanadine function should not exhibit less ganglionic blockade than the corresponding alkyl derivatives. Attempts to prepare WR 1988 in larger quantity have met with difficulties. We anticipate that the pharmacology of these agents should be comparable to the AET series. We are not at the present time exploiting this series further in spite of its demonstrated activity in order to develop more variations in the specific areas selected for emphasis.

On Figure 22 is reported a series of heterocyles which demonstrate interesting protective activity. These compounds had remarkable differences between convulsant and depressant properties, for example, 1989 compared to 1990. WR 2217 demonstrated some protective activity at levels far removed from the approximate LD_{50}. WR 2217 did produce delayed liver and renal damage but this damage might be eliminated through changes in the substitutions on the nitrogen. This promising series of compounds is not being developed further at the present time. Figure 23 represents a review of organo metalic chelates. The first three compounds are characteristic by containing 4 ligands and three metal ions. The remarkable

thing about this series is that they offer protection at .09 or less mM/kg. The nickel compounds produced depression. The second group of compounds contains 6 ligands and 3 metal ions. These compounds offer protection at .02 mM/kg which is 1/100th of the level required for activity with MEA. The third group of compounds also offer protection at levels lower than would be anticipated if MEA alone were present. While heavy metals always present problems of toxicity, the protective activity of these compounds is such that combination with other metals using the most effective thiols or combinations with other unsoluble salts might give rise to long term protection.

B. Oral Absorption in Mice

Oral administration of candidate antiradiation agents in mice is summarized in Figures 24 thru 27. Some protective activity is obtained with n-nonyl amino ethyl mercaptan if the compound is administered at 10 times the intraperitoneal dose. This compound is a potent convulsant and is not suitable for administration to higher animals. The same evidence suggestive of poor absorption is also shown by 2576. WR 2650 offers good protection at levels comparable to those used in intraperitoneal injection. This sulfonic acid group is not metabolized but will favor excretion of the agent. On the other hand conversion to sulfonamide may well lead to an auspicious series. WR 848 offers activity orally at levels comparable to the intraperitoneal dose. On Figure 25 hydroxyl derivatives are reviewed. In general it can be seen that these compounds offer some activity when administered by mouth although the dose levels are so high as to offer some question as to how easily this series may be extended to larger animals. On the other hand, chronic administration may offer some build-up of the agents. The best agent in this series is 2496 which has been ordered in large quantity from the preparations laboratory.

Figure 26 contains a review on the effectiveness of thiophosphate derivatives in protecting against radiation injury when given by mouth. With the exception of the lipid soluble 2294 all of the thiophosphate derivatives are effective. The aminoalkylamino derivatives appear to be the best absorbed and last for the longer period of time. This class of agents is receiving further development.

On Figure 27 there is a similar review for Bunte salt derivatives. It should be pointed out however that this series of compounds emphasizes the lipid soluble series in which there is essentially no oral protection. A direct comparison may be made, however, between 361 and the thiophosphate 638. Both of these chemicals are effective although 638 appears to be better absorbed. The same may be said in a comparison between 1488 and 1490.

We feel that the development of heterocycles insulated from the nitrogen of MEA or MEA Bunte may well provide compounds which are effective by mouth. We are therefore attempting to exploit this series with the expectation that we will be able to develop compounds effective at low millimolar levels when administered orally.

C. Combinations

On Figure 28 and 29 combinations of candidate anti-radiation compounds with mercaptoethylamine are reviewed. When combined with a lipid soluble derivative, 2690, there is no enhanced protection and in fact the protection activity of 2690 is essentially eliminated by the small amount of MEA. MEA does combine well with the water soluble thiols as can be seen by inspection of the results with 2694, 2347, 2846, and 2824. In terms of ability to protect against radiation, combinations with the alkyl isothioureas, 334 and 539, are superior. The most effective combination is the combination with 539 (Figure 29). Combination with the phenbutyl derivative, 2691, resulted in very little increased protection in spite of the fact that 2691 was administered at a level in the combination four times the level when given alone. The combination with the amino alkyl amino derivatives (1755) in which the carbon chain was long did not result in any increased protection over that provided by MEA alone. On Figure 30 the ability of MEA to detoxify 1607 is reviewed in detail. The LD_{50} for 1607 when given alone is approximately 10 mg/kg. Almost all animals die at a dose level of 15 mg/kg. As can be seen by the combination study 25 mg/kg of MEA results in all animals receiving 1607 surviving. The mechanism of this survival is uncertain. We feel that the compound acts by antagonizing the central depressant activity of 1607 probably by the formation of an unsymmetrical disulfide. Further combinations with 1607 are reviewed in Figures 31 and 32. Most of these studies were done using 1607

at the ineffective dose of 2.5 mg/kg or the moderately effective dose of 5 mg/kg. Combination with the lipid soluble derivatives 2691, 1818 and 2754 resulted in additive protective action. Excellent protection was obtained at 1607 if 1607 is combined with the alkyl isothiourea 539. We feel that this combination works by virtue of a pharmacologic mechanism. Combination with water soluble derivatives 215, 361, 2389 and 2347 resulted in no enhanced protection. The only activity which is observed is that which might be anticipated from the water soluble thiol alone. WR2347 does detoxify 1607 as can be seen from the data on the bottom of Figure 31. The phenomena observed with MEA and 1607 is therefore also observable with other water soluble thiols.

On Figure 32 a miscellaneous combination is presented showing that paraminopropriophenone augments the protective action of the 2-octyl Bunte. This compound is a convulsant and is not suitable for administration to larger animals, but the nature of this combination is essentially the same as that reported on Figure 31 for 1607 in combination with 539, namely anoxia is superimposed upon the protection offered by the Bunte derivative and does result in good protective action.

In Figure 33 combinations with s-ethylisothiourea are reviewed. This compound is a potent vasopressor agent but combines well to offer rodent protection with almost any anti-radiation compound. Inspection of the amount of protection offered by the individual agents alone indicates that there is a relatively small amount of protection. Combination with both water insoluble and water soluble thiols results in good protection. The pharmacology of these combinations in large animals is reviewed in Section J.

D. Dose Reduction Studies

Dose reduction studies on a number of antiradiation agents are summarized in Figures 34, 35 and 36. The conditions under which the dose reduction studies were conducted are reviewed in Figure 34. All treated animals were jointly housed with controls under the standardized conditions employed in the rest of the rodent test program. In Figure 35 dose reduction

factors of the lipid soluble Bunte derivatives are summarized. The dose reduction factors of the newly discovered derivatives 2754, 2491 and 2926 are greater than those of members of this series discovered earlier. This improvement in the dose reduction factor is a phenomenon we had not anticipated when we embarked on the development of this lipid soluble series typified by 1607 and gives rise to the hope that improved protection may be obtained even with the lipid soluble compounds. The dose reduction factors of these derivatives is now on the same order of magnitude as that obtained using MEA. On Figure 36 dose reduction factors for water soluble derivatives are reviewed. In general it can be seen that these compounds tend to have a slightly higher value than the lipid soluble compounds. The dose reduction factor for 2389 is greater than the factor for 2347. In the development of compounds for man these two compounds demonstrate the difference between the dose reduction factor and the index of interest elaborated for the discovery of interesting agents. WR 2389 has an index of 4.9 and offers no protection below the top tolerated dose. The data is reviewed in Figure 11 of this year's report. WR 2347 has an index of 13 and is reviewed on Figure 8 of last year's progress report. WR 2347 is therefore a compound exhibiting considerably greater safety than 2389. WR 2347 can be administered to dogs in amounts necessary to achieve protection (250 mg/kg) whereas 2389 is not tolerated at the level necessary to protect mice. See Figures 42 and 43. These findings demonstrate the limited value of dose reduction factors as compared to the index in developing an antiradiation agent for man.

The combination of 539 with MEA results in a dose reduction factor of two. We feel that if these combination studies were pushed it might be possible to obtain dose reduction factors in excess of two comparable to the dose reduction factors in dogs and reported in Section K. The last line on Figure 36 referring to the instability of the compounds refers our guess that the water soluble alcohol derivatives may ring close to give products which are not effective in protecting against radiation injury. Results with these combinations are therefore not reportable.

E. Ganglionic Blockade

The Large Animal Testing Section has initiated screening of candidate agents for ganglionic blocking activity -

utilizing the cat superior cervical ganglion-nictitans preparation as a test system. Ganglionic blocking activity may contribute to the hypotensive action of some protective agents. The structural modifications of existing compounds required to reduce or eliminate this side effect have been characterized. The results of evaluation are tabulated in Figures 37 thru 40. Quaternary nitrogen compounds and guanidines (e.g. AET) are well known for their blocking action. The group of agents examined in these studies are secondary and tertiary amines. Dimethyl or trimethyl substitution on the carbon in the alpha position resulted in potent ganglionic blocking activity. The addition of any larger substituents in the case of the tertiary carbon almost completely abolished blocking activity, while in the case of the quaternary carbon in the alpha position, increasing the size of the cationic head greatly reduced but did not abolish action. The effect of main chain length and constitution on ganglionic blocking activity is currently under study. These results are in general agreement with those of Mizzoni who studied the structure activity relationships of certain tris-dialkylaminoethyl amines in 1954.

F. Protection Against HN_2

A selected group of radioprotective thiols have been tested for protective action against lethal doses of nitrogen mustard (10 mg/kg mustargen) in mice. Chemicals were injected i.p. with doses known to provide protection against radiation death. Time schedules, concentrations of solutions, vehicle, pH, etc. were the same as those used in antiradiation studies.

The results are shown in Figure 41 and indicate that the water soluble thiols offer good protection against nitrogen mustard lethality. MEA is reported in the literature as having poor protective effect; we are able to confirm that it does have poor activity. The alcohol derivatives offer good protection. The lipid soluble derivatives are not effective. Protection against nitrogen mustard is considered to be an important part of the Antiradiation Drug Development Program. Nitrogen mustard therapy is used in the treatment of malignancy so that protection of normal tissues using antiradiation agents may well benefit patients receiving mustards. The administration of these drugs to such patients will provide us with much needed information on

the pharmacology of these agents in man. Such administration can take place only after preliminary animal pharmacology is established and after the demonstration (such as reported in Figure 41) that these chemicals will be useful in protecting against nitrogen mustard lethality.

G. Large Animal Testing

1. Dogs

Candidate antiradiation agents are selected for study in dogs or monkeys, if they have an index in the mouse indicating that protection can be achieved with less than the maximum tolerated dose. As a general rule the maximum tolerated dose in the dogs is approximately 1/4 of the dose tolerated in mice although there are instances when the dog tolerates as much as the mouse. Agents are also selected for large animal evaluation if they constitute a new chemical series in need of characterization. To date we have not yet found agents which exhibit protective action in dogs or monkeys when administered at levels which are ineffective in protecting mice against radiation injury. We therefore eliminate some agents for radiation testing if they appear to be poorly tolerated in dogs or monkeys.

Original problems attended with dog protection were convulsions, histamine release, hypotension, cardiac toxicity and emesis. The original agents in dogs produced convulsive activity, histamine release, hypotension or hypertension and myocardial toxicity. The compounds still produce emesis. Accordingly, a special screen for central emetic activity has been initiated. This screen involves the direct injection of candidate chemicals into the lateral ventrical of the brain. As little as 2 mg/kg of MEA introduced in this manner produces emesis. We, therefore, expect to initiate a structure versus activity emetic test in order to see if structural modification can be used to eliminate emetic activity.

The results of drug toxicity studies conducted in unanaesthetized dogs are tabulated in Figures 42 and 43. Dominant clinical observations are indicated as well as the acute mortality. In all cases the compounds were administered slowly over a 4 to 5 minute interval to maximize tolerance. Pharmacologic studies in anaesthetized dogs were also performed to characterize

the effects of the agents on arterial and venous pressure, electrocardiogram, and breathing pattern. A 4-channel Sanborn physiologic recording system was utilized for these measurements. Special studies were initiated as indicated, for example, blood oxygen saturation, blood histamine levels, etc. Gross examination of the major organs was performed in most cases following completion of the experiments. Dogs surviving the acute administration of an agent were observed for a least one week.

Irradiations were performed at the National Institutes of Health utilizing the 2.5 Mev. Van der Graaf accelerator operated as an x-ray generator with a gold target. Radiation doses are measured at the midline in air at a distance of 2 meters from the target in the case of dogs, and 1.5 meters in the case of monkeys. Dose rates of approximately 75r/min and 150 r/min respectively are obtained. Animals are always irradiated in pairs in a two compartment restraining cage without anaesthesia, one drug treated and one untreated control animal per pair. Pre- and post-irradiation clinical observations and routine hematologic examinations are performed at frequent intervals.

The results of radiation protection studies in the dog and monkey are shown in Figures 44 and 47.

WR 638 is tolerated at a slightly higher millimolar dose than MEA itself. This compound is capable of producing all of the clinical symptomatology of MEA, but the signs are delayed in onset and prolonged. The protective activity is similarly prolonged. Good protection is obtained in the dog at least as long as 60 minutes following administration before the onset of hypotension which may be either abrupt or gradual.

WR 1616 is tolerated in the dog at about the same doses as the other N-substituted alcohols. Good protection has been observed in the dog at high dose levels. It possesses ganglionic blocking activity even at sub-protective levels.

WR 2347 provides excellent protection in the dog at maximally tolerated doses. At least 15 minutes is required for full protective activity to be manifested. It lacks the large therapeutic index observed in the mouse. Preliminary studies indicate some protective activity in the monkey as well. Hypotensive

activity is present in productive doses, but appears not to be related to endogenous histamine release. This compound induces severe, prolonged emesis in dog.

WR 2578 induces hypotension, emesis, depression and convulsions which are delayed in onset and prolonged in duration. Post-mortum findings are suggestive of endogenous histamine release. Good protective activity is observed in the dog at 30 minutes post injection. The thiophosphate is much better tolerated than the corresponding thiol.

WR 2754 exhibits no evidence of protective activity in the dog at 30 mg/kg. Higher drug doses induce severe convulsant action. A great disparity exists between the protective dose for the mouse and the maximum non-convulsant dose in the dog.

2. Monkeys

The toxicity studies in monkeys are summarized in Figure 48. It can be seen that monkeys tolerate antiradiation drugs at least as well as dogs; in fact it is our impression that they generally tolerate the drugs better. Ganglionic blockade which is a predominant feature in the dog appears less obvious. In the monkey both a lipid soluble agent such as 1607 and a water soluble agent such as 2347 is well tolerated. As might be expected, monkeys are also much less prone to emesis.

Radiation studies in the monkeys are shown in Figure 49. The results of exposure of untreated animals is on the bottom of the table and as part of the regular runs. As can be seen from inspection of the data, animals survive 1000 roentgens. Radiation exposures for drug protection are now being conducted at 1200 roentgens. There is some variability of survival of control animals. We believe this variability to be the result of differences in radiation dosimetry.

Protection with 2347 was achieved although since many controlled animals survived 800 roentgens, the protection is not significant. There were two runs made using paraaminopropriophenone, parahydroxydiphenyl, MEA and cysteine.

In the first run involving an exposure of 1000 roentgens, five out of six control animals survived so that the survival in the treated animals is not significant. In the second run at 1200 roentgens all control animals died while 6 out of 10 treated animals survived. This study constitutes the first data of which we are aware indicating protection of monkeys under moderately well controlled conditions.

Summary and Conclusions:

Progress continues to be made in the development of a chemical agent to protect man against radiation injury. The toxicity associated with the lipid soluble derivatives has been reduced. At the same time there has been an increase in their dose reduction factors. The water soluble class of antiradiation agents has been expanded to include two new functional groups offering good protection, thereby increasing the flexibility of the program. Long term studies on selective agents have been initiated in preparation for studies in man.

SUMMARY OF MOUSE TESTING AT WRAIR

Total number of new agents tested	426
Agents tested by oral administration	75
Agents tested at 1/4 and 1/8 of maximum tolerated dose	14
Agents tested for duration of action	27
Agents tested for dose reduction	20
Agents tested in combination	28
Special uses (including germ free)	7
Long term administration in diet	10
Protection against nitrogen mustard	12
TOTAL (not including repeats, toxicity, etc.)	619

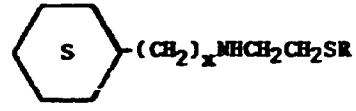
Figure 1

RESULTS ON NEW COMPOUNDS TESTED AT WRAIR

		No. of Compounds	%
1.	Good Protection vs LR (over 45% survival)	42	10
2.	Fair Protection vs LR (26-44% survival)	25	6
3.	Some Protection vs LR (less than 26%)	77	18
4.	No protection vs LR	282	66
	TOTALS	426	100

Figure 2

Figure 3

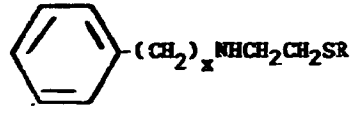


R = H

x	WR No	mM/Kg	Approx LD_{50}	Tested Dose	LD_{50}/ Tested	% Surv	Index
0	227	0.5	85	60	1.4	0	0
1	2639	0.2	75	30	2.5	0	0
2							
3							
4							
5							
6	2576	0.12	45	30	1.5	94	2.9
				15	3.0	7	3.21

R = SO_3H

x	WR No	mM/Kg	Approx LD_{50}	Tested Dose	LD_{50}/ Tested	% Surv	Index
0	892	0.49	175	100	1.75	0	0
1	2864	0.49	375	125	3.00	20	3.6
2	2571	0.62	80	50	1.6	27	2.0
		0.31		25	3.2	20	3.8
3	2942	0.36	150	100	1.5	72	2.5
		0.18		50	3.0	73	5.1
4	2691	0.07	30	20	1.5	73	2.6
		0.03		10	3.0	67	5.01
				5	6.0	13	6.8
5	2906	0.02	15	5	3.0	0	0
6	2866	0.02	15	5	3.0	53	4.6



x	WR No	mM/Kg	Approx LD_{50}	Tested Dose	LD_{50}/ Tested	% Surv	Index
0	1891	1.9	400+	300	1.3	0	0
1	217	0.54	125	90	1.4	20	1.7
2							
3	2298	0.13	75	25	3.0	0	0
4	2397	0.12	80	25	3.2	0	0
5							
6							

x	WR No	mM/Kg	Approx LD_{50}	Tested Dose	LD_{50}/ Tested	% Surv	Index
0							
1	2230	0.61	275	150	1.8	0	0
2	2456	0.2	150	50	3.0	0	0
3	2229	0.15	175	50	3.5	40	4.9
4	2754	0.4	300	120	2.5	94	4.8
				60	5.0	87	9.4
5							
6							

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$RNHCH_2CH_2SH$

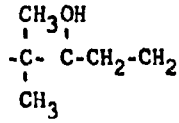
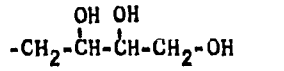
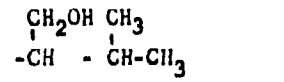
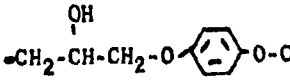
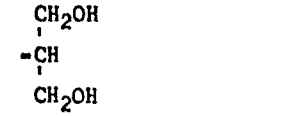
R	WR No	mM/Kg	Approx LD_{50}	Tested Dose	% Survival	Index
	2465	0.26	120	50	0	0
	2664	3.35	800+	600 300	87 53	2.5 4.1
	2846	8.3 4.2	1675	1500 750	60 33	1.8 3.0
	2922	0.8	275	200	27	1.7
	2816	0.55	225	90	0	0
	2863	0.61	125	100	0	0
	2854	0.28	150	50	0	0
	2855	0.12	75	25	0	0
	2739	0.23	150	60	0	0
	2753	3.97	1400	600 300	100 30	4.7 7.5

Figure 4

Figure 5

$H_2N(CH_2)_xNHCH_2CH_2SR$

x	R = H WR No	mM/Kg	Approx LD_{50}	Tested Dose	LD_{50}/ Tested	% Surv	Index	x	R = PO_3H_2 WR No	mM/Kg	Approx LD_{50}	Tested Dose	LD_{50}/ Tested	% Surv	Index
2	884	3.3	525	400	1.3	70	2.2	2	2578	4.0	1200	800	1.5	100	3.0
		1.7		200	2.6	20	3.1			2.0		400	3.0	100	6.0
		0.8		100	5.25	10	5.8			1.0		200	6.0	27	7.6
3	1065	1.1	225	150	1.5	85	2.8	3	2721	2.8	700	600	1.2	86	2.2
										1.4		300	2.3	87	4.3
										.7		150	4.7	40	6.5
										.35		75	9.3	20	11.2
4	1727	0.7	175	100	1.75	Sig	1.75	4	2822	1.8	800+	400	2.0	100	4.0
										0.9		200	4.0	80	7.2
5	1729	0.8	190	125	1.5	5	1.6	5	2823	2.5	900	600	1.5	87	2.8
		0.4		62.5	3.0	5	3.2			1.3		300	3.0	94	5.8
6	1562	0.7	170	125	1.4	60	2.2	6	2824	0.9	675	200	3.4	40	4.8
7	1730	0.5	150	100	1.5	0	0	7							
8	1692	0.5	175	100	1.75	5	1.8	8							
9	1731	0.5	140	100	1.4	0	0	9							
10	1732	0.3	120	75	1.6	0	0	10							
11	1755	0.2	125	50	2.5	0	0	11							
12	1893	0.2	90	50	1.8	0	0	12							

NOTE: 6 carbon Bunte showed 0% protection at 200 mg/Kg (LD_{50} 450)

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$R\text{-}NHCH_2CH_2NHCH_2CH_2SH$

R	WR No	mM/Kg	Approx LD_{50}	Tested Dose	LD_{50}/ Tested	% Survival	Index
		3.3		400	1.3	70	2.2
		1.7		200	2.6	20	3.1
H-	884	0.8	525	100	5.3	10	5.8
CH_3-	858	2.2	375	300	1.2	30	1.6
$CH_3(CH_2)_3$-	778	0.2	75	25	3	0	0
$CH_3(CH_2)_5$-	2415	0.1	45	25	1.8	0	0
$CH_3(CH_2)_7$-	2220	0.1	100	30	3.3	33	4.4
$C_2H_5\text{-}O\text{-}CH_2\text{-}CH_2$-	2477	0.1	100	20	5	0	0
 $\text{-}CH_2\text{-}CH_2$-	2404	0.09	35	20	1.8	0	0
$(C_2H_5)_2$-	857	0.2	100+	35	2.9	0	0
$HOCH_2CH_2$-	1751	3.7	800+	600 300	1.3 2.6	75 35	2.3 3.6
$CH_3CH(OH)\text{-}CH_2$-	1079	3.1	725	550	2.2	5	2.3
$CH_3\text{-}CH_2\text{-}CH(CH_2OH)$-	2414	1.3	300	250	1.2	0	0

$R\text{-}NHCH_2CH_2NHCH_2CH_2SH$

R	WR No	mM/Kg	Approx LD_{50}	Tested Dose	LD_{50}/ Tested	% Survival	Index
$(HOCH_2)_3C$-	2416	0.4	130	90	1.4	0	0
		1.8		300	1.8	73	3.1
$H_2NCH_2CH_2$-	2472	0.9	550	150	3.7	20	4.4
$H_2N(CH_2)_6$-	2363	0.1	75	25	3	0	0

Figure 7

$HSCH_2CH_2NH\text{-}R\text{-}NHCH_2CH_2SE$

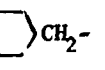
R	WR No	mM/Kg	Approx LD_{50}	Tested Dose	LD_{50}/ Tested	% Survival	Index
$-(CH_2)_2-$	1728	0.3	120	50	2.4	0	0
$-(CH_2)_3-$	2665	0.8	250	150	1.7	93	3.3
				75	3.3	20	4.0
$-CH_2-CH-$ CH_3	2802	0.3	150	60	2.5	40	3.5
		0.15		30	5.0	27	6.4
$-(CH_2)_4-$	2209	0.6	180	125	1.4	70	2.4
		0.3		62.5	2.9	46	4.2
$-CH-CH-$ $CH_3\ \ CH_3$	2847	0.3	70	60	1.2	7	1.2
$-(CH_2)_5-$	2715	0.4	150	100	1.5	27	1.9
$-(CH_2)_7-$	2497	0.2	75	50	1.5	0	0
$-(CH_2)_8-$	1894	0.2	100	50	2.0	0	0
$-(CH_2)_9-$	2239	0.07	80	20	4.0	0	0
$-(CH_2)_{10}-$	2384	0.09	65	25	2.6	0	0
$-(CH_2)_{11}-$	2606	0.08	65	25	2.6	0	0
$-(CH_2)_{12}-$	2235	0.1	65	38	1.7	0	0
$-CH_2$  CH_2-	2238	0.1	45	25	1.8	7	1.9

Figure 8

Figure 9

$HSCH_2CH_2NH\text{-}R\text{-}NHCH_2CH_2SH$

R	WR No.	mM/Kg	Approx LD_{50}	Tested Dose	LD_{50}/ Tested	% Survival	Index
$-CH_2-\underset{OH}{CH}-CH_2-$	1901	0.7	250	150 75	1.67 3.33	73 13	2.9 3.8
$-CH_2-\underset{O-CH-CH_3}{CH}-CH_2-$	2701	0.3	95	60	1.6	0	0
$-CH_2-\underset{OH}{CH}-\underset{OH}{CH}-CH_2-$ MESO	342	0.8	300	200	1.5	75	2.6
$-CH_2-\underset{OH}{CH}-\underset{OH}{CH}-CH_2-$ DL	2494	0.2	150	50	3.0	0	0
$-CH_2-\underset{OH}{CH}-CH_2-O-\underset{OH}{CH}-CH_2$	2495	0.2	75	50	1.5	0	0
$-CH_2-CH_2-O-CH_2-\underset{OH}{CH}-CH_2-$	2666	0.2	140	50	2.8	7	3.0
$-(CH_2)_6-O-CH_2-\underset{OH}{CH}-CH_2-$	2750	0.06	35	20	1.75	0	0
$-(CH_2)_3-O-(CH_2)_2-O-(CH_2)_2-O-(CH_2)_3-$	2383	0.2	80	50	1.6	7	1.7

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R_1	R_2	WR No	mM/Kg	Approx LD_{50}	Tested Dose	LD_{50}/ Tested	% Survival	Index
CH_3-	-H	186	1.9	300	175	1.7	20	2.0
CH_3CH_2-	-H	147	1.4	300+	150	2	33	2.66
$CH_3CH_2CH_2$-	-H	621	0.8	140	100 50	1.4 2.8	60 60	2.2 4.5
$(CH_3)_2CH$-	-H	651	1.3	160	150	1.1	50	1.6
$CH_3(CH_2)_5$-	-H	781	0.2	75	25	3	0	0
$CH_3(CH_2)_{16}$-	-H	2811	0.3	350	80	4.4	20	5.2
$(CH_3)_2CHCH_2$-	$-PO_3H$	2378	0.5	250	100	2.5	0	0
$HOCH_2$-	$-SO_3H$	1986	4.3	1200	800	1.5	13	1.7
HO-CH_2-CH_2-	-H	1996	4.1	800	500	1.6	80	2.9
HS-CH_2-CH_2-	-H	2704	0.3	70	40	1.8	7	1.9
H_2NCHCH_2-S-SO_3H	$-SO_3H$	2717	1.3	500+	400	1.2	7	1.8
HO_3S_2-CH_2-	NH_2 CH-CH_2-CH_2- $-SO_3H$	2933	0.2	175	60	2.9	7	3.1

Figure 10

	WR No	mM/Kg	Approx LD_{50}	Tested Dose	LD_{50}/ Tested	Z Survival	Index
$H_2N-C(CH_3)_2-CH_2SH$	339	1.0	350	100	3.5	Sig	3.5
$H_2N-C(CH_3)_2-CH_2-SH$ $CH_2CH_2CH_3$	2720	0.45	150	100	1.5	0	0
$H_2N-C(CH_3)_2-CH_2-S_2O_3H$ $CH(CH_3)_2$	2293	0.8	400+	150	2.7	0	0
$H_2N-C(CH_3)_2-CH_2S_2O_3H$ $(CH_2)_5CH_3$	2381	0.4	180	100	1.8	0	0
$H_2N-C(CH_2OH)_2-CH_2SH$	2389	7.3	1600+	1000 500	1.6 3.2	94 53	3.1 4.9

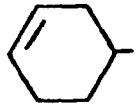
Figure 11

	WR No	mM/Kg	Approx LD_{50}	Tested Dose	LD_{50}/ Tested	% Survival	Index
	2562	0.5	125	50	2.5	0	0
	2649	1.4	800	300	2.7	87	5.0
				150	5.3	47	7.8
	2648	0.7	150	100	1.5	0	0
	2579	0.9	300	200	1.5	40	2.1
				100	3	7	3.2

Figure 12

Figure 13

$NH_2CH_2-\underset{\displaystyle R}{\underset{|}{C}}H\ SH$

R	WR No	mM/Kg	Approx LD_{50}	Tested Dose	LD_{50}/ Tested	% Survival	Index
CH_3- d,l form	166	3.1 1.6	425	300 150	1.4 2.8	66 40	2.3 4.0
CH_3- l form	2470	2.2	650	200	3.2	33	4.3
CH_3- d form	2452	2.2	375	200	1.9	53	2.9
CH_3CH_2-	780	1.9	400+	200	2.0	Sig	2
$CH_3(CH_2)_2$-	1554	0.6	160	75	2.1	5	2.2
$CH_3(CH_2)_3$-	1568	0.4	120	50	2.4	0	0
$CH_3(CH_2)_4$-	1569	0.3	85	50	1.7	0	0
$CH_3(CH_2)_5$-	847	0.3	75	50	1.5	5	1.6
$CH_3(CH_2)_6$-	1696	0.3	120+	60	2	Sig	2
$HS(CH_2)_3$-	2540	0.3	90	50	1.8	7	1.9
	2123	0.2	45	25	1.8	0	0

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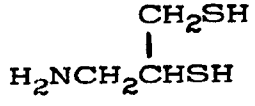
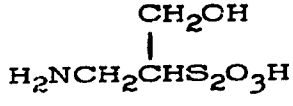
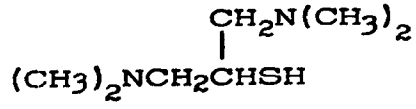
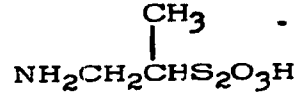
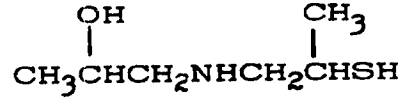
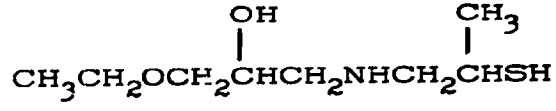
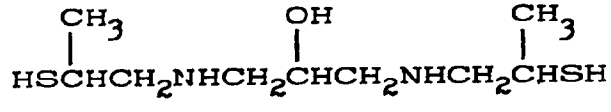
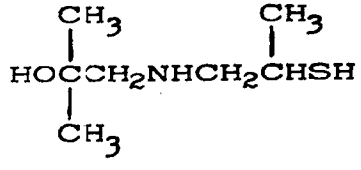
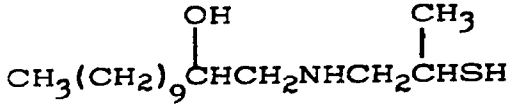
	WR#	mM/Kg	Approx. LD_{50}	Tested Dose	LD_{50} Tested	% Surv	Index
	2623	0.4	65	50	1.3	0	0
	2145	4.8	1100	900	1.2	7	1.3
	2622	0.03	20	6	3.3	0	0
	2748	1.2	200+	200	1	0	0

35

Figure 14

Figure 15

	WR#	mM/Kg	Approx. LD_{50}	Tested Dose	LD_{50}/ Tested	% Surv	Index
	2516	4.7	800+	800	1	7	1.1
	2277	1.3	350	200	1.8	7	1.9
	2624	1.0	375	200	1.9	0	0
	2557	0.4	250	100	2.5	7	2.7
	2455	1.8	500+	300	1.7	13	1.9
	2703	0.3	90	25	1.3	7	1.4

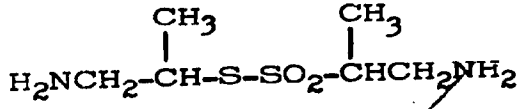
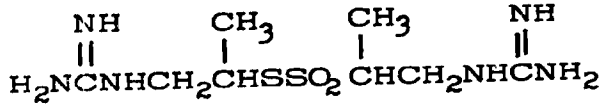
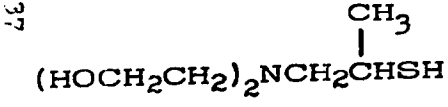
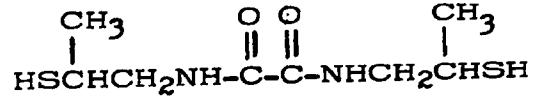
	WR#	mM/Kg	Approx. LD_{50}	Tested Dose	LD_{50}/ Tested	% Surv	Index
	2225	0.9	275	200	1.4	0	0
	2801	0.2	250+	50	5	0	0
	2454	2.8	1000+	500	2	7	2.1
	2278	2.1	1200+	500	2.4	0	0

Figure 16

	WR No	mM/Kg	Approx LD_{50}	Tested Dose	LD_{50}/ Tested	% Survival	Index.
	1553	1.0	350	100	3.5	Sig	3.5
	1726	0.8	240	100	2.4	0	0
	2127	0.9	240	100	2.4	0	0
	1987	0.2	75	30	2.5	0	0
	2086	0.6	140	75	1.9	0	0
	2088	0.3	120	40	3.0	0	0

Figure 17

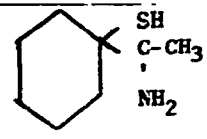
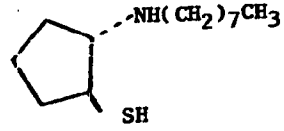
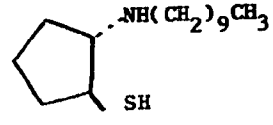
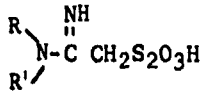
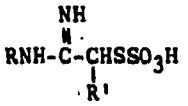
R_1	R_2	WR No	mM/Kg	Approx LD_{50}	Tested Dose	LD_{50}/ Tested	% Survival	Index
CH_3-	-CH_3	364	2.8	350	300 150	1.2 2.3	30 10	1.5 2.6
CH_3CH_2 CH_3 >	-H	2203	0.6 0.3	125	75 37.5	1.7 3.4	0 7	0 3.2
$CH_3CH_2CH_2$-	-CH_3	1819	0.4	130	50	1.6	0	0
$CH_3(CH_2)_3$-	-CH_3	2091	0.3	60	40	1.5	0	0
$CH_3(CH_2)_2$-	-CH_2CH_3	2004	0.17	75	25	3.0	0	0
$(CH_3CH_2)_2$-	-CH_3	2205	0.5	110	75	1.5	0	0
H-	CH_3 CH_2SH >	1621	0.06	15	7.5	2.0	0	0
		2207	0.6	150	100	1.5	0	0
		2872	0.2	90	50	1.8	0	0
		2871	0.1	60	30	2.0	0	0

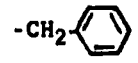
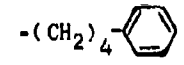
Figure 18

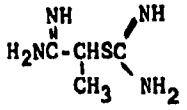


R (R' = H)	WR No	mM/Kg	Approx LD_{50}	Tested Dose	% Surv	Index
H	1551	0.18	60	30	Sig	2.0
$-CH_3$	1757	0.41	100	75	40	1.8
$-C_2H_5$	1758	0.50	190	100	60	3.0
$-(CH_2)_2CH_3$	2708	0.4	150	90	27	2.1
$-CH(CH_3)_2$	2848	0.5	150	100	0	0
$-(CH_2)_3CH_3$	2726	0.2	75	50 25	27 7	1.9 3.2
$-CH_2CH(CH_3)_2$	2727	0.4	175	100	53	2.7
$-{}_nC_5H_{11}$	2049	0.21	85	50	33	2.2
$-(CH_2)_8CH_3$	2706	0.008	4	2.5	0	0
$-(CH_2)_9CH_3$	2608	0.006	3.5	2	0	0
$-CH_2$- 	1756	0.10	85	25	Sig	3.4
$-(CH_2)_2$- 	1867	0.07	35	20	45	2.0
$-(CH_2)_3$- 	2707	0.1	30	12.5	0	0
$-(CH_2)_4$- 	2743	0.07	35	20 10	33 47	2.3 5.1
R = R'						
$-CH_3$	1868	0.45	140	90	Sig	1.7
	2185	0.42	125	100	0	0

Figure 19

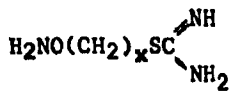


R	R'	WR No.	mM/Kg	Approx LD_{50}	Tested	% Survival	Index
H	$-CH_3$	1552	0.54	160	100	60	2.5
	$-CH_3$	2187	0.18	110	50	0	0
	$-CH_3$	2050	0.47	160	100	0	0
H		2186	0.16	45	40	0	0
	$-CH_3$	2946	0.04	20	12.5	0	0
$-(CH_2)_3CH_3$	$-CH_3$	2947	0.4	125	100	0	0



1870	2.53	425	375	0	0

Figure 20



x	WR No.	mM/Kg	Approx LD_{50}	Tested	% Survival	Index
2	1550	1.85	450	250	85	3.3
3	1988	1. ..	500	200	33	3.2
		0.67		100	7	5.3
4	2248	2.75	600	450	7	1.4
5	2249	2.26	450	400	7	1.1
6	2343	0.26	125	50	0	0

Figure 21

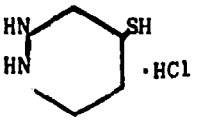
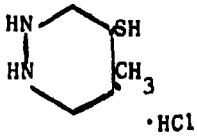
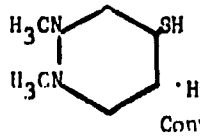
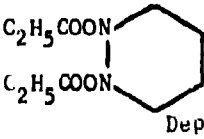
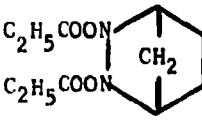
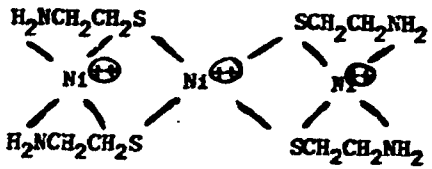
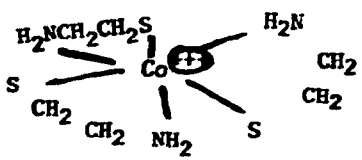
	WR No.	mM/Kg	Approx LD_{50}	Tested Dose	% Surv.	Index
 Depressant	1989	6.6	800	800 750 375	83 73 33	1.8 1.8 2.8
 Convulvant	1990	0.3	75	40	0	0
 Convulsant-Depressant	2217	0.3	700	50	7	15
 Depressant	2215	0.4	700	100	0	0
 Convulsant-Depressant	2212	0.6	275	150	0	0

Figure 22

Figure 23

WR No.	Compound	Tested Dose mM/kg	Tested Dose mg/kg	Results	Sol.	Sx
1169		0.054	25	Sig 600	Sl. Sol.	No Convul. Ataxia Depression
1282		0.016	7.5	No	Sol.	Convul. Depression
1283		0.086	40	20%	Sol.	Depression Lacrimation
1285	6 MEA 	.011	7.5	Sig 600	Sol.	Convul. Cyanosis
1284	6 MEA 	.017	10	20%	Sol.	Late depression
1171		0.24	50	20%	Sl. Sol. in hot H_2O	Late depression
1170		0.35	100	50%	Sl. Sol. in hot H_2O	Sl. depression Liver tox.

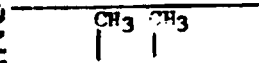
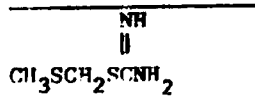
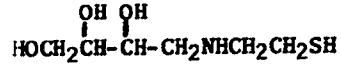
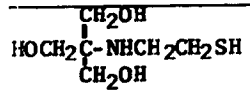
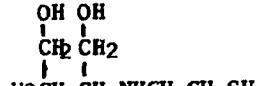
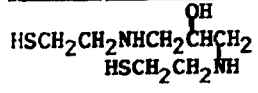
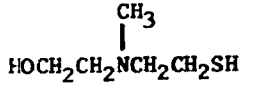
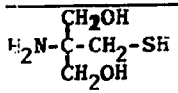
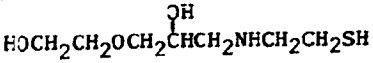
	WR No	IP Approx LD_{50}	IP Dose	IP Time	IP %	PO Approx LD_{50}	PO Dose	PO Time	PO %
$CH_3NHCH_2CH_2SH$	2113	400+	200	30	94	1225	500	30 60	0 0
$CH_3(CH_2)_8NHCH_2CH_2SH$	1547	125	25	15	80	300+	250	15 30	27 40
	2576	45	30	15	94	800	250	30	10
$H_2NCH_2CH_2SSCH_2CH_2NH_2$	2676	275	150	30	80	900	500	15 30	7 46
	2189	525	350	15	94	1250	500	60 120	0 0
$NH_2CH_2CH_2SO_2SCH_2CH_2NH_2$	728	750	300	15	90	3000	1000	30 60	40 30
$NH_2CH_2CH_2NHCH_2CH_2NHCH_2CH_2SH$	2472	550	300	15	73	3500	2000	30 60	7 0
$HSCH_2CH_2NH(CH_2)_3NHCH_2CH_2SH$	2665	250	150	30	93	400	250	15 30	0 26
$HSCH_2CH_2NHCH_2CH_2CH_2SO_3H$	2650	2000+	2000	30	53	3000+	3000	30	60
	848	125	100	15	70	450	200	15 30	0 20

Figure 24

Figure 25

	WR No	IP Approx LD_{50}	IP Dose	IP Time	IP %	PO Aporox LD_{50}	PO Dose	PO Time	PO %
	727	475	300	15	75	2000	750	15 30 60	0 10 10
	2753	1400	600	30	100	2500	2000	30 60 120	90 30 0
	2846	1650	1500	30	90	3000	2000	30 60	0 0
	1616B	1000+	750	30	60	5000	4000	30 60 120	0 0 0
	2347	1500	1200	30	100	5000	3000	15 30 60 120	7 0 0 0
	2664	800+	600	30	87	1800	1000	15 30	27 0
	1901	250	150	15	73	800	400	30 60 90	20 33 7
	888B	500+	250	15	50	1200	700	15 30 60	10 0 0
	2389	1500	1000	15	94	4500	2000	15 30 60	6 42 10
	2496	1400	1000	15	67	5500	3000	30 90	55 40

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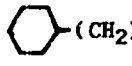
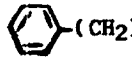
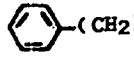
	WR No	IP Approx LD_{50}	IP Dose	IP Time	IP %	PO Approx LD_{50}	PO Dose	PO Time	PO %
$H_2NCH_2CH_2SPO_3H_2$	638B	700	400	30	100	900	500	15 30	13 33
$H_2NCH_2CH_2NHCH_2CH_2SPO_3H_2$	2578	1200	800	30	100	3500	1500	30 60 120	70 50 20
$H_2NCH_2CH_2CH_2NHCH_2CH_2SPO_3H_2$	2721	700	600	30 60	86 100	1400	700	60 120 180	90 60 50
$H_2NCH_2CH_2CH_2NHCH_2CH_2SPO_3H_2$	2822	800+	400	30	100	800	600	15 30 60 120	0 14 40 20
$H_2N(CH_2)_5NHCH_2CH_2SPO_3H_2$	2823	900	600	30	86	900	750	15 60	9 76
$H_2NC(CH_3)_2-CH_2SPO_3H_2$	1490	750	600	30	95	3000	1500	30 60	27 20
$CH_3(CH_2)_9NHCH_2CH_2SPO_3H_2$	2294	125	50	15	67	550	250	60 120	0 0

Figure 26

Figure 27

	WR NO	IP Approx LD_{50}	IP Dose	IP Time	IP %	PO Approx LD_{50}	PO Dose	PO Time	PO %
$H_2NCH_2CH_2SSO_3H$	361	750+	450	15	55%	2000+	1000	15 30	53 27
$H_2NC(CH_3)_2CH_2SSO_3H$	1488	800+	600	15	80	4000+	3000	30 60	0 7
$CH_3(CH_2)_8NHCH_2CH_2SSO_3H$	1818	25	15	30	85	1750	1000	30	55
$CH_3(CH_2)_5CH(CH_3)NHCH_2CH_2SSO_3H$	1606	135	75	30	100	2000+	1500	30 60	0 0
$CH_3(CH_2)_6CH(CH_3)NHCH_2CH_2S_2O_3H$	2709	50	22.5	30	94	800+	800	30 60	0 20
$CH_3(CH_2)_6CH(CH_3{-}CH_2)NHCH_2CH_2SSO_3H$	2390	125	25	15	80	500	400	60 120	20 0
	2691	35	10	15	67	800+	800	30 60	0 0
	2229	150	50	30	40	600+	400	120	0
	2754	200	120	30	93	3000+	2000	30 60	0 0

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COMBINATIONS WITH 347

$H_2NCH_2CH_2SH$

Structure	WR No	Individual Agents Mg/Kg	% Prot	Combined Mg/Kg	% Prot
$H_2NCH_2CH_2SH$	347	75	20		
					10
$CH_3CH_2C(CH_2CH_3)_2CH_2NHCH_2CH_2S_2O_3H$	2690	50	40		
	347	75	20		
					50
$HSCH_2C(NH_2)(O)C(=O)OH\cdot HSCH_2$	2694	50	10		
	347	75	20		
					67
$CH_2=C(CH_3)CH_2-S-C(=NH)-NH_2$	334	30	0		
	347	75	10		
					50
$HO-CH(CH_2OH)-CH(CH_2OH)-NHCH_2CH_2SH$	2347	300	0		
	347	75	20		
					60
$HOCH_2CH(OH)CH(OH)CH_2NHCH_2CH_2SH$	2846	375	20		
	347	75	10		
					100
$H_2N(CH_2)_6NHCH_2CH_2SPO_3H_2$	2824	200	60		

Figure 28

COMBINATIONS WITH 347

$H_2NCH_2CH_2SH$

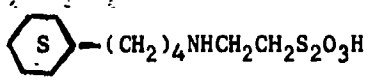
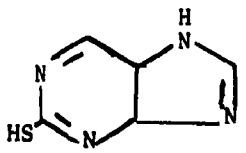
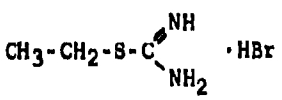
Structure	WR No	Individual Agents MR/kg	% Prot	Combined MR/kg	% Prot
$H_2NCH_2CH_2SH$	347	75	0	75	50
	2691	10	30	40	
	347	75	20		40
	1137	200	0		
$H_2N(CH_2)_{11}NHCH_2CH_2SH$	347	75	30		30
	1755	39	0		
	347	75	20		100
	539	15	0		

Figure 29

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(IP) Toxicity WR 1607

Mg/Kg	10-d Mortality
15	$32_{/35}$
10	$7_{/15}$
5	$0_{/40}$

Toxicity of WR-1607 + MEA

Mg/Kg 1607	Injection Time prior to MEA	Mg/Kg MEA	10-d Mortality
5	15 min	75	0/5
		150	0/5
10	15 min	75	0/5
		150	0/5
	60 min	75	0/5
		50	0/5
15	15 min	25	0/5
		50	0/5
		75	0/5
		150	0/5
15	15 min	250	2/5
	control	250	2/5
15	30 min 60 min 90 min	150	0/5 0/5 2/4

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Figure 30

COMBINATIONS WITH 1607

$CH_3(CH_2)_9NHCH_2CH_2S_2O_3H$

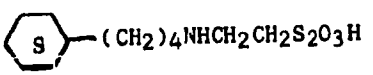
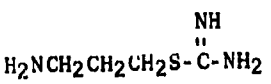
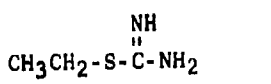
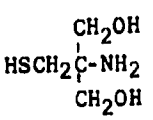
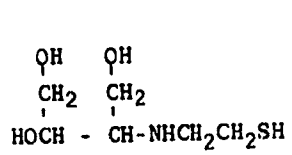
Structure	WR No	Individual Agents Mg/Kg	% Prot	Combined Mg/Kg	% Prot
$CH_3(CH_2)_9NHCH_2CH_2S_2O_3H$	1607	2.5	0		71
	2691	10	78		
	1607	2.5	10		40
	215	50	30		
	1607	2.5	10		80
	539	5	0		
$H_2NCH_2CH_2S_2O_3H$	1607	2.5	10		10
	361	100	0		
$CH_3(CH_2)_8NHCH_2CH_2S_2O_3H$	1607	2.5	0		60
	1818	2.5	20		
	1607	5	40		20
	2389	250	10		
	1607	5	90	50	10
	2347	300	0	300	

Figure 31

COMBINATIONS WITH 1607

$CH_3(CH_2)_9NHCH_2CH_2S_2O_3H$

Structures	WR No	Individual Agents Mg/Kg	% Prot	Combined Mg/Kg	% Prot
$CH_3(CH_2)_9NHCH_2CH_2S_2O_3H$	1607	2.5	0		70
	2754	50	40		

MISCELLANEOUS COMBINATION

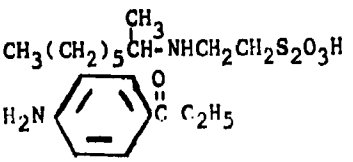
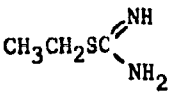
	1606	25	30		90
	302	25	20		

Figure 32

COMBINATIONS WITH 539



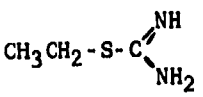
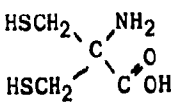
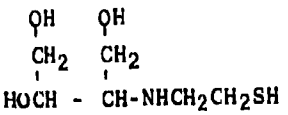
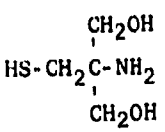
Structures	WR No	Individual Agents Mg/Kg	% Prot	Combined Mg/Kg	% Prot
	539	20	0	90	
	2694	50	10		
					
	539	5	0	80	
$CH_3(CH_2)_9NHCH_2CH_2S_2O_3H$	1607	2.5	10		
	539	25	0	80	
	2347	150	0		
	539	15	0	100	
	2347	300	20		
	539	15	0	80	
	2389	125	0		

Figure 33

TABLE OF EXPOSURE CONDITIONS FOR DOSE REDUCTION STUDIES

Control Points		R_X	
X	γ	X	γ
550r	600r	825r	1000r
600	650	925	1100
650	700	1025	1200
700	750	1125	1300
750	800	1225	1400
	850		1500
	900		1600
	950		1700
	1000		1800

Rate of Rad: 25-50r/min. 300KV 2mm Cu x-ray
100r/min. Co^{60}

Animals/point 10-20 depending on drug availability

$LD_{50/30}$ X = 580r
γ = 750r

Drug Administration: Acute IP under conditions identical to inital test. at the maximum drug level tolerated.

Figure 34

SUMMARY OF DOSE REDUCTION FACTORS

$RNHCH_2CH_2S_2O_3H$

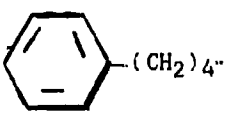
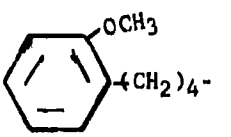
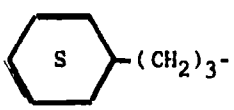
R-	WR No	DRF
$CH_3(CH_2)_4CH(CH_3)-$	1606	1.5
$CH_3(CH_2)_5CH(CH_3)-$	2709	1.5
$CH_3(CH_2)_5CH(C_2H_5)-$	2926	1.8
$CH_3(CH_2)_9-$	1607D	1.4
	2754	1.85
	2941	1.80
	2942	1.4

Figure 35

SUMMARY OF DOSE REDUCTION FACTORS

COMPOUND	WR No	DRF
$HOCH_2CH_2NHCH_2CH_2SH$	698-E	1.6
$CH_3CH(OH)CH_2NHCH_2CH_2SH$	727	1.52
$(HOCH_2)_2CHNHCH_2CH_2SH$	2753	1.7
$H_2NC(CH_2OH)_2CH_2SH$	2389-B	1.87
$HOCH_2CH(OH)CH(CH_2OH)CH_2NHCH_2CH_2SH$	2347-C	1.53
$H_2N(CH_2)_2NH(CH_2)_2SPO_3H_2$	2578-A	1.86
$CH_3CH_2SC(=NH)NH_2$	539	1.6
$CH_3CH_2SC(=NH)NH_2$ (539) + $H_2NCH_2CH_2SH$ (347)	539 + 347	2.0

Five additional DRF's were determined but the stability of the chemical is questioned.

Figure 36

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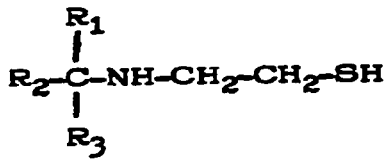
Figure 37

BIOASSAY FOR GANGLIONIC BLOCKING ACTIVITY

(Superior Cervical Ganglion of the Cat)



WR#	R_1	R_2	R_3	ACTIVITY	
2003	$-CH_2CH_3$	CH_2OH	CH_2OH	Ganglionic Block	10 mg/Kg
2347	$-CH(OH)-CH_2OH$	CH_2OH	H	Ganglionic Block	100 mg/Kg
1787	$-C(CH_3)_2-NH-C(CH_3)_2-CH_2OH$	H	H	Ganglionic Block	3 mg/Kg

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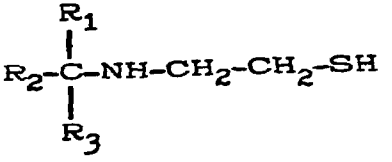
BIOASSAY FOR GANGLIONIC BLOCKING ACTIVITY

(Superior Cervical Ganglion of the Cat)



WR#	R_1	R_2	R_3	ACTIVITY	
1616	CH_2OH	CH_2OH	CH_2OH	Ganglionic Block	35 mg/Kg
1788	CH_2OH	CH_2OH	CH_3	Ganglionic Block	15 mg/Kg
1780	CH_2OH	CH_3	CH_3	Ganglionic Block	15 mg/Kg
1512	CH_3	CH_3	CH_3	Ganglionic Block	1 mg/Kg
2753	CH_2OH	CH_2OH	H	Partial Block	75 mg/Kg
2112	CH_2OH	CH_3	H		
691	CH_3	CH_3	H	Ganglionic Block	3 mg/Kg
698	CH_2OH	H	H	No Block	
889	CH_3	H	H		

Figure 38

BIOASSAY FOR GANGLIONIC BLOCKING ACTIVITY

(Superior Cervical Ganglion of the Cat)

WR#	R	ACTIVITY	
662	H	No block	
1616	$-CH_2-CH_2-SH$	Ganglionic Block	35 mg/Kg
2416	$-CH_2-CH_2-NH-CH_2-CH_2-SH$	Ganglionic Block	

Figure 39

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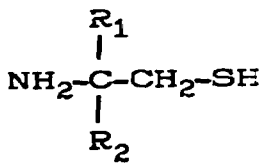
BIOASSAY FOR GANGLIONIC BLOCKING ACTIVITY

(Superior Cervical Ganglion of the Cat)



WR#	R_1	R_2	ACTIVITY - Dose to Cause 50% Relaxation of Electrically Stimulated Nictitans	
2389	CH_2OH	CH_2OH	Ganglionic Block	25 mg/Kg
339	CH_3	CH_3	Spontaneous Contraction of Unstimulated Nictitans	5 mg/Kg
186	CH_3	H		
347	H	H	Partial Block	100 mg/Kg

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Figure 40

PROTECTION AGAINST NITROGEN MUSTARD LETHALITY

(All Animals Received 10Mg/Kg Nitrogen Mustard IP)

WR No	Compound	Mg/Kg	% Surv
347	$H_2NCH_2CH_2SH$	150	0
298	$H_2NCH_2CH_2SC(=NH)NH_2$	150	80
2389	$H_2NC(CH_2OH)_2CH_2SH$	1000	86
698	$HOCH_2CH_2NHCH_2CH_2SH$	400	87
2753	$(HOCH_2)_2CHNHCH_2CH_2SH$	10	86
2347	$HOCH_2CH(OH)CH(CH_2OH)NHCH_2CH_2SH$	1000 500 250	86 67 20
1616	$(HOCH_2)_3C\text{-}NHCH_2CH_2SH$	750	86
1901	$HOCH(CH_2NHCH_2CH_2SH)_2$	150	33
2209	$HSCH_2CH_2NH(CH_2)_4NHCH_2CH_2SH$	125	20
1547	$CH_3(CH_2)_8NHCH_2CH_2SH$	25	0
228	$CH_3(CH_2)_9NHCH_2CH_2SH$	25	0
1552	$HN\text{-}C(=NH)\text{-}CH(CH_3)\text{-}S_2O_3H$	100	20

Figure 41

TOXICITY OF CANDIDATE ANTIRADIATION CHEMICAL AGENTS

IN THE DOG

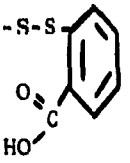
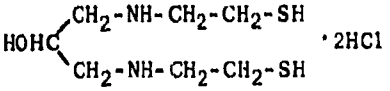
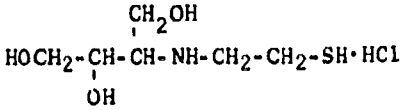
WR No	Compound	Dose Mg/Kg (Free Base)	Route	Vehicle	pH	Number of Deaths	Emesis	Diarrhea	Hypotension	Hypertension	Depressant	Convulsant
65	$H_2N-CH_2-CH_2OH$	100	IV	Water	7.5	0/1				+		
		200				0/1	+	+		+	+	
		300				0/1				+		
		400				0/1				+		
		600				0/2	+	+		+		
		650				1/1				+	+	
88	CH-CH-CH_2-SO_3Na (SH SH)	100	IV	Water	7.4	0/1			+		+	+
		150				1/1	+		+		+	+
		200				3/3	+		+		+	+
		300				1/1	+		+		+	+
		400				1/1	+		+		+	+
638	$H_2N-CH_2-CH_2-SPO_3H$	300	IV	Water	7.4	0/1	+		+		+	+
		400				1/1	+	+	+		+	+
698	$HO-CH_2-CH_2-NH-CH_2-CH_2-SH \cdot HCl$	200	IV	Water	7.2	0/2	+		+		+	+
		300				2/2	+		+		+	+
1616	$(HOH_2C)_3-C-NH-CH_2-CH_2-SH \cdot HCl$	300	IV	Water	7.4	0/3	+		+		+	+
		350				1/1			+		+	+
		500				1/1	+		+		+	
1886	$H_2N-CH_2-CH_2-S-S-$ 	75	IV	Water	7.0	1/2			+		+	+
		100				1/1			+		+	+
		150				1/1			+		+	+
1901	 $\cdot 2HCl$	50	IV	Water	7.4	0/1	+		+		+	
		60				1/1	+		+		+	
		75				1/1	+		+		+	+
		100				1/1			+		+	
2347	 $\cdot HCl$	250	IV	Water	4.0	0/2	+		+		+	
		300			or	2/5	+				+	
		350			6.5	1/3	+		+		+	
		400				1/1	+		+		+	

Figure 42

TOXICITY OF CANDIDATE ANTIRADIATION CHEMICAL AGENTS

IN THE DOG

CONFIDENTIAL

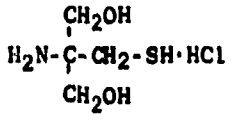
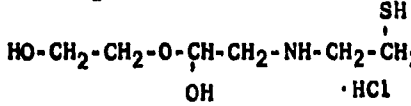
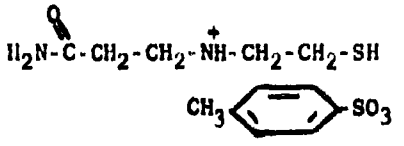
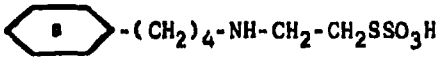
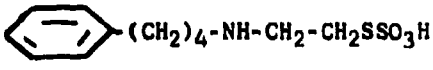
WR No	Compound	Dose Mg/Kg (Free Base)	Route	Vehicle	pH	Number of Deaths	Emesis	Diarrhea	Hypotension	Hypertension	Depressant	Convulsant
2389		500	IV	Water	5.6	2/2	+		+		+	
		750				1/1	+		+		+	+
2496		300	IV	Water	6.5	0/1	+		+		+	
		285	Oral	Water	6.5	0/1	+					
2529		150	IV	Water	7.2	0/1	+		+		+	
		200				2/2	+		+		+	+
		250				1/1	+		+		+	±
		250	Oral	Water	7.2	0/1	+					
2578	$H_2N-CH_2-CH_2-NH-CH_2-CH_2-SPO_3H$	300	IV	Water	8.0	0/2	+	+	+		+	
		400				1/1	+		+		+	
		500				1/1	+		+		+	
		600				2/2	+	+	+		+	
		700				0/1	+		+		+	
		800				1/1	+	+	+		+	
2691		10	IV	MC	Unadj	0/2	+	±	+		+	
2754		30	IV	Water	11.8	0/1	+			+	+	
		45				0/1	+			+	+	+
		60				1/1	+		+		+	+

Figure 43

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RADIATION PROTECTION STUDIES IN THE DOG

450r WHOLE-BODY 2.5 MEV. X-IRRADIATION

(1 July 1963 to 30 June 1964)

WR No	Chemical Structure	Dose of Agent (Mg/Kg)	Route	Time Before Rad	Deaths Due to Chemical Toxicity	Time of Death (Days Post Rad) Controls	Protected	30-Day Surv Cont	Prot
638	$H_2N-CH_2-CH_2-SPO_3H$	300	IV	30 min	1/5	12,12,14 14,15	0	0/5	4/4
1616	$(HOH_2C)_3-C-NH-CH_2-CH_2-SH$	250	IV	5 min	1/6	12,13,14, 15,15	0	0/5	5/5
2347	$HOH_2C-\underset{\underset{OH}{|}}{CH}-\underset{\underset{CH_2OH}{|}}{CH}-NH-CH_2-CH_2-SH$	150	IV	15 min	0/6	9,9,12, 13,14,19	8,15,15, 16,16,20	0/6	0/6
		200	IV	15 min	0/6	8,10,12, 13,14,19	16,21	0/6	4/6
		250	IV	15 min	2/6	10,12,12, 12,12,12	1,1	0/6	4/4
		250	IV	3 min	1/6	13,13,13, 14,14,16	0,16	0/6	4/5
2578	$H_2N-CH_2-CH_2-NH-CH_2-CH_2-SPO\ H$	300	IV	30 min	1/5	11,11,13, 19	1	0/5	4/4
2754	 $-(CH_2)_4-NH-CH_2-CH_2SSO_3H$	30	IV	15 min	0/6	10,12,12, 14,14,16	11,12,13 14,15,15	0/6	0/6

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Figure 44

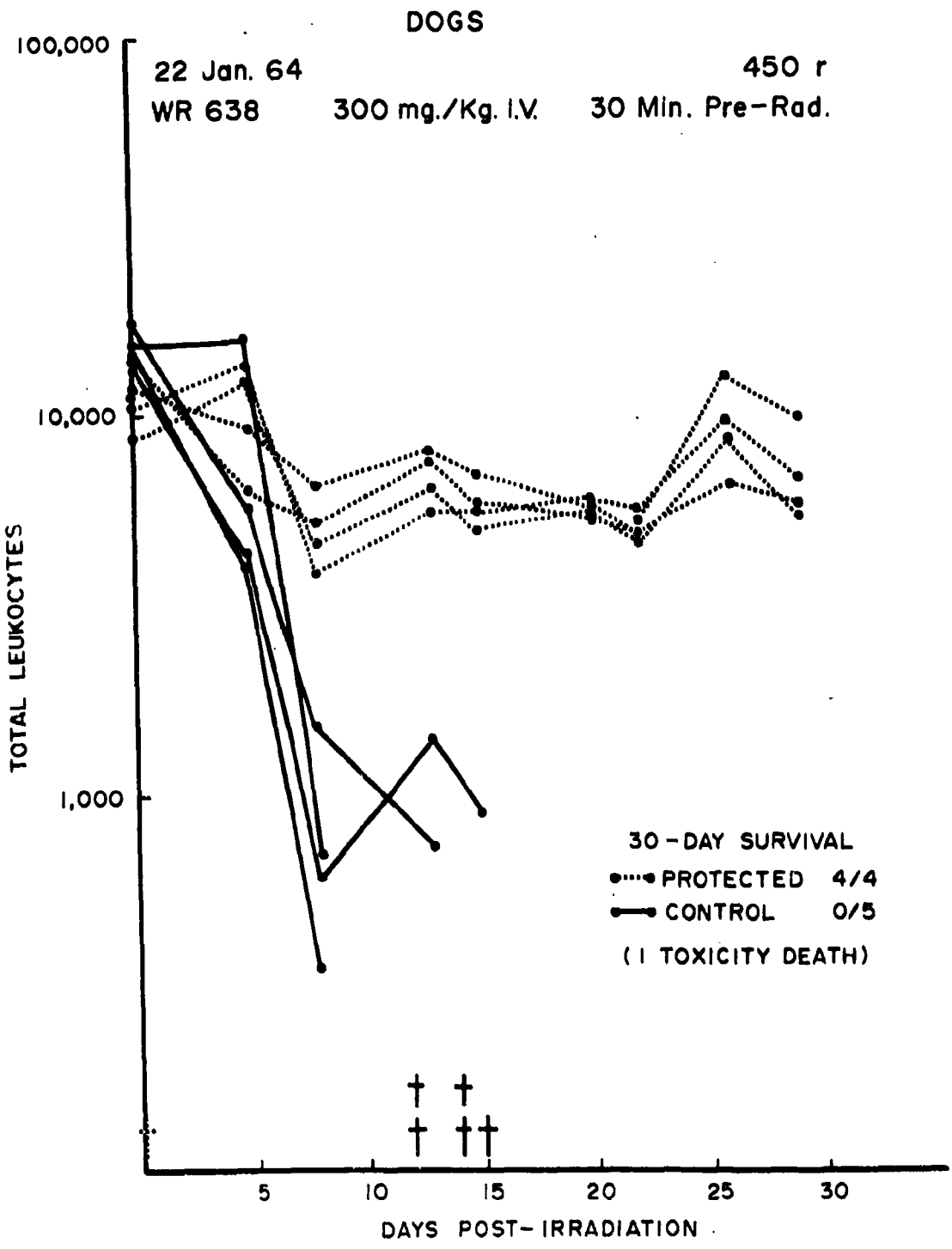
DOGS
22 Jan. 64
WR 638
300 mg./Kg. I.V.
450 r
30 Min. Pre-Rad.
100,000
10,000
1,000
TOTAL LEUKOCYTES
30-DAY SURVIVAL
PROTECTED 4/4
CONTROL 0/5
(1 TOXICITY DEATH)
5
10
15
20
25
30
DAYS POST-IRRADIATION

Figure 45

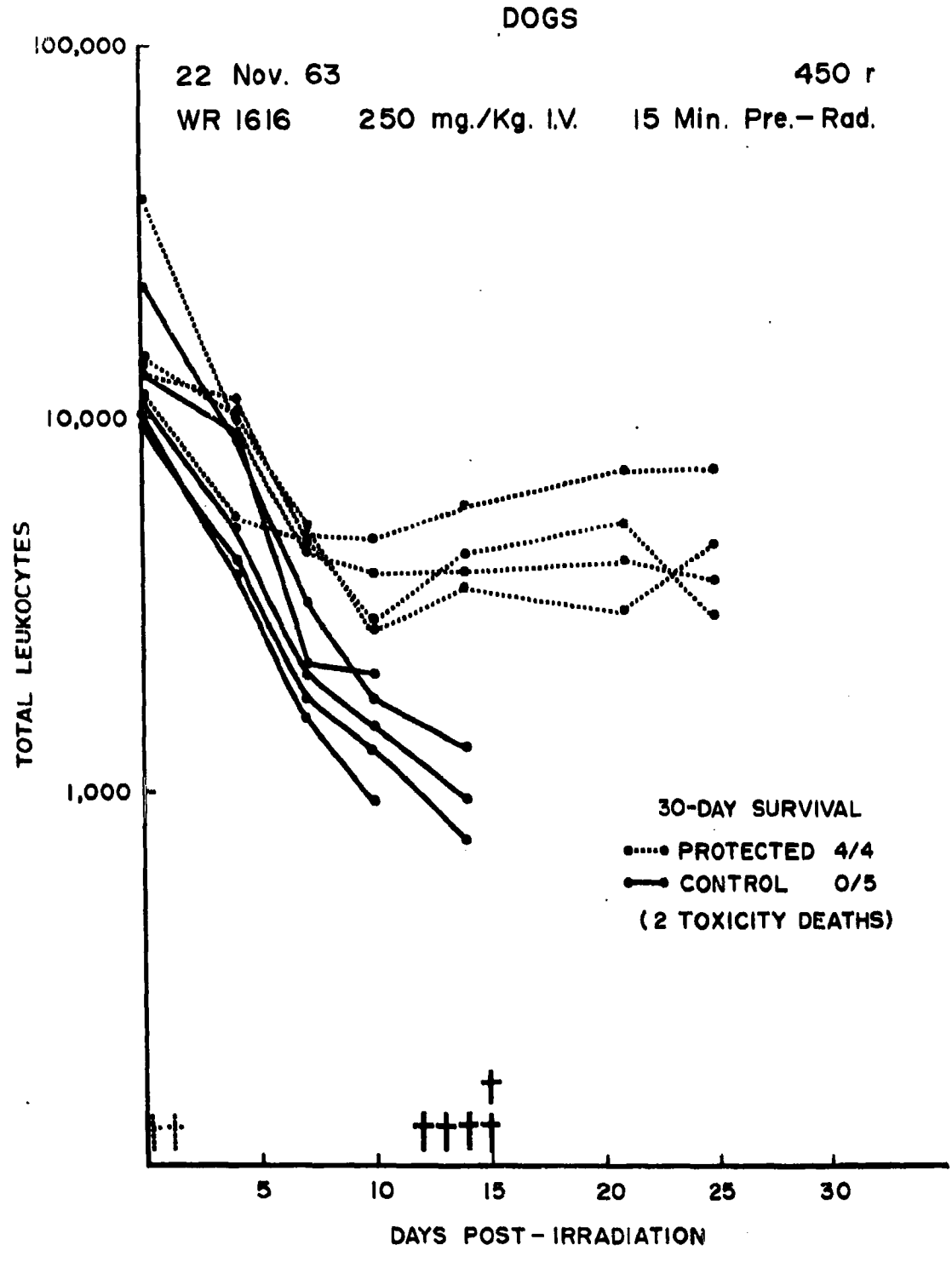
DOGS
22 Nov. 63
450 r
WR 1616
250 mg./Kg. I.V.
15 Min. Pre.– Rad.
100,000
10,000
1,000
TOTAL LEUKOCYTES
30-DAY SURVIVAL
PROTECTED 4/4
CONTROL 0/5
(2 TOXICITY DEATHS)
5
10
15
20
25
30
DAYS POST – IRRADIATION

Figure 46

DOGS

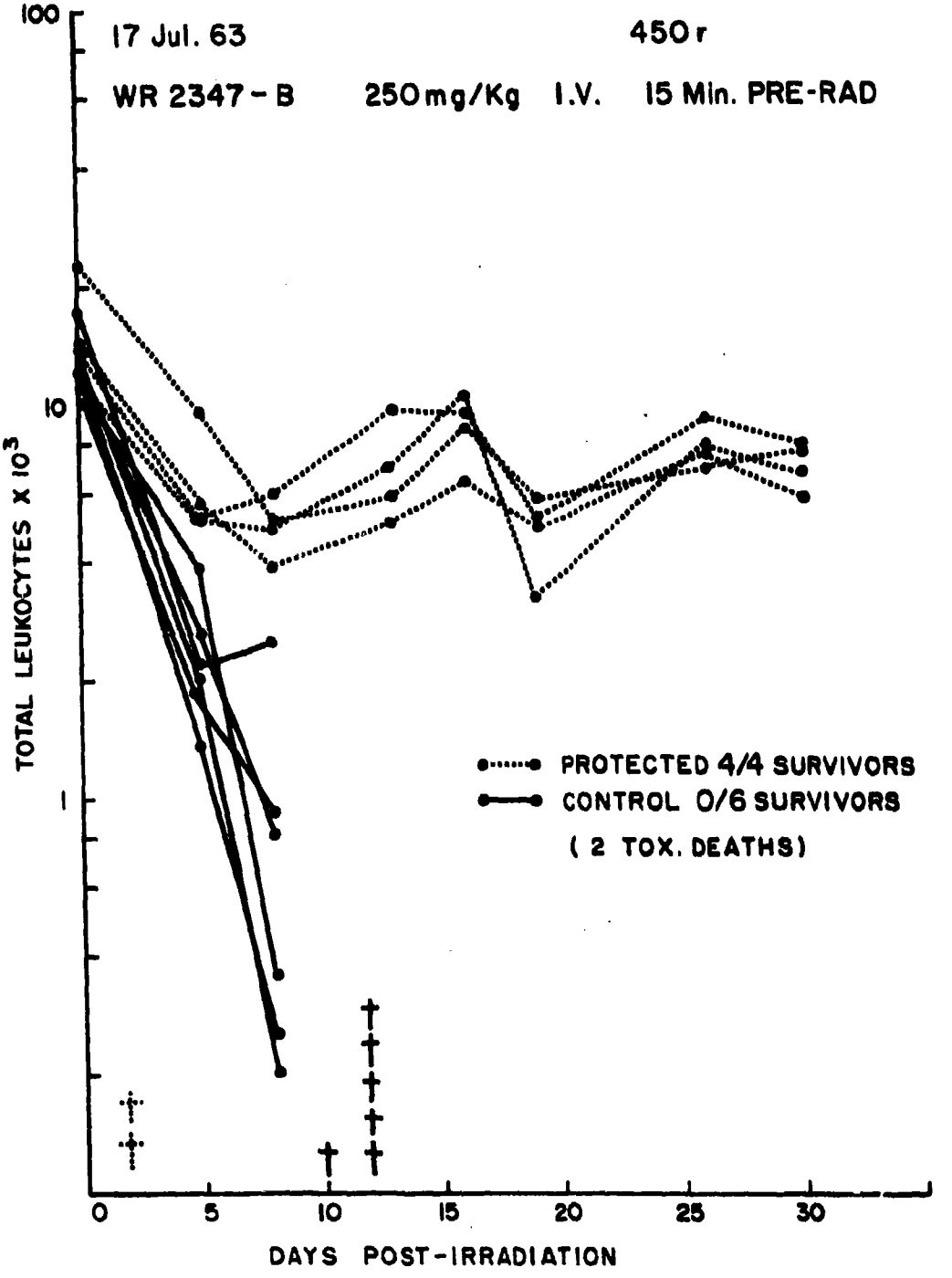

Figure 47

TOXICITY OF CANDIDATE ANTIRADIATION CHEMICAL AGENTS

IN THE RHESUS MONKEY

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WR No	Compound	Dose Mg/Kg (Free Base)	Route	Vehicle	pH	Number of Deaths	Emesis	Diarrhea	Hypotension	Hypertension	Depressant	Convulsant
215 (APT)	H_2N, HN, C-S-CH_2-CH_2-CH_2-NH_2	45	IV	Water	7.4	0/1	+		+		+	
		67				0/1			+		+	+
		80				1/1	+		+		+	+
298 (AET)	H_2N, HN, C-S-CH_2-CH_2-NH_2	42	IV	Water	7.2	1/1			+		+	+
		55				0/1	+		+		+	
		83				1/1			+		+	
		110				1/1	+		+		+	
1607	CH_3-(CH_2)$_9$-NH-CH_2 CH_2-SSO_3H	30	IV	Water	11.0	0/3	±			?	±	
2347	HOH_2C-CH(CH_2OH)-CH-NH-CH_2-CH_2-SH, OH	300	IV	Water	6.5	0/2	+		+		+	

Figure 48

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RADIATION PROTECTION STUDIES IN THE RHESUS MONKEY

2.5 MEV. WHOLE-BODY X-IRRADIATION

(1 July 1963 to 30 June 1964)

WR No	Chemical Structures	Dose of Agent (Mg/Kg)	Route	Time Before Rad	Rad Dose (r)	Deaths Due to Chemical Toxicity	Time of Death (Days Post Rad) Controls	Protected	30-Day Survival Cont	Prot
2347	$HOH_2C-CH(CH_2OH)-CH(OH)-NH-CH_2-CH_2-SH$	300	IV	15 min	800	2/12	12,13,14, 16,19,20	0,0	5/11	10/10
302	PAPP	5	IV	45 min						
1795	POHQQ	10			1000	0/6	20	-	5/6	6/6
347	MEA	100	IV	2-4 min						
348	CYSTEINE	500								
	(Atropine 0.2 Mg/Kg pre. methylene blue 3 Mg/Kg post)									
302	PAPP	5	IV	45 min			5,7,8,9,9, 10,12,12,	1,5,9,20,26		
1795	POHQQ	10			1200	1/11	15,17,19		0/11	6/10
347	MEA	100	IV	2-4 min						
348	CYSTEINE	500								
					1300		6,7,10,10 11,11,12	-	0/7	-
	CONTROLS				1200		5,7,8,9,9,10 12,12,15,17,18	-	0/11	-
					1000		20	-	5/6	-
					900		17	-	0/1	-
					800		19	-	3/4	-
					450		12,13,14,20	-	2/6	-

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Figure 49

Section H

ON THE POSSIBLE BETA-ADRENERGIC BLOCKING PROPERTIES OF 2-(n-DECYLAMINO)-ETHANETHIOSULFURIC ACID (WR 1607)

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Washington, D.C. 20012

INTRODUCTION

The intriguing observation of Fromherz (1946) that pilocarpine converted the depressor response of isoproterenol to a pressor effect captured the interest of many investigators. Subsequently, several agents were found to have this action. Nash, et al. (1961) reported that vasopressin reversed the normal isoproterenol vasodepression. These investigators presented evidence which indicated that vasopressin selectively blocked the peripheral vasodilating receptors without concomitant blockade of cardiac stimulator receptors.

During a study of the pharmacology of a series of substituted aliphatic amines, it was found that the newly synthesized compound, 2-(n-decylamino)-ethanethiosulfuric acid (WR 1607), reversed isoproterenol vasodepression and antagonized the positive inotropic action of isoproterenol on the isolated guinea pig atrium. These actions of WR 1607 could be explained on the basis of beta-adrenergic blockade. The present study concerns experiments designed to test this hypothesis by observing the antagonistic action of WR 1607 against catecholamines. The following parameters were observed: the blood pressure of the dog, cardiac contractile force of the dog

heart in vivo and the isolated guinea pig atrium, the ileal intraluminal pressure of the dog in vivo, and the spontaneous contractions of the isolated rat uterus. Whenever possible dichloroisoproterenol (DCI) was used for purposes of comparison.

MATERIALS AND METHODS

WR 1607[1] is a white, fluffy powder which is insoluble in water and stable in cold alkali; therefore, the sodium salt was prepared by the dropwise addition of 1 N NaOH and dissolved in 0.9% NaCl at room temperature. This resulted in a solution having a pH of 10.8 to 11.2. Neutralizing this solution precipitated the acid; therefore, whenever possible, solutions of 0.9% NaCl at pH 11 were used to control for pH effect.

The formula of WR 1607 is:

$$CH_3-(CH_2)_8-NH-CH_2CH_2S_2O_3-H$$

Experiments on Dogs.

Mongrel dogs of either sex were anesthetized by the intravenous injection of pentobarbital sodium (30 mg/kg). A catheter was introduced into the abdominal aorta through the femoral artery and connected to a Sanborn 267-B pressure transducer for arterial pressure measurements. Intraluminal pressure of the ileum was measured by the technique described by Alquist and Levy (1959). Recordings were made on a Sanborn polygraph, Model 350. All injections were given into the femoral vein through an indwelling catheter which was washed with 0.9% NaCl after each injection.

[1] This compound was synthesized by Dr. Daniel L. Klayman, Department of Medicinal Chemistry, this institute.

Three experiments were performed. In the first, the blood pressure responses to rapid injections of epinephrine HCl (1 to 2 μg/kg) norepinephrine bitartrate (1 to 2 μg/kg of the base) and isoproterenol HCl (0.25 μg/kg) spaced at least five minutes apart, were obtained in 17 dogs before and after the administration of WR 1607 (10 to 17.5 mg/kg, 5 or 10 min injection period). Then phenoxybenzamine HCl (15 mg/kg) was infused over a 30 minute period. One hour after the end of the phenoxybenzamine infusion, the dogs were again challenged with the catecholamines. A second identical dose of WR 1607 was given and the catecholamine challenges were repeated. Three of these dogs were challenged with nitroglycerin (0.3 to 1.13 mg/kg) in addition to the catecholamines. The same procedure was carried out on two additional dogs except that DCI hydrochloride (15 mg/kg) was substituted for WR 1607 (a dose approximately equimolar to 17.5 mg/kg of WR 1607).

In a second acute experiment, cardiac contractile force changes as measured by a strain gauge arch sutured to the right ventricle (Boniface, et al. 1953) and arterial pressure response to epinephrine HCl (1 μg/kg) or isoproterenol HCl (0.5 μg/kg) were recorded before and after multiple doses of WR 1607 in two vagotomized, atropinized (1 mg/kg) dogs pretreated with phenoxybenzamine HCl (15 mg/kg).

In a third experiment, ileal intraluminal pressure responses to epinephrine HCl and isoproterenol HCl (0.25 to 4 μg/kg) before and after WR 1607 (5 mg/kg) were observed in five dogs. DCI hydrochloride (5 mg/kg) was substituted for WR 1607 in two additional dogs.

Experiments on isolated guinea pig atria.

Adult guinea pigs were stunned by a blow to the back of the neck. The right atrium was quickly removed and placed in a five ml organ bath containing Tyrode solution at 37.5° C with a mixture of 95% oxygen and 5% carbon dioxide bubbling through the bath. The spontaneous contractions were measured by means of a Statham force transducer and recorded on a Sanborn polygraph. Isoproterenol or epinephrine were given in concentrations that elicited an increase in contractile force was approximately 50% of the maximum increase that could be induced by the respective catecholamine. Calcium chloride concentrations were adjusted so that the contractile force increases (without contracture) nearly matched those induced by the catecholamines. The increased contractile force induced by the catecholamines was compared before and after the addition of 0.1 ml of a solution of WR 1607 (final dilution: 1 to 4 μg/ml) or 0.1 ml of a solution of DCI (final dilution: 1 to 10 μg/ml) to the bath. The same comparison was made with respect to calcium chloride responses. The Tyrode solution containing DCI or WR 1607 was left in contact with the atrium 5 to 10 minutes then replaced with fresh Tyrode solution prior to testing with catecholamines and calcium chloride. Catecholamines and calcium chloride were left in contact with the atrium for 1 minute then replaced with fresh Tyrode solution. An equal volume (0.1 ml) of alkaline saline was introduced into the bath for 10 minutes to control for pH effect of WR 1607 solutions.

Experiments on isolated rat uteri.

Segments of rat uteri were suspended in a six ml muscle bath containing a modified Tyrode solution of the following concentrations: NaCl (7.95 g/L), KCl (1.13 g/L), $CaCl_2$ (0.05 g/L), $MgCl_2$ (0.004 g/L), NaH_2PO_4 (0.04 g/L), $NaHCO_3$ (0.8 g/L) and glucose (1 g/L). The temperature of the bath was maintained at 37° C. Spontaneous contractions were detected by a Sanborn Linearsyn displacement transducer and recorded electronically. The minimal amount of epinephrine required to abolish the spontaneous contractions for 2 minutes was the endpoint measurement. This parameter was compared before and after the introduction of 0.05 ml of a solution of WR 1607 final dilution: 1.67 μg/ml) to the bath for five minutes. The Tyrode solution containing the chemical was replaced with fresh Tyrode solution and the muscle was examined for epinephrine responsiveness. An equal volume (0.05 ml) of alkaline saline was introduced into the bath for five minutes to control for pH effects of WR 1607 solutions.

RESULTS

Experiments on dogs.

In a series of 21 dogs anesthetized with pentobarbital sodium, WR 1607 caused a 22 ± 7 mm Hg[a] rise in mean arterial blood pressure for 30 to 70 minutes. The intensity of the hypertension was not dose dependent. There was a rise in diastolic pressure with little or no change in a systolic pressure, resulting in a decreased pulse pressure (Fig. 1A). The animals' tongues

[a] All values are stated as the mean ± SE, unless otherwise indicated.

appeared cyanotic. Pretreatment of the dogs with phenoxybenzamine prevented narrowing of the pulse pressure and cyanosis but it did not prevent a rise in mean arterial pressure (Fig. 1B). However, the mean blood pressure of the phenoxybenzamine treated dogs was only 107 ± 8 mm Hg. Although WR 1607 increased the pressure to 142 ± 11 mm Hg, this was the same as the pre-phenoxybenzamine infusion blood pressure which was 135 ± 6 mm Hg.

The blood pressure response to the catecholamines are shown in Table 1 and Figure 2. WR 1607 synergized with the pressor effects of epinephrine and norepinephrine and abolished the depressor component of the epinephrine which was present in the control period. The depressor response to isoproterenol was significantly reduced in all dogs and in 3 out of 6 dogs it was eliminated. The isoproterenol effect was converted to a pressor action. This reversal lasted for approximately 30 minutes at which time a diphasic response appeared. Normal depressor responses to isoproterenol were observed at approximately 60 minutes after the administration of WR 1607.

Classical epinephrine reversal was produced and the norepinephrine pressor response was reduced in all dogs which were treated with phenoxybenzamine (Fig. 2). WR 1607 restored the pressor response to epinephrine and synergized with the norepinephrine pressor effect. The vasodepressor responses of nitroglycerin were essentially unmodified by WR 1607 or DCI in both the normal and phenoxybenzamine treated dog.

Figure 3 shows a recording of two dogs' blood pressure and cardiac contractile force responses to epinephrine and isoproterenol before and after WR 1607 administration. WR 1607 diminished the cardiac contractile force.

The onset of this effect was immediate and lasted for about 60 minutes. WR 1607 had some inhibitory action on the positive inotropic effect of the catecholamines.

In the dog, WR 1607 did not cause a decrease in tone or motility of the ileum whereas DCI produced intestinal inhibition. Both drugs had no effect on the intestinal response to epinephrine while the inhibitory response to isoproterenol was consistently antagonized (Fig. 4). This antagonizing action of WR 1607 to isoproterenol inhibition of the motility of the dog ileum lasted for over 80 minutes, long after its antagonizing action to the isoproterenol vasodepressor response had passed. On the other hand, DCI antagonized both the vasodepressor effect of isoproterenol and the intestinal inhibitory response for over two hours. Larger doses of isoproterenol (μg/kg) could "override" the effects of WR 1607 or DCI and would produce the typical intestinal inhibition.

Experiments on isolated guinea pig atria.

WR 1607 was found to depress the contractile force of the atrium ($-17 \pm 3\%$, $p < 0.001$). The chemical consistently inhibited the positive inotropic effect of isoproterenol ($-35 \pm 4\%$, $p < 0.001$) in the 13 atria tested, whereas it caused little or no effect on the calcium chloride response ($-9 \pm 5\%$, $p > 0.05$). WR 1607 inhibited the positive inotropic response to epinephrine in two experiments (-32% and -55%) while the positive inotropic response to calcium chloride was not affected (-3% and 0%). It was observed that higher concentrations of the catecholamines could overcome the inhibition of the positive inotropic effect. In three experiments in which DCI

was introduced instead of WR 1607, the suppression of the positive inotropic response to isoproterenol was -32 ± 5% while the positive inotropic effect of calcium chloride was not suppressed. Alkaline saline in equal volumes did not depress spontaneous contractions or antagonize the responses to catecholamines or calcium chloride.

Experiments on isolated rat uteri.

Epinephrine (8.4×10^{-5} to 1.2×10^{-3} μg/ml) abolished the spontaneous contractions of the uterus for two minutes. In a paired experiment WR 1607 prevented this inhibitory response to epinephrine and 6.3 times as much[3] epinephrine was required to abolish the spontaneous contractions. It should be noted that WR 1607 caused an increase in the tone of the uterus while the chemical was in contact with the tissue; however, the tone returned to control levels when WR 1607 was washed out and epinephrine testing was started. Alkaline saline in equal volumes did not produce a measurable change in the pH of the bathing solution nor did it depress the response of the uterus to epinephrine.

DISCUSSION

These data demonstrate that WR 1607 is one of several agents which antagonize adrenergic vasodilation and restore the pressor effect of epinephrine following alpha-adrenergic blockade. This could be explained by

[3] The data were normally distributed as the logarithm of the doses. Thus, the analysis of the log of the ratios of the doses by the Student "t" test was performed ($p < 0.001$, the range was 2.5 to 16 times).

the concept put forth by Lands, et al. (1950), i.e., that this agent may be exerting its action by means of causing generalized vasoconstriction. Since WR 1607 causes a rise in mean arterial blood pressure, a decrease in pulse pressure and cyanosis, it appears to be a vasoconstrictive agent. However, vasoconstriction per se does not satisfactorily explain isoproterenol reversal. Nash, et al. (1961) found that the duration of the isoproterenol reversing action of vasopressin is much shorter than its vasoconstrictive effect. These investigators found that not all vasoconstrictors would reverse the depressor effect of isoproterenol. Indeed, dihydroergocornine which is not a strong vasoconstrictor is able to produce reversal of isoproterenol. In addition, they found that nitroglycerin still exhibits its hypotensive action while isoproterenol reversal is present. In the present study, nitroglycerin elicited depressor responses after WR 1607, although hypotension from isoproterenol and the depressor component of the epinephrine response were either eliminated or significantly diminished. Phenoxybenzamine pretreatment prevented or diminished to a large degree any gross evidence of vasoconstriction such as narrowing of the pulse pressure and cyanosis. WR 1607 caused an increase in the mean arterial pressure in these dogs pretreated with phenoxybenzamine; however, it did not cause the mean arterial pressure to rise above the pre-phenoxybenzamine levels. It appears, therefore, that the mechanism of the isoproterenol reversal and the restoration of the epinephrine pressor response after alpha-adrenergic blockade, may be independent and not related to generalized

vasoconstriction in the case of WR 1607, but due rather to a selective inhibition of the vasodepressor responses to the catecholamines.

WR 1607 had some inhibitory action on the positive inotropic effect of isoproterenol and epinephrine in the dog. This action was not of the same order of magnitude that Moran and Perkins (1958) reported in experiments with DCI. There was usually some positive inotropic effect of the catecholamines after WR 1607. If WR 1607 blocked the beta-adrenergic receptor sites of the vascular smooth muscles and if the chemical caused only a partial blockade of the positive inotropic effect, one would expect a pressor response to isoproterenol. In Figure 3 where there was complete inhibition of the positive inotropic effect of isoproterenol, there was no pressor action. Phenoxybenzamine pretreatment cancels out much of the general vasoconstriction action of WR 1607. This leads to the implication of vasoconstriction acting to augment blockade of the dilator action in producing the hypertensive response to the catecholamines.

WR 1607 depressed cardiac contractile force in doses above 4 mg/kg in the dog. Moran and Perkins (1958) found that DCI in doses of 4 to 16 mg/kg caused a similar prolonged cardiac depression. The dose of WR 1607 could not be increased above 20 mg/kg without causing serious toxic reactions. Therefore, further study of the direct cardiac effects of the compoung was conducted on the isolated guinea pig atrium. WR 1607 selectively blocked the positive inotropic action of isoproterenol and epinephrine, leaving this effect of calcium chloride essentially unchanged. High

concentrations (10 μg/ml) seriously depressed the strength of spontaneous contractions, whereas DCI could be administered in concentrations up to 10 μg/ml without causing depression of the contractile force.

Levy and Tozzi (1963) have shown that the adrenergic receptors of the smooth muscle of the rat uterus are only of the beta type. Since WR 1607 antagonized the epinephrine inhibition of the motility in this preparation, this action may be explained on the basis of beta-adrenergic blockade. The effect of WR 1607 to stimulate contraction of this tissue was eliminated when the chemical was replaced by fresh Tyrode solution; therefore, it is felt that this effect of WR 1607 is not important in the mechanism of epinephrine antagonism. Studies could not be performed with DCI in this test system because this compound had a serious inhibitory effect on both the motility and tone.

There are differences in the pharmacological actions of WR 1607 and DCI, however, both chemicals selectively inhibit the vasodepressor action and the positive inotropic effect of isoproterenol and epinephrine in the dog. They selectively antagonize the inhibitory effect of isoproterenol on the dog ileum and selectively inhibit the positive inotropic effects of isoproterenol and epinephrine on the isolated guinea pig atrium. Further, WR 1607 antagonizes epinephrine inhibition of the motility of the isolated rat uterus. However, until blockade of the positive chronotropic effect of catecholamines can be demonstrated, WR 1607 cannot be classified as a typical beta-adrenergic receptor site blocking agent. Experiments are being

conducted in this laboratory to test this effect. Nevertheless, the simi- [illegible] nd WR 1607 should be of interest, since the latter is a straight chained aliphatic [illegible]ine containing a thiosulfate group while other chemicals reported to have beta-adrenergic actions are aromatic amines.

SUMMARY

The newly synthesized compound, 2-(n-decylamino)-ethanethiosulfuric acid (WR 1607) was found to synergize with the pressor action of epinephrine and norepinephrine and abolish or significantly attenuate the vasodepressor response to isoproterenol and the vasodepressor component of the epinephrine response in the normal and phenoxybenzamine treated dog. WR 1607 caused a rise in diastolic pressure with little or no change in systolic pressure resulting in a narrowing of the pulse pressure and an elevated mean arterial pressure. Because of the fact that the vasodepressor response to nitroglycerin was unaltered by the chmical and the fact that phenoxybenzamine pretreatment prevented the narrowing of the pulse pressure, it is felt that the selective inhibition of the vasodepressor action of isoproterenol is independent of the generalized vasoconstriction. WR 1607 was also observed to restore the pressor response to epinephrine after alpha-adrenergic blockade. In addition, the chemical depressed the contractile force of the heart and inhibited the positive inotropic action of epinephrine and isoproterenol on the in vivo dog heart and selectively inhibited the

positive inotropic action of these catecholamines in the isolated guinea pig atrium while the calcium chloride responses were essentially unchanged. WR 1607 selectively abolished the intestinal inhibition by isoproterenol in the dog. Further, WR 1607 antagonized epinephrine inhibition of the motility of the isolated rat uterus. Comparisons with DCI were made whenever possible. Additional studies are in progress to determine whether WR 1607 antagonizes the positive chronotropic action of the catecholamine so that a final conclusion can be made as to its specific site of action. Pending positive findings in these studies, WR 1607 can be tentatively assigned beta-adrenergic blockading properties.

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LEGENDS FOR FIGURES

Figure 1. Arterial Pressure During WR 1607 Injection

A. Arterial pressure of a dog given 17.5 mg/kg of WR 1607

B. Arterial pressure of a dog pretreated with 15 mg/kg of phenoxybenzamine HCl and given 17.5 mg/kg of WR 1607

Dots indicate the start of WR 1607 injections. Time segment represents 1 min. Blood pressure scale is in mm Hg.

Figure 2. Mean Arterial Blood Pressure Responses to Catecholamines and Nitroglycerin Before and After WR 1607 (17.5 mg/kg) or DCI (15 mg/kg)

A. Mean arterial blood pressure responses of a dog before and after WR 1607 administration

B. Mean arterial blood pressure responses of a dog before and after DCI hydrochloride administration

C. Mean arterial blood pressure responses of a phenoxybenzamine treated dog before and after WR 1607 administration

WR 1607 was injected between 4 and 5

1 and 5: Isoproterenol HCl (0.25 μg/kg)

2 and 6: Nitroglycerin (0.3 mg/kg in A and C, 1.13 mg/kg in B)

3 and 7: Epinephrine HCl (1 μg/kg)

4 and 8: Norepinephrine (1 μg/kg)

Dots indicate injection times. Time segment represents 1 min. Mean areterial pressures were derived by electronic integration. Pressure scale is in mm Hg.

Figure 3. Cardiac Contractile Force and Blood Pressure Responses to Catecholamines Before and After WR 1607 Administration in the Phenoxybenzamine Treated Dog

E: Epinephrine HCl (1 μg/kg)

I: Isoproterenol NCl (0.5 μg/kg)

WR 1607 was given between the first and second isoproterenol responses. The figure represents two dogs. Epinephrine was given to one dog and isoproterenol was given to another. Dots indicate injection times. The time segment represents 1 min. Pressure scales are in mm Hg. The sudden increase in contractile force near the end of the WR 1607 injection is probably due to a sudden release of epinephrine which was observed to occur during this time in another experiment.

Figure 4. Intestinal Motility and Blood Pressure Responses to Catecholamines Before and After WR 1607 (5 mg/kg) in the Dog

A. Responses to 1 μg/kg of epinephrine HCl before WR 1607

B. Responses to 1 μg/kg of isoproterenol HCl before WR 1607

C. Responses to 1 μg/kg of epinephrine HCl after WR 1607

D. Responses to 1 μg/kg of isoproterenol HCl after WR 1607

Top tracing (obliquely rising lines) represents deflections of electronically integrated voltage changes of intestinal motility. Middle tracing represents intraluminal pressure changes (intraluminal pressure minus intra-abdominal pressure). Lower tracing represents mean arterial pressure electronically integrated (scale in mm Hg). Dots represent injection times. Time scale represents 1 min.

Section H

Table 1

Mean Arterial Blood Pressure Responses to Catecholamines
Before and After WR 1607 Administration to Normal Dogs

Treatment	M	PRESSOR RESPONSE mean ± S.E. (mmHg)			DEPRESSOR RESPONSE mean ± S.E. (mmHg)		
		Before WR 1607 P_1	After WR 1607 P_2	P_2/P_1	Before WR 1607 P_1	After WR 1607	P_2/P_1
Epinephrine	7	31 ± 4	65 ± 8	2.1 ± 0.1[1]	-16 ± 4	0	16.0 ±3.7[4]
Norepinephrine	4	39 ± 6	65 ± 9	1.7 ± 0.2[2]	0	0	0
Isoproterenol	6	0.8 ± 0.8[3]	39 ± 4	34.8 ± 4.4[1]	-36 ± 6	-7 ± 4	16.4 ± 5.8[2]

[1] $P < 0.001$
[2] $P < 0.05$
[3] not different from 0
[4] $P < 0.01$

CONFIDENTIAL

Table 2

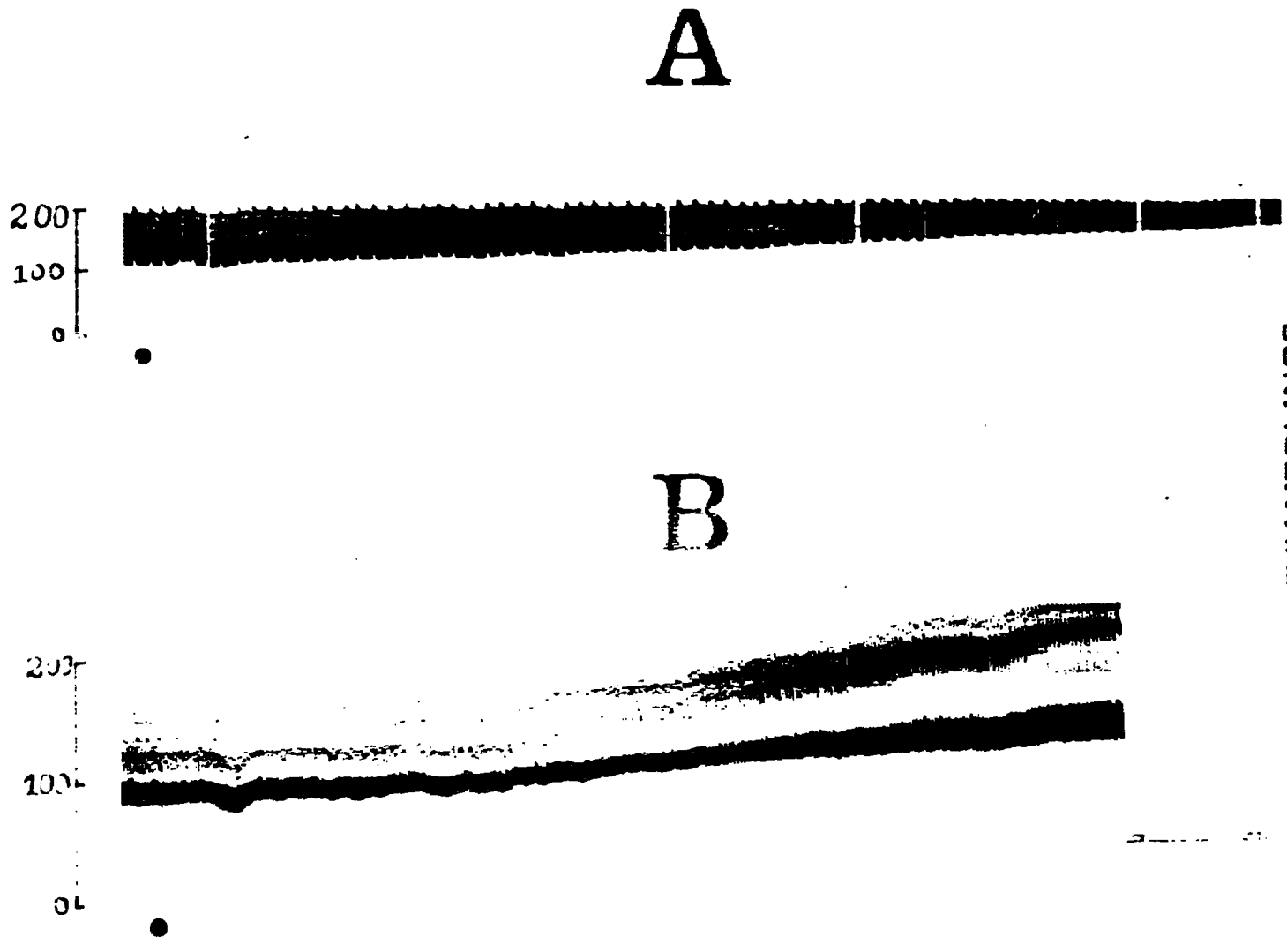
Mean Arterial Blood Pressure Response to Catecholamines Before and After WR 1607 Administration to Phenoxybenzamine Treated Dogs.

Treatment	M	PRESSOR RESPONSE mean ± S.E. (mmHg)			DEPRESSOR RESPONSE mean ± S.E. (mmHg)		
		Before WR 1607 P_1	After WR 1607 P_2	P_1/P_2	Before WR 1607 P_1	After WR 1607	P_1/P_2
Epinephrine	6	8 ± 1	35 ± 4	5.1 ± 0.9[1]	-49 ± 4	-14 ± 2	3.7 ± 0.4[3]
Norepinephrine	6	12 ± 3	41 ± 13	3.3 ± 0.6[2]	0	0	0

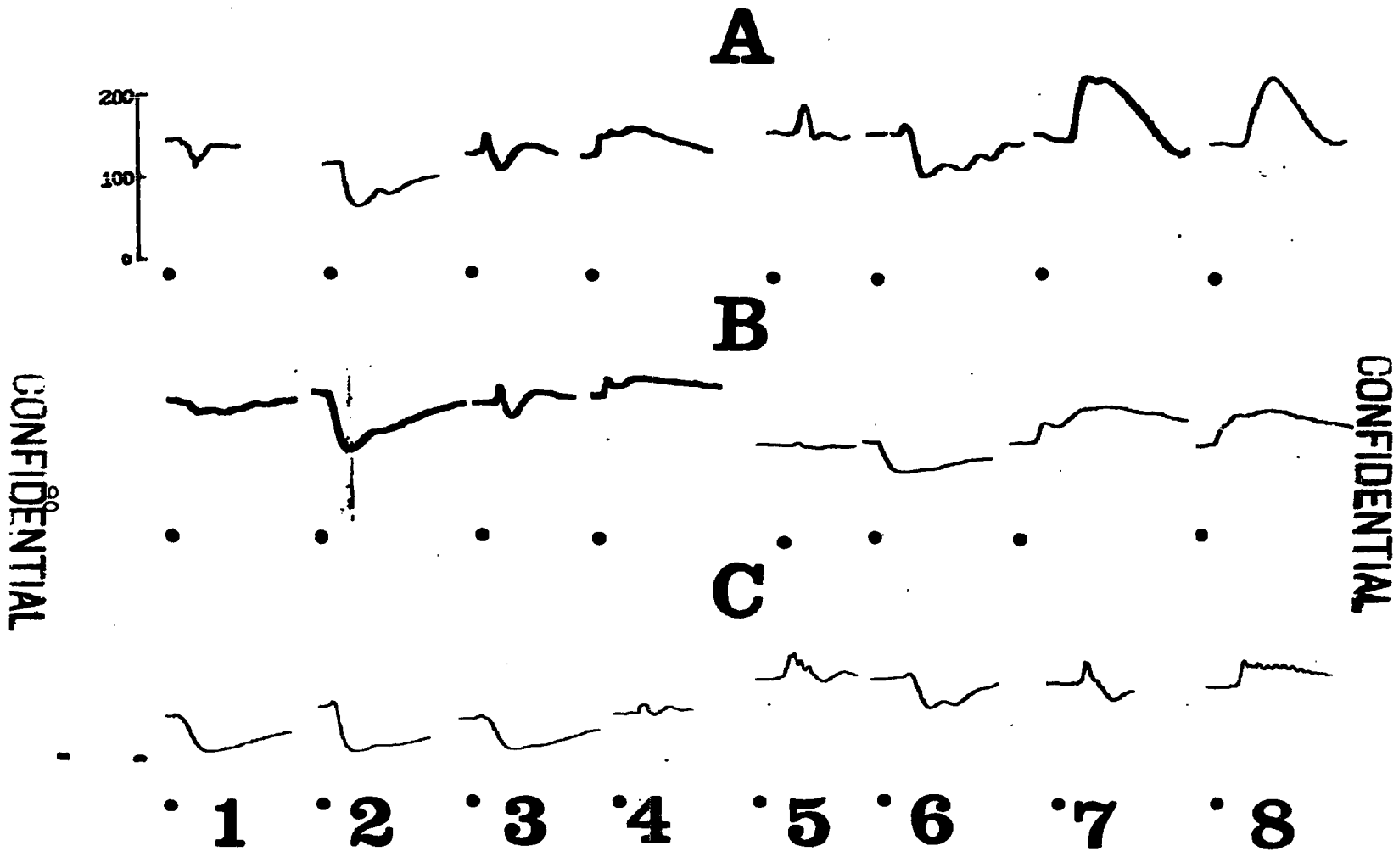
[1] $P < 0.01$
[2] $P < 0.02$
[3] $P < 0.001$

CONFIDENTIAL

Section H
Figure 1



Section H
Figure 2



Section H
Figure 3

Section H
Figure 4

CARDIOVASCULAR SCREEN FOR N-SUBSTITUTED AMINOETHANETHIOSULFATES

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Since many of the pharmacologic actions of WR 1607 could be explained on the basis of beta-adrenergic blockade, cardiotoxicity and direct vasoconstriction, a screen was developed to rank this series of compounds on these parameters.

METHODS

Cardiotoxicity and beta-adrenergic blockade. The left guinea pig atrium was isolated in 7 ml muscle bath containing Krebs-Henzeleit solution at 37 degrees C and aeriated with 95% oxygen and 5% carbon dioxide. The atrium was stimulated at 5 pps at 2 X threshold voltage. Contractions were registered by a Statham force transducer and recorded electronically. The atrium was challenged with 7×10^{-9} gm/ml of isoproterenol and 3×10^{-5} gm/ml of calcium chloride following 10 minutes contact with the agent being tested. The agents were dissolved in 0.5 ml of 1 N NaOH and diluted 1:20, 1:200 and 1:2000; 0.1 ml of each dilution was added to the bath. The concentration of the agents added related to equimolar concentrations of 1, 10 and

100 ug/ml of 1607 in the bath. Normal saline at the pH of the test solutions was used to control for pH and osmotic effect.

Direct vasoconstriction. Rats were anesthetized with pentobarbital and heparinized; the abdominal aorta was cannulated and attached to a perfusion apparatus filled with Tyrode solution at 37 degrees C, aerated and driven by 1.16 atm of 95% oxygen and 5% carbon dioxide. Flow was measured for 10 minutes. Each preparation was standardized to 2×10^{-8} gm/ml of epinephrine hydrochloride. The agents were added at 10^2, 10^3, and 10^4 times the molar concentrations of epinephrine hydrochloride.

RESULTS

Cardiotoxicity and beta-adrenergic blockade. The response parameter, R_i/R_c (R_i: response after each dose of the agent; R_c: response before the initial dose of the agent) was normally distributed for unstimulated contractions, and responses to calcium chloride and isoproterenol. The 99% confidence intervals for the mean in 8 control experiments where alkaline saline substituted for the agent are shown in Table I. Responses falling outside these intervals were considered significant at $p = 0.05$. Statistical comparison of 78 random responses verified this assumption. The results are shown graphically in Figure 1, which is a conventional

three-dimensional display. The responses are represented as planes extending into the x axis; the points nearest the origin are the response parameters to dose x and the points farthest from the origin are the response parameters to dose 100 x with 10 x response parameters falling between. The bands extended on the y axis represent the number of carbon atoms in the side chain. The height of the plane on the z axis represent relative contractile force as parameter R_l/R_c. The bands extending into the x axis are families of related substitutions, beginning with the band nearest the origin are (1) n-alkyl, (2) 2-alkyl, (3) 3-alkyl except WR 2324 which is di-n-heptyl, (4) 4-alkyl, (5) cyclohexyl alkyl, (6) phenalkyl, (7 & 8) miscellaneous branching. The inset represents the effect of adding polar substitutions to lipid side chain of n-decyl substitution:

1607: n-decyl

2865: 10-hydroxy-n-decyl

2907: 1-hydroxy-2-decyl

2857: (formyl,n-decyl)

2850: (acetyl,n-decyl)

2851: (ethanol, n-decyl)

Direct vasoconstriction. Results are shown in Tables 2 & 3.

Definition of vasoconstrictor indices:

Index	Vasoconstrictor power relative to epinephrine (20 μg/L)
1	10^{-4}
2	10^{-3}
3	10^{-2}

Statistical comparison of regression parameters for cardiotoxicity screen:

To provide a basis for estimation of the validity of the differences shown in Figure 1, the following statistical parameters are provided from 20 point regression analyses comparing WR 1607 responses with responses to WR 2390 in a non-paired experiment using eight animals. The responses to these two agents appeared to be different as seen in Figure 1.

RESPONSE TO ISOPROTERENOL

Parameter	Mean	$S_{y \cdot x}$	Regression Coefficient
WR 1607	0.613	0.170	-0.226
WR 2390	0.775	0.167	-0.200
p	>0.05		>0.40

These data suggest that the apparent shift of response parameters in the case of WR 1607 and 2390 is real and that there is about a 20% overlap of response of these two populations.

DISCUSSION

The confidence with which agents can be compared with each other has already been discussed in the preceding section. Some generalizations are immediately possible from inspection of the data. There is a correlation between myocardial toxicity and the ability to antagonize Beta-adrenergic stimulation (beta-blockade). The myocardial toxicity observed in this isolated guinea pig atrium is also observable in the dog and discussed in detail elsewhere. The magnitude of beta blockade of, for example, 1607 is comparable to the amount of beta blockade induced by dichloroisoperterenol (DCI) in equimolar concentrations, although DCI has less myocardial toxicity. Other compounds reported here are more potent in producing beta blockade. This series of compounds represents a major new pharmacologic tool for investigation of beta blockade and constitutes the only series other than DCI capable of producing beta blockade.

From the tables of vasoconstrictor activity and protective indices, it can be seen that there is no correlation between vasoconstrictor action and protective action. This test has therefore provided a very clear lead to be exploited in the synthetic program. The vasoconstriction is less potent than the vasoconstriction produced by 20 micrograms per liter of epinephrine but is sufficiently

potent that hypertension constituted a very significant aspect of the toxicity of 1607 in the intact dog. This discovery of compounds having no constrictor action is therefore of paramount importance.

There is a lack of correlation between vasoconstrictor activity and myocardial toxicity. For example, the cyclohexylhexyl derivative has relatively weak vasoconstrictive action but marked myocardial toxicity. The 3-octyl derivative has no vasoconstrictor activity and appreciable myocardial toxicity. The three most powerful vasoconstrictor compounds, the dodecyl derivative, the tridecyl derivative and the tetradecyl derivative are relatively poor in producing myocardial toxicity in comparison to 1607 or 2866. This semiquantitative test has therefore permitted the development of agents lacking in myocardial toxicity.

The two agents which appear promising from point of view of lack of adverse pharmacologic responses while providing good radiation protection are the cyclohexylbutyl and phenbutyl derivatives. The ring systems of both of these compounds are isolated from the nitrogen function by methylene groups. We feel that other ring systems might be exploited if protected by a similar methylene chain from the nitrogen function. This test system has therefore provided the tools necessary for a major change in the synthesis of potential antiradiation compounds as well as developing agents

which will permit the definitive investigation of beta blockade and additional insight into peripheral vasoconstriction and myocardial toxicity.

SUMMARY

A new test system has been developed which provides information on myocardial toxicity, beta-adrenergic blockade and peripheral vasoconstriction. Protective agents have been found which lack these side effect as measured in this system.

Section I

TABLE 1

99% Confidence Intervals for Control Response Parameter (R_1/R_0)

Dose	Unstimulated Contractions	Isoproterenol Response	Calcium Chloride Response
X	0.70 - 1.00	0.97 - 1.11	0.90 - 1.10
10 X	0.61 - 1.01	0.97 - 1.07	0.84 - 1.14
100 X	0.59 - 0.97	0.95 - 1.17	0.91 - 1.11

Section I

TABLE 2

VASOCONSTRICTOR

INDICES OF N-SUBSTITUTED AMINOETHYLTHIOSULFATES

Direct Smooth Muscle

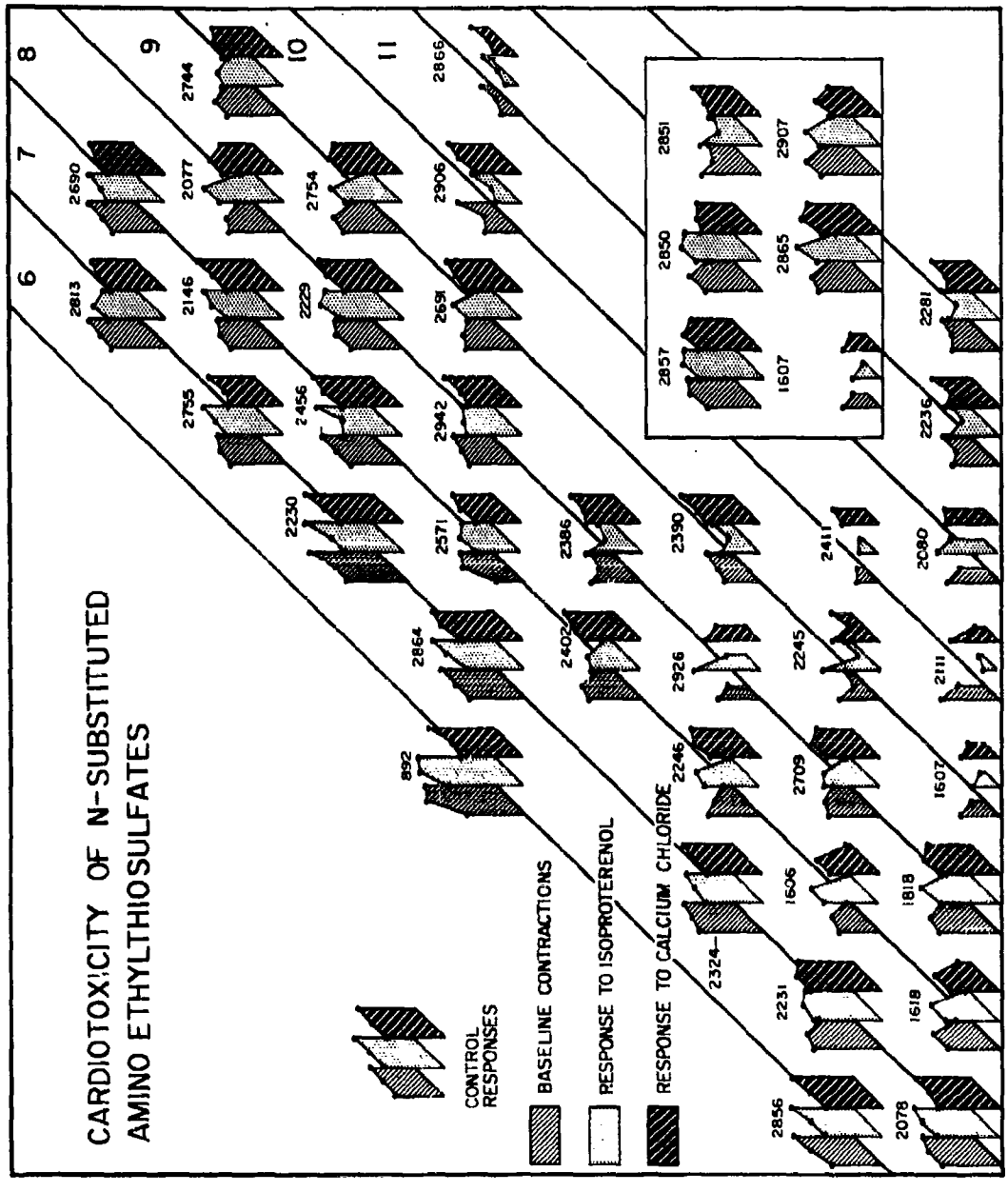
WR NR	SUBSTITUTION	CONSTRICTOR INDEX	PROTECTIVE INDEX
2137	n-hexyl	0	0
2078	n-heptyl	0	4.5
1618	n-octyl	0	0
2231	2-heptyl	0	0
1606	2-octyl	0	3.6
2246	3-octyl	0	4.3
2401	cyclooctyl	0	2.0
892	cyclohexyl	0	0
2864	cyclohexylmethyl	0	3.6
2571	cyclohexylethyl	0	3.8
2691	cyclohexylbutyl	0	6.8
689	diethyl	0	0
2755	dibenzyl	(dilates)	0
2324	di-n-heptyl	0	0
2230	phenmethyl	0	0
2456	phenethyl	0	0
2229	phenpropyl	0	4.9
2754	phenbutyl	0	9.4
2146	phenoxyethyl	0	0

Section I
TABLE 3

VASOCONSTRICTOR INDICES OF N-SUBSTITUTED

AMINOETHYLTHIOSULFATES

WR NR	SUBSTITUTION	INDEX	PROTECTIVE INDEX
2402	4-octyl	1	4.6
2386	4-nonyl	1	5.6
2390	3-decyl	1	11.0
2744	m-methoxyphenpropyl	1	3.2
2709	2-nonyl	1	5.1
2079	2-ethyl,1-hexyl	1	2.2
2813	diphenylmethoxyethyl	1	0
2866	cyclohexylhexyl	1	4.6
2906	cyclohexylpentyl	1	0
2942	cyclohexylpropyl	1	5.1
2857	formyl,n-decyl	1	0
2851	ethanol,n-decyl	2	0
2245	2-decyl	2	5.0
2111	n-undecyl	2	.0
1607	n-decyl	2	6.0
1818	n-nonyl	2	3.1
2080	n-dodecyl	3	0
2236	n-tridecyl	3	6.8
2281	n-tetradecyl	3	3.0



Section I
Figure 1

Section J

THE PHARMACOLOGY OF COMBINATIONS OF RADIOPROTECTANT CHEMICALS

I. S-alkylthiouroniums and Mercaptoalkylamines

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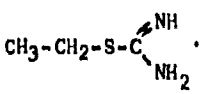
Isothiourea and amidine derivatives with short S-alkyl chains are vasopressor in action and have been used in the treatment of hypotension (1). Certain of the isothioureas have recently been re-examined for radioprotectant activity and have been found to afford significant protection against otherwise lethal radiation in the mouse (2). Since hypotension and other circulatory problems, such as severe hemoconcentration, were found when mercaptoalkylamines were injected in radioprotectant doses (3), a study has been made of the possibility of combining these two agents in a well tolerated radioprotectant mixture.

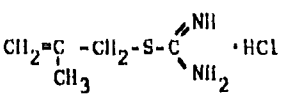
METHODS

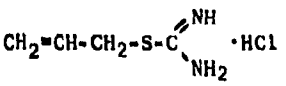
Adult mongrel dogs of either sex, weighing from 10-12kg., were anesthetized by the intravenous injection of 35mg/kg of pentobarbital sodium. Arterial blood pressure was measured in a catheter introduced into the femoral artery and advanced into the abdominal aorta. The catheter was connected to a pressure transducer and the pressure recorded electronically. Respiratory exchange was measured by means of a pneumotachograph head placed in the tracheal air-way and connected to a differential pressure transducer.

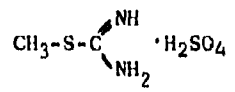
The pressure changes were recorded electronically. Hematocrit determinations were made by centrifuging 1 ml of blood in a Wintrobe tube at 2000 rpm for 30 minutes. Arterial and venous oxygen saturation values were determined with a Colson densiotometer which was calibrated with values determined by the manometric oxygen method of Van Slyke (4). Catechol-amine concentrations in venous blood drawn from the inferior vena cava were determined by the Weil-Malherbe and Bone method as modified by Gray and Young (5). Blood glucose concentrations in venous blood were determined by a potassium ferricyanide reduction method (6). Blood flow through an innervated hind limb was measured in the cannulated femoral artery by means of a Shipley-Wilson rotameter and the flow rate was recorded electronically.

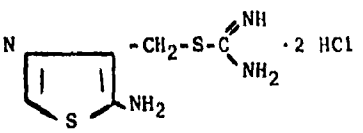
The following chemical compounds were dissolved in distilled water and injected intravenously over a 5 minute period. Dose was calculated as the base.

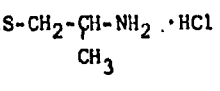
WR 539 

WR 334 

WR 688 

WR 1091 

WR 572 

and the sulfhydryl amine WR 166 

The principles of laboratory animal care as promulgated by the National Society for Medical Research were observed.

RESULTS

I. Physiological Changes.

Table I shows the arterial pressure changes caused by injection of the S-alkylurothiourea compounds into anesthetized dogs. It can be seen that each of the compounds caused an increase in arterial pressure. There was an increase in both systolic and diastolic pressure. The duration of the pressor effect was variable but with the exception of S-ethylisothiourea, where the pressure was 220/170 mm Hg at 60 and 120 minutes, the blood pressure returned to pretreatment levels in 20 minutes. When compound WR 166 alone was injected (120mm/kg) an increase of mean arterial pressure was seen during the 5 minute injection period (+20 mm Hg - average of 5 experiments). This hypertension was followed in approximately 12 minutes by an abrupt hypotension (-90 mm Hg - average of 5 experiments).

When 50 mg/kg or 5 mg/kg of WR 539 was injected along with 120 mg/kg of WR 166, very severe hypertension was produced. Systolic pressures reached over 300 mg Hg even when the WR 166 injection was given 20 minutes after injection of the isothiourea compound. Both animals treated with this combination developed hemorrhagic areas in the right heart and large hemorrhagic patches in the lungs. The same general result was obtained when 30 mg/kg of WR 1091 was combined with a half-dose of WR 166 (72mg/kg). The animal bled from his lungs into the tracheal cannula and the injection was stopped. Systolic arterial pressure reached levels of over 400 mg Hg.

There were no marked hematocrit changes (Table II) in the animals treated with the thiouronium compounds. The small increases that did occur came on early and did not become more severe at two hours. Compound WR 166 caused a marked increase in hematocrit which became more severe with time.

When the thiourea compounds were injected, they caused a marked increase in rate and depth of respiration.

Measurement of blood flow through the innervated hind limb of the dog following close intra-arterial injection of compound WR 539 was made. It was found that 0.28 mg of this compound caused a decrease in flow greater than that produced by 0.25 ug of norepinephrine injected in a like manner. Larger doses of WR 539 failed to reduce the flow more than the 0.28 mg dose.

II. Biochemical Changes.

Table III shows oxygen saturation values in arterial and venous blood following the administration of several of the isothiouronium derivatives and combinations of them with compound WR 166. These compounds (possible exceptions, WR 572 and WR 688) caused a relative hypoxia in the animals. This was more severe in the case of the mixtures. The hypoxia was present along with hypertension and adequate ability to exchange air.

Table IV shows catechol-amine and blood glucose values for animals treated with the thiourea compounds and WR 166. Compound WR 572 produced a clear-cut hyperglycemia which was sustained for 2 hours. The other thiourea compounds were less active in this respect. WR 539 and WR 334 showed a tendency to increase blood glucose levels; however, the limited number of experiments did not allow a statistical evaluation of this point. Compound WR 166 caused a strong hyperglycemia which was still evident at 2 hours. The preliminary survey of catechol-amine levels showed that there was no strong or sustained release of these materials following injection of the S-alkylisothiouronium compounds. WR 166 caused a sustained increase in epinephrine level at the one and two hour periods. Norepinephrine levels were increased over the control value in this one experiment. However, the increased norepinephrine

levels seen in this one experiment were within normal limits found in our laboratory.

DISCUSSION

The pressor activity of the S-alkylthiouronium compounds has been recognized for a long time and a study of the compounds with alkyl chain lengths of 1-9, 11, 13 and 15 carbons has been made (7). The first ten members of the series show a steady gradation in pressor activity while the last three members of the group are inactive due to poor water solubility. It was found that increase of chain length caused a significant depressor component to be expressed and indeed only the first three members of the series are reported to be consistently pressor in anesthetized animals. Our results do not agree with these findings because each of the compounds did cause hypertension. Our series is too limited to estimate potency as a function of chain length; but compound WR 334 which had the longest alkyl chain and compound WR 572 with the 2-amino-ethioxylmethyl function both produced strong pressor action with no evidence in 2 hours of a depressor component. Fastier reported on smaller doses of the series (1-10mg/kg) and perhaps this accounts for the differences in our results.

The combination of the S-alkylthiouroniums with WR 166 led to the production of lethal hypertensive reactions. It is possible that release of epinephrine by WR 166 potentiated the vasoconstrictive power of the thiouroniums and that inhibition of the epinephrine destructive enzymes by the thiouroniums potentiated the pressor reaction. Mundy et al. (8) have shown that beta-mercaptoethylamine (MEA) is a strong releaser of epinephrine and Blaschko and Duthier (9) have examined the monoamine oxidase inhibiting powers of the thiouroniums. These compounds are potent inhibitors of both monoamine and diamine oxidases (10). It should be noted that small (5mg/kg) or large

(50mg/kg) doses of compound WR 539 plus 120 mg/kg of WR 166 caused explosive hypertensive reactions.

The hematocrit increases observed in these experiments with S-alkylthiouroniums were minor in nature, developed early and did not progress. It is probable that splenic contraction may have accounted for the changes because it is well known that barbiturate anesthesia causes splenic engorgement and that sympathetic discharge, or more likely sympathetic potentiation in this case, can cause the spleen to contract and discharge cell rich blood into the circulation. WR 166 caused a large increase in hematocrit which continued to rise up to 2 hours. Hemoconcentration has been described following MEA administration (3) and the loss of plasma from the circulation may be prevented by antihistaminics (11). Since WR 166 is a close chemical congener of MEA, the possibility of a similar mechanism of hemoconcentration must be considered.

The femoral blood flow studies, although preliminary in nature, showed that WR 539 was a potent peripheral vasoconstrictor. Fastier and Reid (12) have described a similar pressor action in the pithed rat hindquarters and also reported that S-alkyl chain lengths up to 3 carbons greatly increased the sensitivity of the vasculature to catechol-amines. As has already been pointed out, this increased sensitivity to catechol-amines may have contributed to the extreme hypertension produced with combinations of S-alkylisothioureas and WR 166.

Each of the S-alkylisothiourea derivatives used caused some degree of venous hypoxia. Arterial sampling was scanty, but in the one compound studied (WR 688) there was no decrease in arterial saturation. The most probable explanation for the decrease in venous oxygenation might be that the peripheral vasoconstriction caused by these agents slowed blood flow, thereby extending oxygen extraction time in the tissues. When the two agents were combined, there was lowering of

arterial and venous oxygen saturation. It should be remembered that these animals experienced extreme hypertension and bleeding into the respiratory tract. Right heart and pulmonary damage found in these cases suggest hemodynamic changes which might have prevented optimum oxygenation in the lungs.

Amidine derivatives have the property of lowering blood sugar and, indeed, a number of them have been proposed as "oral insulins." The more effective agents are the diguanidine compounds and even these agents may cause an early hyperglycemia which is followed by a hypoglycemia when the body is nearly depleted of glycogen (13). The clinical use of compounds such as decamethylene-diguanidine (Synthalin) and 2 phenylethyl-diguanide (Pherformin) has been discontinued because of their toxic effect on the liver (13). The short chain S-alkylthiouronium compounds have received little attention as hypoglycemic agents and the results of our experiments suggest that at least in the doses employed, they may not be very potent in this respect. In fact, they tend to produce increases in blood glucose level. The short period of the experiments may have not allowed time for the hypoglycemic action to be manifested. WR 166, caused a strong and sustained hyperglycemia. This action is similar to that shown by MEA (8).

The limited survey of the effects of these agents upon peripheral catecholamine levels show that they do not produce a strong or prolonged release of catechols. Had the samples been taken at an earlier time after administration of the S-alkylisothioureas, higher catechol levels might have been found. WR 166 caused an increase in epinephrine level which persisted at 60 and 120 minutes. Again, this result would be expected and agrees with studies on MEA (8).

SUMMARY

This survey study has dealt with two S-alkylthiouronium compounds WR 539 and WR 1091 which have been well studied (1); and with three compounds WR 334,

WR 572 and WR 688 which have not been described in the pharmacological literature. The physiological changes caused by the former compounds were those already described; however, Fastier's (1) thesis that longer and larger side chains lead to significant depressor activity was not confirmed in this limited series. Perhaps the functional groups never reached the length or size requirements for depressor action.

Combinations of a sulfhydrylamine, WR 166, which has many of the properties of beta-mercaptoethylamine (MEA), with the S-alkylthiouroniums, precipitated a lethal hypertensive reaction. This reaction was produced with very small doses of the S-alkylthiouroniums. Peripheral blood flow studies have confirmed the peripheral pressor potency of S-ethylthiouronium in the dog. Extremely low concentrations of this agent produce a maximum constriction of the vasculature of the innervated hind limb. The fact that WR 166 increases the peripheral level of epinephrine and that the S-alkylthiouroniums potentiate epinephrine action, possibly through inhibition of breakdown mechanisms, may account for the lethal nature of the combinations studied. Because of these facts, very small doses of the S-alkylthiouroniums in conbination with sulfhydrylamines will lead to as much difficulty as larger ones.

The S-alkylthiouronium compounds caused vasoconstriction and in some cases a relative venous hypoxia in the dog. When they were combined with a sulfhydryl amine, both parameters were markedly affected. Thus their mode of radioprotection may be of the anoxic or 5-hydroxytryptomine type.

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ABSTRACT

A study has been made of the feasibility of combining pressor amidine radioprotectant compounds with depressor sulfhydrylamines in a well tolerated radioprotectant mixture. Four S-alkylthiouroniums and a methyl-betamercaptoethylamine were used. All of the S-alkylthiouroniums were pressor agents, with no depressor component, when injected intravenously in doses of 50-90mg/kg in the dog. When these agents were combined with a methyl-betamercaptoethylamine (72-120mg/kg) at doses from 5 to 50mg/kg a severe hypertension ensued. Systolic pressures of up to 400mm Hg were sometimes observed. This severe hypertension may have been the result of potentiation of the effects of catechol-amines released by the sulfhydrylamine and aided by the fact that the S-alkylthiouroniums inhibited breakdown mechanisms responsible for catechol destruction.

The S-alkylthiouroniums tended to reduce venous oxygen saturation and when combined with the sulfhydrylamine reduced saturation in both arterial and venous blood. It is possible that their mode of protection falls into the "anoxic" or 5-hydroxytryptamine class. These compounds tended to produce mild hyperglycemia and very little change in peripheral catechol-amine levels.

It is concluded that combinations of S-alkylthiouronium and sulfhydrylamine protectants are not a practical method of reducing toxicity of these two classes of compounds.

TABLE I

ARTERIAL PRESSURE CHANGES INDUCED BY THIOUREA COMPOUNDS IN THE DOG

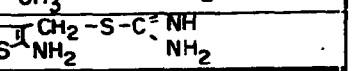
Compound-WR# Dose mg/Kg	Control Period	Maximum During Injection	+5 Minutes	+20 Minutes	+60 Minutes	+120 Minutes	Formula
539 50 mg/Kg	150/110	200/150	130/85	160/125	220/170	220/170	$CH_3-CH_2-S-C(=NH)-NH_2$
1091 50 mg/Kg	200/155	240/175	220/170	200/155	200/155	190/140	$CH_3-S-C(=NH)-NH_2$
688 56 mg/Kg	180/130	195/140	210/160	185/150	185/142	185/142	$CH_2=CH-CH_2-S-C(=NH)-NH_2$
334 63 mg/Kg	230/140	320/200	230/180	180/135	180/130	180/130	$CH_2=C(CH_3)-CH_2-S-C(=NH)-NH_2$
572 90 mg/Kg	160/120	230/160	230/160	185/140	165/130	155/110	
COMBINATIONS WITH WR-166 PROPYL MEA							$HS-CH_2-C(CH_3)-NH_2$
539 50 mg/Kg 166 120 mg/Kg	185/120	After Thiourea 230/160	Maximum After SH Cpd 320/240				Cpd 166 given 20 minutes after Cpd 539. Lung damage — right heart damage.
1091 30 mg/Kg 166 72 mg/Kg	180/140	First Infusion 435/300	Second Infusion 420/250	Simultaneous injection			Blood gushed from tracheal cannula-stopped injection then continued.
539 5 mg/Kg 166 120 mg/Kg	170/110	During Injection 340/220		"		"	Bled into tracheal cannula.

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TABLE II

HEMATOCRIT VALUES IN ANIMALS TREATED WITH THIOUREA DERIVATIVES AND WR 166

COMPOUND	CONTROL VALUE	1 HOUR AFTER ADM.	2 HOURS AFTER ADM.
539	41.0	44.0	43.0
1091	38.0	41.5	41.5
688	40.3	43.5	43.5
572	32.0	39.0	39.0
334	48.0	51.5	53.0
166	36.5	43.5	49.0

Note that the hematocrit value for 166 (sulfhydryl amine) shows a progressive sharp increase while the other compounds cause a sharp minor rise which is not, in general, progressive.

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TABLE III

BLOOD OXYGEN VALUES FOLLOWING ADMINISTRATION OF THIOUREA DERIVATIVES AND WR 166

COMPOUND	CONTROL		15 MIN.		30 MIN.		60 MIN.		120 MIN	
	A	V	A	V	A	V	A	V	A	V
539 50 mg/Kg.		85		67		48	Min. +90 = 59		Min +210 = 65	
1091 50 mg/Kg		68		51		--		28		32
334 63 mg/Kg		69		51		--		52		45
572 90/mg/Kg		68		76				66		58
688 56 mg/Kg	73	67	86	66			84	52	87	58
166 120 mg/Kg	90	75	90	51	77	22	84	29	80	25
166 (120) 1091 (30)	92	62	60	15	+ 85 MIN. A-64, V-20					
166 (120) 539 (50)		84				60		32	+221 V-65	

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TABLE IV

BLOOD CATECHOL AMINE (micrograms/L plasma) AND BLOOD GLUCOSE (mg/100 ml) VALUES FOLLOWING ADMINISTRATION OF THIOUREA DERIVATIVES AND WR 166

DRUG	CONTROL			60 MINUTES			120 MINUTES		
	GLUCOSE	E	N	GLUCOSE	E	N	GLUCOSE	E	N
539	103	–	–	120	–	–	118	–	–
1091	113	0	14.4	109	0	18.7	104	0.8	8.1
334	78	0	5.8	92	0.3	7.6	102	0.3	6.4
572	77	0.4	3.7	98	0	9.3	134	0.5	9.8
688	84	–	–	74	–	–	88	–	–
166	106	0.1	1.5	216	1.9	7.6	184	2.5	4.0

E = Epinephrine
N = Norepinephrine

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Section K

STUDIES OF ANTI-RADIATION CHEMICAL AGENTS IN THE DOG

SUMMARY REPORT

Project 6X60-16-001 Ionizing Radiation Injury - Prevention and Treatment (Chemical Protection Against Total Body Radiation)

Reporting Installation: Walter Reed Army Institute of Research
Walter Reed Army Medical Center
Washington 12, D. C.

Department of Radiobiology
Division of Nuclear Medicine

Period Covered by Report: May 1957 through December 1962

Principle Investigator: David P. Jacobus, M.D.

Data Compiled by: David E. Davidson, Jr., Capt., VC

Reports Control Symbol: MEDDH-228

Security Classification: Confidential

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SUMMARY REPORT OF CHEMICAL PROTECTION STUDIES IN THE DOG

I Introduction

The dog has been used infrequently in other laboratories as an experimental animal for investigating the protective effects of antiradiation chemical agents. Rodents have been, and will probably continue to be, the principle mammalian species used in the screening of protective chemicals, since mice may be used in large numbers, with a relatively smaller expenditure of space, labor and funds.

It has long been known that mice can tolerate on a weight basis extremely large quantities of pharmacologically active chemical agents compared to man or to other large mammals. The dog appears to resemble man in that the presently available antiradiation agents are poorly tolerated in protective doses.

It is thus of the greatest importance that the study of radioprotective agents be extended to the dog, and to other large mammals in order that a more reasonable extrapolation to man might eventually be possible. This report is a summary of approximately five years of investigations in chemical protection in the dog.

II Materials and Methods

The Van de Graaff accelerator at the National Institutes of Health, Bethesda, Md., has been reserved on a continuing basis for the irradiation of dogs one afternoon per week in connection with these studies. The accelerator may be operated at up to 2.5 MVP and 10 MA, generating, with the use of a gold target x-ray dose rates up to 200r/min at distances from the target sufficiently great to assure a nearly uniform radiation field for exposing dogs. Usually, a target to midline distance of two meters is employed. At shorter distances, dose rates up to 500r/min can be attained, sacrificing little in field uniformity.

The x-ray beam is directed downward. The dogs are exposed inside of a metal twin-compartment restraining cage without rotation. The gold target is located 285cm above the floor of the exposure room. Machine malfunctions have been frequent during the course of these experiments. Shorting of the high voltage in the tube due to water vapor in the filling gas or electronic malfunction has been the most frequent problem. These interruptions in radiation dose delivery can be reduced, but not entirely eliminated by reducing the voltage and current through the tube. This significantly reduces the x-ray dose rate, but this does not appreciably detract from the experimental procedures.

The dogs are irradiated in pairs for the chemical protection studies. Each pair consists of a control and a chemically pre-treated dog, irradiated simultaneously. Customarily, five or six dog pairs can be irradiated in a single afternoon. The dogs and equipment are transported to and from the irradiation facility in a three ton truck (covered) on each exposure day.

The Van de Graaff accelerator is designed so that the entire x-ray beam passes initially through an ionization chamber. As the radiation dose is delivered counts are recorded on a display panel in the control room. Thus an integrated total dose, proportional to the number of counts can be determined for each exposure. This should not be affected by fluctuations in voltage, current or interruptions in the delivery of the dose. Before irradiation, the dose per count at any point in the dog irradiation cage can be determined by exposing one or more Victoreen thimble chambers inside of the cage for an arbitrary number of counts.

The normal pre-irradiation dosimetric procedure is to expose simultaneously two chambers, 500r and 1000r capacity, for about 5 minutes. One chamber is placed centrally on top of the radiation cage, and the other is placed inside

of the cage at the estimated midline of one of the dog compartments. At the end of the test exposure, the corrected (s.t.p.) dosimeter readings and the total number of counts are recorded. This procedure is repeated until 3 essentially identical results are obtained. All subsequent dog exposures for that day are based on this dose per count determination. A further check on the reproduceability of the dose actually delivered to the dogs is made by placing one thimble chamber on top of the cage during each dog exposure. These chamber readings can then be compared to the previous readings made during the test exposures.

The dog irradiation cage is rectangular in shape and constructed of sheet aluminum, with some steel fittings (hinges, hasps, etc.) A vertical aluminum panel divides the cage into two compartments of equal size for the two dogs. Each compartment measures 30" L x 24" H x 12" W.

Solutions of the chemical agents are ordinarily prepared fresh on the morning of irradiation. Soluble agents are usually prepared in water, so that the final concentration is isotonic with the plasma (155 x 10^{-3}meq./liter). Sparsely soluble agents are dissolved in physiological saline solution, or prepared as a suspension in 0.3% methyl cellulose solution. Insoluble chemical agents are usually prepared as a suspension in 0.3% methyl cellulose, although occasionally other vehicles have been used. When the chemical nature and stability of the compound permits, the solution is adjusted to a physiological pH(7.45).

The chemical agents are administered according to the body weight of the dog. The doses of all agents are expressed as milligrams of the free base per kilogram of body weight.

Chemical agents have been administered intravenously, orally, intramuscularly, intraperitoneally or subcutaneously. Agents expected to induce marked physiologic

changes when given intravenously, e.g. MEA, are customarily injected slowly over at least a five minute interval. Less pharmacologically active compounds, e.g. PAPP, may be injected more rapidly. The cephalic vein in the foreleg or lateral saphenous vein in the hindleg are the usual injection sites. A quarter inch diameter rubber or plastic stomach tube and a wooden mouth gag are utilized for giving solutions by mouth. Solutions are prepared in such a way as to avoid gross bacterial contamination, but the instability of many of the chemical agents precludes heat sterilization before administration. Infection at the site of injection has never been a problem, although phlebitis and even local tissue necrosis has been observed when some of the more irritating chemical compounds have inadvertantly been injected perivascularly.

Healthy, mature mongrel dogs of either sex, weighing 6 to 11 kg. are used in the radiation studies. In a limited number of the earlier studies, dogs weighing up to 20 kg were used.

Prior to issue, the dogs at this laboratory are held for observation in a central facility for at least two weeks, during which time they are immunized for canine distemper, hepatitis and rabies with the commercial modified live virus vaccines, and when indicated, given anthelmintics. Upon issue, the dogs are placed indoors in individual metal cages and held for a minimum of five to seven additional days for observation. During this time they are examined clinically for gross defects or disease, and pre-irradiation blood counts and body weights are taken. The dogs are paired for irradiation with another of similar body weight, and when possible with one of like sex. The member of the pair to receive the chemical treatment is randomly selected. No food is given to the dogs on the day of irradiation.

The dogs are retained in the indoor cages for at least thirty days following

irradiation. During this time the dogs are examined regularly for the appearance (or lack of appearance) of the signs of radiation injury. Approximately twice weekly, blood samples are drawn for hematologic studies. At the time of the bleedings the body weight is recorded. If death occurs, the survival time is noted. Post-mortem examinations are sometimes performed in both the dogs dying after irradiation, and in the dogs surviving beyond 30 days.

Space for the long term holding of about seventy dogs is available in the outdoor runs at the Forest Glen section of this laboratory. Both control irradiated and chemically protected survivors have been sent to Forest Glen for the observation of the possible late effects of radiation. Some dogs have been maintained for as long as 5 years following irradiation. In the past two years few new dogs have been sent to this facility, since the runs have long since been filled with the survivors of the earliest protection studies.

Prior to the use of a new chemical agent it is necessary to perform preliminary toxicity tests. Extensive studies of toxicity have been performed on very few compounds. The main purpose of the initial toxicity studies is to determine the approximate maximum dose of the chemical which can be administered without causing the death of the dog or producing an unduly severe toxic reaction. The administration of the agent to five or six dogs is usually sufficient to gain this information, although the "maximum tolerated dose" so determined is admittedly an estimate. At the time of the toxicity study, observations of the major clinical signs exhibited by the animal are recorded. Food is ordinarily witheld on the day in which a toxicity study is performed

More precise pharmacological studies of the actions of radioprotective compounds in both anaesthetized and unanaesthetized dogs are conducted by the Pharmacology section of this department. Close coordination is maintained with this group of investigators.

III Results

A. Control Irradiations

A large number of unprotected, untreated dogs have been irradiated during the course of the dog protection program. In addition to the control irradiated dogs paired with protected dogs, a limited series of untreated dogs has been irradiated at various doses solely for the purpose of characterizing the radiation syndrome and patterns of lethality produced under the conditions of whole-body irradiation in this laboratory. The pooled survival data from these two groups of dogs is presented in Table I. Survival times at various radiation dose levels are also illustrated in Figures 1 through 4.

No deaths occurred within 30 days after the whole body irradiation of nine dogs at 200r. Acute mortality was observed after all doses of 300r or more. At 300r, two of ten dogs died within 30 days, one at 12 and one at 20 days post-irradiation. Sixty per cent mortality within 30 days was observed in ten dogs exposed to 350r, with deaths occurring between the twelfth and twenty-third days. At 400r, mortality was near ninety per cent, and deaths all occurred between six and twenty days after irradiation. 450r was 92% lethal in 137 dogs irradiated. Deaths were observed between the seventh and twenty-sixth post-irradiation days. All of ten dogs died within 18 days after whole body exposure to 500r, and after 700r, death within 13 days was observed in all of 4 dogs. A large series of dogs was irradiated at 775r, and only one out of the entire group of 112 dogs survived beyond 30 days. All other deaths occurred by the sixteenth post-irradiation day, and one dog died as early as the third day.

Thus at doses up to 775r, death is principally the result of radiation injury in the hematopoietic tissues. The characteristic hematopoietic syndrome characterized by pancytopenia, hemorrhage, anemia, secondary infection, and death in one to four weeks was observed in the dogs irradiated in this dose range. The percent 30-day

mortality increases as the radiation dose is increased from 200 to 500r. The $LD_{50/30\ days}$ appears to be slightly less than 350r. The $LD_{99/30\ days}$ is apparently near 500r. One dog has survived 775r during the course of these experiments. This was a very large dog, and at this early stage in these experiments (1957), dosimetry was based solely on the integrator reading of the Van de Graaff. No Victoreen chambers were exposed with each dog pair, so that the accuracy of the radiation dose in this case is uncertain.

In the hematopoietic dose range, the survival time tends to decrease as the radiation dose is increased. At 350 to 450r the mean survival time is about 14 days, while at 500 to 775r, the mean survival time is 11 to 12 days. At 700 to 775r no dog has ever survived beyond the sixteenth post-irradiation day, except for the single 30-day survivor mentioned previously. This decreasing survival time is believed to be the result of the increasing dominance of intestinal radiation injury at the higher doses. At 775r, a few deaths were observed within 3 to 6 days after irradiation, the survival time associated with the acute intestinal radiation syndrome in the dog.

At radiation doses above 1200r in this laboratory, dogs invariably died within the first week after whole body irradiation. These deaths are the result of radiation injury in the epithelium of the gastrointestinal tract. Denudation of the intestinal epithelium, vomiting, diarrhea, loss of fluids and electrolytes and death in two to six days after irradiation are characteristically observed. At 1500r, two dogs of the thirty-six irradiated survived eight days. This is somewhat longer than the expected survival time for the acute intestinal syndrome in the dog. No histopathologic examinations were conducted in these two dogs, so that the reason for the delayed deaths cannot be stated with certainty. Apparently some recovery of the intestinal epithelium was possible at this level of irradiation in these animals.

No signs of radiation injury referable to the central nervous system were observed in two dogs exposed to 8000r, but at 9000r each of two irradiated dogs exhibited the typical signs of CNS injury. Nearly all dogs exposed to whole body doses of 9000 to 18,000r exhibited the CNS syndrome, and death occurred within a few hours to four days after irradiation. Three of the dogs exposed to 12,000r did not exhibit the characteristic signs of deep scleral injection, nystagmus, ataxia, extensor rigidity and clonic-tonic convulsions. At the time when these dogs were irradiated, however, the Van de Graaff accelerator was operating improperly, and the radiation dose delivered may have been inaccurate.

B. Toxicity Studies

The results of the chemical toxicity studies conducted in the dog are summarized in Tables II, III, IV, V, and VI.

Two chemical compounds, 2-mercaptoethylamine HCl (WR 347 MEA) and 2-aminoethanethiosulfuric acid (WR 361 - the Bunte salt of MEA) have been studied in considerable detail. The toxic effects of these two compounds are summarized in Tables II and III respectively. The incidence of vomiting, hyperactivity and convulsions following the administration of the chemical agents has been indicated, but no indication of the severity of these signs is presented. This must be considered in interpreting these tables.

In the tables, the signs of both retching and vomiting are included under the category of "Vomiting", since food was withheld prior to most of these studies. The term "hyperactivity" has been used to include a variety of hyperactive states including excitement, episodes of twitching or jerking, severe restlessness, tremors, muscular fasciculations, exaggerated reflex activity, hypersensitivity to external stimuli, etc. Convulsions of clonic, tonic or mixed type have been observed. In addition to the above mentioned signs the dogs given these chemical agents in toxic doses characteristically exhibit cyanosis, pallor of the visible mucous

membranes, hyperventilation and tachycardia. Inability to walk and loss of consciousness may be observed after high drug doses.

Atropine is a frequently used pre-medication in many of these studies. The principle reason for its use is to reduce bronchial secretions following the use of many of these agents, particularly MEA. Atropine also eliminates the hypersalivation associated with drug action. Atropine appears to have little or no additional beneficial influence on the toxicity of most of the agents studied in this laboratory. Atropine does not measureably influence the cardiovascular effects of MEA. Atropine has been given subcutaneously or intramuscularly in doses of 0.05 to 0.22 mg/Kg 15 minutes to several hours before the subsequent administration of the chemical agents.

The maximum tolerated dose of MEA in the dog is about 100 mg/Kg. Even at this dose occasional drug-induced mortality is observed. The mortality rises sharply if the dose is increased to 125 or 150 mg/Kg. The rate of injection of MEA is critical. If injected intravenously in much less than a five minute interval, the agent is poorly tolerated.

Pentobarbital Sodium (Nembutal) administered prior to or following the administration of MEA prevents the development of convulsant activity in sedative or anaesthetic doses, however Numbutal potentiates the hypotension associated with MEA toxicity, and thus reduces the dose of MEA tolerated by the dog.

Ten mg/kg of Dibenzyline (Phenoxybenzamine), an adrenergic blocking agent, is effective, administered orally or intravenously several hours before MEA, in preventing or reducing the hypotension associated with MEA toxicity. This work was performed in the Pharmacology Section at Forest Glen. Too few dogs are included in Table II to demonstrate this sparing effect of Dibenzyline on MEA toxicity.

Drug induced mortality has rarely been observed in dogs receiving the chemical mixture containing 100 mg/Kg of MEA and 300 mg/Kg of Cysteine. This mixture seems in fact to be slightly less toxic than 100 mg/Kg of MEA by itself. The rationale

for using this particular chemical mixture is that MEA and Cysteine induce toxicity by different mechanisms. The common pathologic lesions after MEA administration are found in the liver and the kidney. The toxicity of the two agents together is not additive. Furthermore, the toxicity of this mixture in the dog is not greatly increased by the prior oral or intravenous administration of 5 mg/Kg of para-aminopropiophenone (PAPP) and 10 mg/Kg of para-phenylphenol (POHØØ).

Neither MEA or Cysteine can be administered intravenously in the dog in the same mg/Kg doses as those required to protect the mouse. 100 mg/Kg of MEA is one half of the mg/Kg dose required to protect the mouse. 300 mg/Kg of Cysteine is likewise half of one mouse protective dose. Thus one full protective dose of thiol, equivalent to that required on a weight basis to protect the mouse can be attained in the dog.

Mea is absorbed after oral administration, but it induces vomiting, even in sub-protective doses. Chlorpromazine (Thorazine) does not prevent the vomiting. Oral doses of MEA as high as 400 mg/Kg do not cause death, since the agent is expelled from the body by the vomiting.

The Bunte salt of MEA (WR 361) does not produce acute mortality after intravenous doses up to 350 mg/Kg, although delayed deaths due to chemical toxicity were observed after 80 mg/Kg, and after 300 mg/Kg in single instances. Clinical signs similar to those produced by MEA itself are observed following the administration of the Bunte salt.

Oral administration of the Bunte salt usually causes vomiting and self-elimination of the agent. Emesis cannot be prevented by the administration of any of a variety of anti-emetics.

As much as 60 mg/Kg of the Bunte salt can be administered intravenously without mortality in combination with 5 mg/Kg PAPP, 10 mg/Kg POHØØ, 100 mg/Kg of MEA,

and 300 mg/Kg Cysteine. No dog has survived 75 mg/Kg or more of the Bunte salt in this combination.

The toxicity of other single chemical agents is summarized in Table IV. In this table, the estimated maximum tolerated dose is indicated where possible from the available data. Clinical observations were also made in most of these studies, but these data have not been included in this report. An asterisk after the value of the maximum tolerated dose indicates that the stated value was the highest dose administered. In many cases, even though the dog survived the highest tested dose of the drug, the signs of toxicity were so severe that further increasing the dose was considered to be unprofitable. Some of the compounds for which toxicity information is presented have not been tested against radiation in the dog, since the maximum tolerated dose is so low as to make protection unlikely.

As a general rule, the maximum tolerated dose of chemical agents in dogs is considerably lower than the dose tolerated by mice. The exceptions of course are the orally administered compounds, most of which are readily rejected in the dog by vomiting. One notable exception is n-decylaminoethane thiosulfuric acid (WR 1607). The maximum tolerated dose of this agent in the mouse is 5 mg/Kg, while the dog tolerates at least 20 mg/Kg. The mouse has an apparent sensitivity to the central depressant effects of this agent, and death is the result of respiratory paralysis. The dog does not exhibit this effect.

Tables V and VI are a summary of the toxicity of chemical agents in combination. The maximum tolerated dose of the agent in the indicated combination is presented.

C. Radiation Protection Studies

The results of all the chemical protection studies in the dog conducted in this laboratory since 1957 are tabulated in Tables VII and VIII.

Four hundred fifty Roentgens has frequently been used as the test dose of radiation in the dog protection studies in this laboratory. Occasional control

survivors are expected at this dose of whole-body radiation. This nearly 100 per cent lethal dose provides a very sensitive test system for discovering agents which protect, even though the protection may be slight in terms of actual radiation dose reduction. Because of the steepness of the radiation dose - mortality curve in dogs and other mammals which has been observed in this and other laboratories, chemical agents having only slight or moderate protective activity may produce very high survival rates in treated dogs at a dose of radiation which is just barely supralethal. Using a radiation test dose this close to the steep portion of the dose - mortality curve, however, requires careful control of the radiation factors to insure proper delivery of the dose. Careful dosimetry, including dose measurements during each dog exposure, is important. The simultaneous irradiation of a control dog with each protected dog provides an additional check on the validity of each exposure.

775r has also frequently been used as the radiation test dose. At this supralethal dose, survival of any control dog is an extremely rare occurence. No dog in the 7-11 Kg weight range has ever survived this dose. Significant 30 day survival in chemically pre-treated dogs is possible only if the protective agent or combination of agents possesses considerable protective activity. 775r is somewhat more than twice the $LD_{50/30\ days}$.

The 1500r dose is above the threshold for the intestinal syndrome and death is expected to occur in the dog within 2½ to 5 days. Survival of chemically pre-treated dogs into the second post-irradiation week is indicative of protection of the intestinal epithelium, even though death within 30 days due to hematopoietic injury is observed. Thirty day survival after 1500r implies protection of both hematopoietic and intestinal tissues. Only the most potent chemical protective mixtures have produced 30-day survival after 1500r in the dog.

The threshold dose for induction of the Central Nervous System syndrome in dogs in this laboratory is about 9000 to 10,000r. Evidence of protection in dogs

given such radiation doses is based on the failure of the typical clinical signs of Central Nervous System radiation injury to develop in the protected dogs. To date no unprotected dog has ever survived beyond the fourth post-irradiation day at doses above 9000r. The peak mortality occurs on the second post-irradiation day.

1. MEA and Combinations of MEA

Administered intravenously, 100 mg/Kg of MEA provided excellent protection against 450r whole-body radiation, although one of three dogs died as the result of the toxic effects of this chemical agent. The irradiation was performed within one to six minutes after the completion of the administration of the MEA. The Bunte salt of MEA (WR361) did not protect against 450r when administered intravenously one to nine minutes before irradiation in doses of 300 or 350 mg/Kg. The higher drug dose was poorly tolerated and produced drug deaths in four of six dogs.

A mixture of MEA and Cysteine provided excellent protection when administered within a few minutes before irradiation. At 775r, 100 mg/Kg of MEA plus 500 mg/Kg of Cysteine protected six of the seven dogs which survived the toxic effects of the mixture. These high doses were poorly tolerated, and the drug-induced mortality rate was high. These same two agents in combination at lower doses of each (50/150 mg/Kg) provided excellent protection against 400r without any deaths due to drug toxicity.

The administration of 5 mg/Kg of para-aminopropiophenone intravenously one half to two hours before and 100 mg/Kg of MEA + 300 mg/Kg of Cysteine intravenously 1 to 3 minutes before 700 or 775r provided excellent protection without any deaths due to toxicity. Methylene blue was administered (1-3 mg/Kg) intravenously following irradiation to reverse the PAPP-induced methemoglobinemia. This same combination of 3 agents increased the survival time of all dogs into the second post-irradiation week after 1500r. Given orally, however, no protection was observed after 700r.

In mice para-phenylphenol (POHØØ), a cytochrome-oxidase inhibitor, has been shown to potentiate the protective activity of PAPP. For this reason it has frequently been used in the dog whenever PAPP is administered. The protective activity of POHØØ has not been investigated in the dog, but at doses of 10 mg/Kg it does not seem to measureably increase the toxicity of 5 mg/Kg of PAPP.

5 mg/Kg of PAPP + 10 mg/Kg of POHØØ intravenously 30 minutes to 2 hours before irradiation in combination with 100 mg/Kg of MEA and 300 mg/Kg of Cysteine provided 30-day survival in 18 of 22 dogs. Only one dog succumbed due to the toxic action of the chemical agents. Some 30-day survivors were also observed even after 1200 or 1500r, and all but one of the non-survivors lived until the second post-irradiation week. Excellent protection was also observed after 775r, when the PAPP and POHØØ portion of the combination were given orally, but little or no protection was observed when all four agents were given by mouth at either 775r or 450r, although one dog did survive the lower radiation dose.

The combination of PAPP, POHØØ, MEA and Cysteine given intravenously appears to confer excellent protection in the dog. In order to estimate the dose-reduction factor for this combination in the dog the mortality data from all experiments in which these four agents were included in the pre-irradiation treatment have been pooled. In some cases one or more additional agents were included in the combination, but none of these have been shown to increase the protection afforded by the other four agents. These pooled data are tabulated below. The dose mortality curves for both control and protected dogs are presented in Figure 5. Comparison of these two curves indicates a dose-reduction factor of over three for this combination of protective agents.

Number of Protected Dogs	Rad. Dose	Per Cent 30-Day Mortality in Protected Dogs
31	700-775r	14%
28	1500r	77%

Excellent protection against 450r was afforded by the combined intravenous administration of PAPP, POH@@, and 300 mg/Kg of the Bunte salt of MEA, even though the Bunte salt exhibited no protective activity in previous experiments. In the earlier studies, however, the Bunte salt was administered within ten minutes before irradiation. This suggested that a longer time may be required between the administration of the Bunte salt and irradiation.

2. Analogues of MEA

WR 1607 (n-decylaminoethanethiosulfuric acid) exhibited good protective activity against 450r. Since this compound is insoluble in water, it has been administered in hot propylene glycol as a vehicle, or converted to the calcium or sodium salt by the addition of calcium or sodium hydroxides, in which form it is water soluble. This agent protected dogs at doses of only 10 to 20 mg/Kg, a very small amount of material on a molar basis. Good protection has been noted whether the agent has been given 5 minutes or 30 minutes before irradiation.

WR 1606 (n-methylheptylaminoethanethiosulfuric acid) conferred no protection in the dog when administered intravenously 2 to 8 minutes before 450r. This compound is a good protective agent at higher levels in the mouse, but it is a convulsant in the dog at these doses.

Twenty mg/Kg of WR 1607 in combination with PAPP and POH@@ produced excellent protection given intravenously 4 to 17 minutes before 450r irradiation, but no protection and several toxic deaths were observed when this same dose was given 25 minutes before irradiation. The reason for the poor results in this latter study are not known.

WR 2078 (n-heptylaminoethanethiosulfuric acid) at 35 mg/Kg in combination with PAPP and POH@@ produced no significantly greater 30-day survival than is ordinarily observed with PAPP and POH@@ alone at 450r.

WR 166B (1-amino-2-propanethiol or "Russian MEA") provided excellent protection given intravenously 2 minutes before 450r in combination with PAPP and POH00. This agent appears to afford protection in the dog comparable to that of MEA itself, although MEA is given at a lower millimole per kilogram dose. WR 166B in combination with PAPP, POH00 and Cysteine also protected all of three dogs against 775r at a dose of 150 mg/Kg.

WR 147 (2-aminobutanethiol) at 75 mg/Kg in combination with PAPP, POH00 and Cysteine failed to protect against 775r.

3. Isothiuronium Compounds

AET is one of the group of isothiuronium compounds which at a physiological pH, transguanylates to yield mercaptoethylguanidine. Thus the protective activity of AET can be explained on the basis of the usual sulfhydryl theories; eg. radical trapping, mixed disulfide formation, etc.

WR 334, 539, and 572, are examples of isothiuronium compounds which are incapable of transguanylation, and thus their protective activity is usually attributed to their pharmacological activity, although there is the remote possibility that they might be converted to the corresponding thiol in vivo by the splitting off of the urea portion of the molecule.

AET (WR 298) exhibited no protective activity whatsoever against 450r at 50 mg/Kg I.V. WR 334 (2-methylallylisothiuronium) exhibited only slight protective activity, and then only at doses of the agent in which it produced toxic deaths.

WR 539 (S-ethylisothiuronium) provided excellent protection against 450r at 75 mg/Kg. One toxic death was observed at this dose of the compound. No dog which survived the toxic effects of this chemical agent either alone or in combination with other agents has ever failed to survive 450r during the course of these experiments. The pharmacology of this agent in the dog and other mammals has been studied here and in other laboratories. Its hypertensive activity has

been reported to be the result of a direct action on the smooth muscle of the vascular system. It has been used in humans to control surgical shock, but at much lower doses than are required for radiation protection. The hypertensive effect of this agent is potentiated by epinephrine. MEA is an epinephrine releaser in vivo and for this reason combinations of WR 539 with MEA, and perhaps other thiols are poorly tolerated, although this mechanism might be responsible for the potent protective activity of this kind of combination.

WR 572 (2-amino-4-thiazolylmethylisothiuronium) also conferred good protection against 450r. Pharmacological studies undicate that this compound has less hypertensive activity than the S-ethylisothiuronium.

Good protection was usually observed when isothiuronium compounds were used in combination with other protective agents. Against 775 and 1500r, 100 mg/Kg of AET intravenously in combination with PAPP, POH00, and 500 mg/Kg of Cysteine was poorly tolerated, but appeared to give protection comparable to that observed when MEA was used in this combination.

WR 539 in combination with MEA or with WR 1607 (n-decylaminoethanethiosulfuric acid) produced a large number of toxic deaths, but provided excellent protection in those dogs which survived the drug administration.

AET and PAPP (200/7 mg/Kg) given by mouth 2 hours before irradiation offered no protection against 775r.

4. Phenols and Phenones

15 mg/Kg of PAPP I.V. or 20 mg/Kg I.V. of 2,4-dinitrophenol in combination with PAPP, POH00, MEA and Cysteine were not tolerated. No dogs survived the administration of these agents.

WR 134 (4,4-dichlorobenzophenone) at 200 mg/Kg intraperitoneally in combination with MEA and Cysteine intravenously provided good 30-day survival after 775r, but in so few dogs, it would be impossible to say whether the protection was better than that observed with MEA and Cysteine alone. 200 mg/Kg of WR 134 and 10 mg/Kg of

POH00 orally in combination with MEA and Cysteine intravenously before 775 or 1200r offered good protection. All protected dogs survived beyond 30 days at 775r, and at 1200r the survival time was extended into the second post-irradiation week.

The addition of the protective agent diphenylamine to the usual combination of PAPP, POH00, MEA and Cysteine, prolonged survival of all protected dogs into the second post-irradiation week, although no 30-day survival was noted in this study. Methylene blue was given intravenously at 25 mg/Kg after irradiation in this study. No drug induced lethality was noted.

PAPP and POH00 given intravenously at 5 and 10 mg/Kg respectively before 400 or 450r produced 40% survival in the treated groups. One dog died as the result of drug toxicity in spite of the administration of Methylene blue intravenously after irradiation. In one additional study, the radiation dosimetry was undoubtedly improper, as 5 of 6 irradiated controls, and all of the 6 protected dogs survived.

WR 1798 (ω mercaptoacetanelidecarbamate) given by mouth did not increase the protective activity of the combination of PAPP, POH00, MEA and Cysteine.

5. Serotonin

Deaths due to the chemical toxicity of Serotonin have been observed after intravenous doses as low as 15 mg/Kg. In spite of the fact that Serotonin protects mice at far less than lethal drug doses, no protection whatsoever has been observed in dogs given 775r. No protection was observed after either intravenous or intraperitoneal administration.

In combination with other radioprotective chemical agents, no protection was observed over and above that normally expected from the other protective agents in the combination.

6. Carbamates

WR 638 (N-beta-mercaptoethyldithiocarbamic acid) orally at 500 mg/Kg

did not exhibit significant protective activity against 400r. In combination with Cysteine or with PAPP and POHØØ, orally or intravenously, WR 48A produced no significant additional protection.

7. Colchiceine, Colcemide

Neither of these mitotic inhibitors have showed any protective activity in the dog, either alone, or in combination with other radioprotective agents.

8. Miscellaneous

200 mg/Kg of Tyramine orally did not increase the protection afforded by PAPP, POHØØ, MEA, and Cysteine against 1500r, and one of three dogs died due to the toxic effects of the chemical combination.

A high dose of Reserpine and Marsalid did not protect against 775r.

No protection was afforded by 5-amino-1,3,4-triazole against 1500r which could not be attributed to the additional administration of PAPP, POHØØ, MEA and Cysteine.

D. Protection against the CNS Syndrome

The results of chemical protection studies in the dog at radiation doses of 10,000 to 18,000r are summarized in Table VIII.

The "protected" dogs received 5 mg/Kg of PAPP plus 10 mg/Kg of POHØØ intravenously one to three hours before irradiation and 100 mg/Kg of MEA plus 300 mg/Kg of Cysteine one to three minutes before irradiation. Most of the dogs were premedicated with 0.1 mg/Kg of Atropine subcutaneously, and 1 to 3 mg/Kg of Methylene blue was usually administered intravenously shortly after irradiation.

No significant difference in survival time was noted between the protected and unprotected dogs. One protected dog did survive for 9 days after 12,000r. This unlikely occurence undoubtedly represents an error in dosimetry. One of the deaths in a protected dog on the day of irradiation could definitely be

attributed to chemical toxicity, based on clinical and pathologic findings.

Evidence of protection against central nervous system injury in the treated dogs was dramatic at radiation doses of 10,000r. Protection against 12,000r was less dependable. The control dogs exhibited signs of CNS injury developing shortly after irradiation. Nystagmus, deep conjunctival injection, extensor rigidity, and tonic-clonic convulsions were observed in nearly all of the untreated dogs. CNS signs in the protected animals were usually absent or confined to the less incapacitating signs of somnolence, ataxia, nystagmus, etc. At radiation doses above 15,000r, the protected dogs were also incapacitated by the irradiation.

IV Discussion

These experiments with chemical protective agents in the dog illustrate that protection is possible in the larger mammalian species.

Two of the most effective chemical agents in the mouse, however, - AET and Serotonin - are relatively ineffective in the dog. AET, due to its toxicity, cannot be administered at high enough doses to protect by itself, but in combination with other protective agents, particularly PAPP, it is of significant value in inducing survival after supralethal irradiation. AET and PAPP do not, however, protect when given by mouth. We have been completely unable in this laboratory to demonstrate any protection with Serotonin in the dog, either by itself or in combination with other protective agents.

MEA has excellent protective activity in the dog, but unfortunately the margin of safety between toxic and protective doses is extremely narrow. MEA is not effective when given by mouth in the dog, although it is absorbable, since even sub-protective doses of MEA induce vomiting. We have so far been unable to prevent the vomiting by the use of anti-emetics.

Even after intravenous administration, the best protection is achieved when the irradiation is performed within a few minutes of the drug administration, since MEA is rapidly detoxified and excreted.

No protection has been observed in studies in which the Bunte salt of MEA (WR 361) has been given by itself intravenously; however, studies in which the Bunte salt has been given to dogs in combination with other protective agents (ie, PAPP + POH00) indicate that it probably does protect. It is suggested that a longer wait between the administration of the agent and irradiation might be necessary for the demonstration of protection, since the suggested mode of action of this agent is the conversion of the Bunte salt to the thiol in vivo. The Bunte salt is also not tolerated orally, since it induces vomiting in the dog, which cannot usually be prevented by the use of anti-emetics.

The combination of MEA and Cysteine given intravenously at doses of 100 and 500 mg/Kg respectively, provides excellent protection against decidedly supra-lethal irradiation, although at these doses the mixture is poorly tolerated, and drug-induced mortality is high. Tolerance can be increased by the administration of various hypertensive agents such as levarterenol. MEA and Cysteine have been used together at 100 and 300 mg/Kg doses in a variety of combinations of chemical agents. At these reduced doses this mixture produces only occasional deaths due to their toxic effects, and yet they still provide excellent protection. At only 50 and 150 mg/Kg respectively, they provide excellent protection against 400r.

Para-aminopropiophenone (PAPP) is a good protective agent in the mouse. Its protective activity has been attributed to its ability to convert oxyhemoglobin to methemoglobin and thus presumably to reduce tissue oxygen tension. In the mouse, PAPP potentiates the protective activity of thiols, and its activity is potentiated by the addition of para-phenylphenol (POH00), a cytochrome oxidase inhibitor. For these reasons, PAPP has frequently been used in our studies as

a component of many radioprotective mixtures, especially in combination with POHØØ, MEA and Cysteine.

PAPP with POHØØ intravenously does protect dogs against 450r. This protection is not very great, but it is definitely demonstrable. PAPP is absorbable orally, and is well tolerated. It does not usually induce vomiting, and seems to confer slight protection by mouth in the dog. PAPP has an additional merit in that the methemoglobinemia which it produces can be reversed by the administration of Methylene blue. Given after irradiation, the Methylene blue does not influence the protective action of the PAPP.

The most potent protective mixture so far tested in this laboratory has been the combination of 5 mg/Kg of PAPP, 10 mg/Kg of POHØØ, 100 mg/Kg of MEA, and 300 mg/Kg of Cysteine all given intravenously. The PAPP and POHØØ may be administered 30 minutes to 2 hours prior to irradiation, but the MEA and Cysteine must be given within a few minutes before irradiation. Tolerance of the MEA and Cysteine mixture is dependent on <u>slow</u> intravenous administration, over at least a five-minute injection period. It appears that this combination of agents is nearly as effective if the PAPP and POHØØ are given by mouth, but most of the protective activity is lost unless the MEA and Cysteine are given intravenously. These four agents in combination (intravenously) produce 30-day survival in nearly all of the dogs given 700 or 775r. Thirty-day survival even after 1200 or 1500r has also been observed in a few dogs given this mixture, and invariably after such doses of radiation intestinal death has been precluded in the protected dogs. This mixture has also protected dogs against the incapacitating central nervous system effects of multi-kiloroentgen doses of radiation, although the ultimate time in which death occurred was unchanged in all but one dog.

"Russian" MEA (WR 166B) appears to possess protective activity in the dog roughly comparable to that of MEA itself. It is difficult to tell at this time whether this agent has any advantages over MEA itself, although it can be given

in higher molar doses.

The series of MEA analogues in which straight hydrocarbon chains are attached to the nitrogen atom of the MEA molecule have been synthesized in this laboratory and have been studied in detail in the mouse. The effect of chain length on protective activity has been examined. Those compounds with chain length of eight to ten are of particular interest, since they do protect mice, and their lipid solubility raises an interesting question as to their intracellular distribution in vivo. The decyl Bunte derivative (WR 1607) has been studied in some detail in the dog, and it appears that at least at 450r radiation doses it is protective. Furthermore, its protective activity is potentiated by PAPP in the mouse, and apparently also in the dog. This agent protects dogs at molar doses far below those required for any other chemical agent. This agent is insoluble in water, and must be administered in either organic vehicles, such as hot propylene glycol, or in water at a very alkaline pH. The decyl Bunte salt is also extremely toxic, and is tolerated in the dog only at doses of 20 mg/Kg. This, however, is still about four times the mouse tolerated dose on a weight basis. Severe hypertension, cardiac arrest, collapse and death are observed within a few minutes after the administration of a toxic dose in the dog. Dibenzyline (phenoxybenzamine), an adrenergic blocking agent, is effective in increasing the drug tolerance when given orally or intravenously at doses of 10 mg/Kg well in advance of the protective agent.

The heptyl Bunte derivative (WR 2078) is non-protective in the dog. The methylheptyl derivative (WR 1606) is protective in the mouse, but to date protection in the dog cannot be demonstrated. The octyl derivative is protective in the mouse, and at the present time is under study in the dog.

Several isothiuronium compounds are presently being studied in the dog in some detail. The agents under study, S-ethylisothiuronium, 2-allylisothiuronium, 2-methylallylisothiuronium, and 2-amino-4-thiazolylmethylisothiuronium (WR 539, 782,

334 and 572) are all incapable of transguanylation and thus protection by the usual non-pharmacologic mechanisms seems unlikely. At least two of these agents the S-ethyl and 2-amino-4-thiazolyl isothiuronium compounds seem to have marked protective activity in the dog. Such protection has seldom been observed for single agents in the dog in the course of these experiments. These agents are, however, discouraging for their marked toxic effects. Their epinephrine potentiating activity seems to preclude their use in combinations with most of the thiols.

V Conclusions

Investigations of chemical protective agents in the dog have been conducted in this laboratory during the past five years. Anti-radiation chemical agents are in general pharmacologically active and are thus poorly tolerated in the dog. Most chemical agents protect only at drug levels uncomfortably close to the maximum tolerated dose. By the appropriate use of mixtures of chemical agents with non-additive toxicities, total molar doses of protective agents as great on a weight basis as those required to protect mice can be administered to dogs.

Chemical protection of dogs is possible in spite of the toxicity of protective agents. At doses of radiation more than twice the $LD_{50/30\ days}$ (775r) a combination of chemical agents consisting of PAPP, POH00, MEA and Cysteine induces 30-day survival in nearly 80 per cent of the treated dogs. Thirty-day survival can also be observed in a few cases after radiation doses as high as 1500r in dogs protected with this combination of four chemical agents. Under the conditions of irradiation in this laboratory, this represents a dose-reduction factor in excess of three.

At $LD_{90/30}$ to $LD_{100/30}$ doses of whole-body radiation, significantly increased 30-day survival in chemically protected dogs is easy to demonstrate. This does represent definite protection, but does not necessarily require very high dose-reduction factors. Significantly increased 30-day survival rates can be provided in dogs receiving chemical combinations or single chemical agents at these doses

of radiation.

Chemical protection not only against the hematopoietic syndrome, but also against the intestinal and central nervous system syndromes can be demonstrated in the dog. The CNS symptoms can be prevented by the pre-irradiation administration of a protective chemical mixture. The dogs so protected die as the result of intestinal radiation injury. Similarly, the intestinal syndrome can be reduced to the hematopoietic syndrome by protective agents, delaying death until the second post-irradiation week. Occasional 30-day survivors have been observed at doses of radiation sufficient to ordinarily induce intestinal death.

Protection in the dog has usually only been observed when the chemical agents have been given intravenously. With many of the most effective agents, good protection is afforded only if the irradiation is performed within a few minutes after the administration of the chemical agents. Little or no protection has been observed in the dog when protective agents have been administered orally.

TABLE I

SUMMARY OF ACUTE LETHALITY IN UNTREATED CONTROL DOGS EXPOSED TO 2.0-2.5 MVP X-RAYS (WHOLE BODY)

MIDLINE RAD. DOSE (r)	30 DAY SURVIVORS NO. IRRADIATED	TIME OF DEATH (DAYS)	SURVIVAL TIME (DAYS)				% MORTALITY
			MEAN	MEDIAN	MODE	RANGE	
200	9/9	—					0
300	8/10	12,20	16			12-20	20
350	4/10	12,12,12,12,14, 23	14.2	12	12	12-23	60
400	4/29	6,8,10,11,11, 11,11,12,12,13, 13,14,14,14,14, 15,15,15,15,16, 16,16,17,19,19, 20	13.7	14	14-15	6-20	89.7
450	11/137	See Figure 1.	14.3	14	13	7-26	92.0
500	0/10	10,10,10,11,11, 12,12,13,13,18	12	11-12	10	10-18	100
700	0/4	10,12,12,13	11.8	12	12	10-13	100
775	1/112	See Figure 2.	10.9	11	12	3-16	99.1
1200	0/2	6,6	6	6	6	6	100
1500	0/36	See Figure 3.	4.2	4	4	2-8	100
2000	0/1	4	4	4	4	4	100
7000	0/2	4,4 (No CNS signs)	4	4	4	4	100
8000	0/2	4,5 (No CNS signs)	4.5	4-5	4-5	4-5	100
9000	0/2	1,2 (CNS deaths)	1.5	1-2	1-2	1-2	100

TABLE I (cont.)

MIDLINE RAD. DOSE (r)	30 DAY SURVIVORS No. IRRADIATED	TIME OF DEATH (DAYS)	SURVIVAL TIME (DAYS)				% MORTALITY
			MEAN	MEDIAN	MODE	RANGE	
10,000	0/14	1,1,2,2,2,2,2, 3,3,3,3,4,4,4	2.5	2-3	2	1-4	100
12,000	0/14	1,1,2,2,2,2,2, 2,2,3,3,3,4,4,	2.4	2	2	1-4	100
14,000	0/1	1	1	1	1	1	100
15,000	0/1	1	1	1	1	1	100
16,000	0/1	1	1	1	1	1	100
18,000	0/1	1	1	1	1	1	100

TABLE II A

CONFIDENTIAL

TOXICITY OF MEA (WR 347) (2 MERCAPTOETHYLAMINE HCL) IN THE DOG

MEA Mg/Kg	ROUTE	ADDITIONAL MEDICATION Mg/Kg	VOMITING	HYPERACTIVITY	CONVULSIONS	NO.DEAD/ NO. INJECTED
50	IV	-	2/3	2/3		0/3
75	IV	-	1/2			0/2
85	IV	-	3/3	2/3		0/3
100	IV	-	12/15	10/15	2/15	3/15
125	IV	-	10/13	13/13	6/13	7/13
150	IV	-	3/4	4/4	2/4	4/4
175	IV	-		1/1	1/1	1/1
200	IV	-		2/2	2/2	2/2
100	IV	ATROPINE 0.2	4/5	4/5	1/5	1/5(6DAYS)
125	IV		2/3	3/3	2/3	3/3
150	IV		4/4	3/4	2/4	3/4
175	IV		1/1	1/1	1/1	1/1
200	IV		1/1	1/1		1/1

TOXICITY OF MEA (WR 347) (MERCAPTOETHYLAMINE HCL) IN THE DOG

MEA Mg/Kg	ROUTE	ADDITIONAL MEDICATION Mg/Kg	VOMITING	HYPERACTIVITY	CONVULSIONS	NO. DEAD/ NO.INJECTED
50	IV	PREMEDICATION 11 (75 Min)				0/1
80	IV	PREMEDICATION 20 (3 Hr)	1/1	1/1		0/1
100	IV	PREMEDICATION 3 (15-30 Min)	2/2			0/2
125	IV	PREMEDICATION 15 (90 Min)	1/2	1/2		1/2
		PREMEDICATION 30 (10 Min)				1/1
200	IV	NEMBUTAL I. V. 1 (1 Min)	1/1	1/1	1/1	1/1
		NEMBUTAL I. V. 5 (1 Min)				1/1
		NEMBUTAL I. V. 10 (1 Min)				1/1
250	IV	NEMBUTAL I. V. 3 (1 Min)				1/1

CONFIDENTIAL

TOXICITY OF MEA (WR 347) (MERCAPTOETHYLAMINE HCL) IN THE DOG

MEA Mg/Kg	ROUTE	ADDITIONAL MEDICATION Mg/Kg		VOMITING	HYPERACTIVITY	CONVULSIONS	NO. DEAD/ NO. INJECTED
125	IV	NEMBUTAL IV POST-MEDICATION	2 (1 Min)	1/1	1/1		1/1
			5 (1 Min)	1/1	1/1		0/1
			5 (20 Min)	4/4	4/4	2/4	0/4
			5 (1 Hour)	3/3	3/3	2/3	1/3
			10 (2 Min)	3/3	3/3	2/3	1/3
			40 (2-3 Hr)	1/1	1/1		1/1
150	IV	NEMBUTAL I.V. POST-MEDICATION	5 (15 Min)	1/1	1/1	1/1	1/1
			5 (60 Min)	1/1	1/1	1/1	0/1
175	IV		5 (2 Min)		1/1		1/1
			5 (15 Min)	1/2	2/2	1/2	1/1
			5 (30 Min)		1/1		1/1
			12.5 (15-60 Min)		1/1	1/1	1/1
			10 (15 Min)	2/2	2/2	1/2	2/2
200	IV		30 (15-75 Min)	1/1	1/1		0/1
250	IV		10 (10 Min)		1/1	1/1	1/1

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TOXICITY OF MEA (WR 347) (MERCAPTOETHYLAMINE HCL) IN THE DOG

MEA Mg/Kg	ROUTE	ADDITIONAL MEDICATION Mg/Kg	VOMITING	HYPERACTIVITY	CONVULSIONS	NO. DEAD/ NO. INJECTED
200	IV	NEMBUTAL I.V. PRE AND POST 3 1MIN/ 3 30MIN	1/1	1/1		1/1
		5 1 MIN/ 10 20MIN		1/1	1/1	1/1
		10 2 HRS/ 1 20MIN		1/1		1/1
150	IV	CORTISONE 10 ORAL	1/1	1/1	1/1	1/1
		CORTISONE 45 ORAL	1/1			0/1
		CORTISONE 3 ORAL THIRAZINE 1 I.M.	1/1	1/1	1/1	1/1
		DIBENZYLINE 25 ORAL (18 HRS PRE)	1/1	1/1	1/1	1/1
		DIBENZYLINE 10 ORAL (18 HRS PRE) NEMBUTAL IV (5MIN PRE) 5-15	2/2	1/2	1/2	2/2
200	IV	ATROPINE 0.2 DIBENZYLINE 5-10 I.V. ROBAXIN I.V. 75-250	3/3	2/3	2/3	3/3
100	IV	REGITINE I.V. 0.6 PRE+POST	1/1			0/1
150	IV	NEMBUTAL IV 30 (POST) STRICHNINE .13 I.V. (POST)	1/1	1/1	1/1	0/1

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TABLE II B:

ORAL TOXICITY OF MEA AND TOXICITY OF COMBINATIONS OF MEA (WR 347) IN THE DOG

-------Mg/Kg---------									
PAPP	POHØØ	MEA	CYST.	ROUTE	ADDITIONAL MEDICATION Mg/Kg	VOMITING	HYPERACTIVITY	CONVULSIONS	NO. DEAD/ NO. INJECTED
-	-	40	-	ORAL	-				0/1
-	-	75	-	ORAL	CALCIUM GLUCONATE	1/1			0/1
-	-	250	-	ORAL	CALCIUM GLUCONATE	1/1			0/1
-	-	400	-	ORAL	THORAZINE 25 I.M.	1/1			0/1
-	-	100	300	IV	-	1/1			0/1
-	-	150	500	IV	-	1/1			0/1
-	-	125	625	IV	ATROPINE 0.1		1/2		2/2
-	-	100	300	IV	TIGAN ORAL 10	2/2			0/2
-	-	100	300	IV	BENEMID-PROBENECID 100 ORAL (TABS)	2/2			0/2
5	10	100	300	IV	ATROPINE 0.2 METH. BLUE 1		1/1	1/1	1/1
5	10	200	600	ORAL	-	1/2			0/2
10	20	200	600	ORAL	-	1/1			0/1

TABLE III A:

TOXICITY OF 2-AMINOETHANETHIOSULFURIC ACID (BUNTE SALT OF MEA) (WR361) IN THE DOG

WR 361 Mg/Kg	ROUTE	ADDITIONAL MEDICATION Mg/Kg	VOMITING	HYPERACTIVITY	CONVULSIONS	NO. DEAD/ NO. INJECTED
90	IP	-				0/1
40	IV	-				0/1
90	IV	-	1/1			1/1 (7Days)
200	IV	ATROPINE 0.1				0/1
250	IV	ATROPINE 0.1	1/1			0/1
300	IV	ATROPINE 0.1	2/4	1/4		1/4 (6Days)
325	IV	ATROPINE 0.2	1/1	1/1	1/1	0/4
350	IV	ATROPINE 0.2	1/1	1/1		0/1
400	IV	ATROPINE 0.2	2/2	2/2	1/2	1/2
500	IV	ATROPINE 0.2	1/1			1/1

TABLE III B:

ORAL TOXICITY OF 2-AMINOETHANETHIOSULFURIC ACID (WR 361) IN THE DOG

WR 361 Mg/Kg	ROUTE	ADDITIONAL MEDICATION Mg/Kg	VOMITING	HYPERACTIVITY	CONVULSIONS	NO. DEAD/ NO. INJECTED
100	ORAL		1/2			0/2
300	ORAL		1/1			0/1
400	ORAL		1/1			0/1
600	ORAL		2/2			0/2
700	ORAL		1/1			0/1
800	ORAL		2/3	2/3		0/3
1000	ORAL		4/5	1/5		0/5
1200	ORAL		2/2			0/2
1300	ORAL		3/4			0/4
1400	ORAL		2/2			0/2
1500	ORAL		1/1			0/1
1600	ORAL		1/1	1/1		0/1
400	ORAL	TIGAN 10	1/1			0/1
600	ORAL	TIGAN 10	1/1			0/1
800	ORAL	TIGAN 10 PRE 10 WITH	1/1	1/1		0/1
100	ORAL	XYLOCAINE 45				0/1
200	ORAL	XYLOCAINE 40	1/1	1/1		0/1

ORAL TOXICITY OF 2-AMINOETHANETHIOSULFURIC ACID (WR361) IN THE DOG

WR 361 Mg/Kg	ROUTE	ADDITIONAL MEDICATION Mg/Kg	VOMITING	HYPERACTIVITY	CONVULSIONS	NO. DEAD/ NO. INJECTED
400	ORAL	THORAZINE 2	1/1			0/1
500	ORAL	THORAZINE 2	1/1			0/1
600	ORAL	THORAZINE 2	1/1	1/1		0/1
800	ORAL	THORAZINE 2	1/1			0/1
200	ORAL	THORAZINE 4 XYLOCAINE 500	0/1	1/1	1/1	0/1
300	ORAL	THORAZINE 4 XYLOCAINE 30	1/1			0/1
400	ORAL	THORAZINE 4 XYLOCAINE 30	1/1			0/1
600	ORAL	THORAZINE 8 XYLOCAINE 38	1/1			0/1
600	ORAL	THORAZINE XYLOCAINE EMETROL LIQUID	1/1			0/1
300	ORAL	CYCLIZINE 6.5 XYLOCAINE 40	1/1			0/1

TOXICITY OF 2-AMINOETHANETHIOSULFURIC ACID (BUNTE SALT OF MEA) (WR 361) IN THE DOG

WR 361 Mg/Kg	ROUTE	ADDITIONAL MEDICATION Mg/Kg	VOMITING	HYPERACTIVITY	CONVULSIONS	NO. DEAD/ NO. INJECTED
600	ORAL	PYRIDOXINE 40	1/1			0/1
600	ORAL	PYRIDOXINE 40 BONAMINE 7	1/1			0/1
600	ORAL	PYRIDOXINE 40 THORAZINE 2 XYLOCAINE 42	1/1			0/1
600	ORAL	PYRIDOXINE 40 MECLIZINE 6 THORAZINE 6	1/1			0/1
600	ORAL	PYRIDOXINE 40 MECLIZINE 6 XYLOCAINE 38	1/1			0/1
600	ORAL	PYRIDOXINE 40-50 MECLIZINE 6-7 THORAZINE 8-12 XYLOCAINE 30-35	2/2			1/2
600	ORAL	PYRIDOXINE 40 BONAMINE 5 THORAZINE 2 XYLOCAINE 36	1/1			0/1
600	ORAL	MECLIZINE 6 THORAZINE 2 XYLOCAINE 33	1/1			0/1
600	ORAL	MORNIDINE 0.6 (ORAL)	1/1			0/1
600	ORAL	MORNIDINE 0.5 IV (PRE AND POST) EMETROL LIQUID	1/1			0/1
600	ORAL	MORNIDINE 0.6 (PRE AND POST) IV THORAZINE 4 XYLOCAINE 31	1/1			0/1

TABLE III C:

TOXICITY OF COMBINATIONS OF 2-AMINOETHYLTHIOSULFURIC ACID IN THE DOG

Mg/Kg						ADDITIONAL MEDICATION Mg/Kg	VOMITING	HYPERACTIVITY	CONVULSIONS	NO.DEAD/ NO.INJ.
WR 361	PAPP	POHØØ	MEA	CYSTEINE	ROUTE					
800	-	-	-	600	ORAL	-	1/1			0/1
800	-	-	-	300	ORAL		1/1			0/1
700	-	-	-	600	ORAL		1/1			0/1
700	-	-	-	300	ORAL		2/2			0/2
700	-	-	-	200	ORAL		1/1			0/1
100	-	-	-	100	ORAL	XYLOCAINE 32	1/1			0/1
300	-	-	-	100	ORAL	THORAZINE 4 XYLOCAINE 35	1/1			0/1
300	-	-	-	100	ORAL	THORAZINE 65 XYLOCAINE 50	1/1			0/1
250	5	10	-	190	IV	ATROPINE 0.1		1/1		1/1
300	5	10	-	300	IV	ATROPINE 0.1				1/1
25	5	10	100	300	IV	ATROPINE 0.1		1/1		0/1
50	5	10	100	300	IV	ATROPINE 0.1				0/1
60	5	10	100	300	IV	ATROPINE 0.1	1/1	1/1		0/1
75	5	10	100	300	IV	ATROPINE 0.1		1/2		2/2
100	5	10	100	300	IV	ATROPINE 0.1		1/1	1/1	1/1
175	5	10	100	300	IV	ATROPINE 0.1		1/1	1/1	1/1
250	5	10	100	300	IV	ATROPINE 0.1			1/2	2/2
450	5	10	100	300	IV	ATROPINE 0.2				1/1

TABLE IV

TOXICITY OF ANTIRADIATION CHEMICAL AGENTS IN THE DOG

(* HIGHEST DOSE ADMINISTERED)

WR#	NAME OF COMPOUND	Mg/Kg	ROUTE	VEHICLE	pH	NO.OF DEATHS /NO. INJ.	PREMEDICATION Mg/Kg	APPROX. MAX TOLER. DOSE Mg/Kg
XI	BRITISH ANTI-LEWISITE (BAL)	10	IM	WATER	UNADJUSTED	0/1	-	10*
34	METHYLAMINE-HCL	100	IV	WATER	7.8	0/1	-	100*
40	2,4-DINITROPHENOL	5	IV	M.C	5.0	0/1	-	10*
		10				0/1		
		20	ORAL	M.C.	5.0	0/1	-	20*
48A	N,N-MERCAPTOETHYL-DITHIOCARBAMIC ACID	175	IV	M.C.	8.0	0/1	ATROPINE 0.1	275*
		200				0/1		
		275				0/1		
		400	ORAL	M.C	8.0	0/2	ATROPINE 0.1	500*
		500				0/1		
62	SEROTONIN (5-HYDROXYTRYPTAMINE) (CREATINE SULFATE COMPLEX)	10	IV	P.S.S.	7.4	0/1	-	95
		95				0/1		
		220				2/2		
		100	IP	P.S.S.	7.4	0/1	-	100
		200				0/1		
65	2-AMINOETHANOL	65	IV	P.S.S.	7.8	0/1	-	65*
81A	S-ACETYL β MERCAPTOETHYLAMINE- HCL	200	IV	WATER		0/1	ATROPINE 0.1	200
		250				1/1		
		300				1/1		
134	4,4-DICHLOROBENZOPHENONE	150	IP	M.C.		0/1	-	200*
		200				0/1		

TOXICITY OF ANTIRADIATION CHEMICAL AGENTS IN THE DOG

WR#	NAME OF COMPOUND	Mg/Kg	ROUTE	VEHICLE	pH	NO. OF DEATHS / NO. INJ.	PREMEDICATION Mg/Kg	APPROX.MAX TOLER.DOSE Mg/Kg
147	2-AMINOBUTANETHIOL HCL	75	IV	WATER		0/1	-	75
		100				1/1		
		150				1/1		
166B	1-AMINO-2PROPANETHIOL HCL	110	IV	WATER	7.2	1/1	-	225*
		150				0/2		
		225				0/1		
170	COLCEMIDE	4	IV	WATER	UNADJUSTED	2/2	-	< 4
215A	S,3-AMIN PROPYLISO-THIURONIUM-Br·HBr (APT	<25	IV	PSS	7.45	0/3	ATROPINE 0.1	40
		40				0/2		
		50				2/4		
		60				0/1		
228	n-DECYLAMINOETHANETHIOL	13	IV	MC		1/1	ATROPINE 0.1	-
		20				0/1		
230	n-OCTYLAMINOETHANETHIOL	20	IV	MC		0/1	ATROPINE 0.1	20
		30				1/1		
297	3-(2-AMINOETHYL)INDOLE-HCL (TRYPTAMINE)	95	IV	PSS		0/1	-	95*
298	2-AMINOETHYLISOTHIURONUM Br HBr (AET)	50	IV	WATER	7.45	0/1	ATROPINE 0.1	85
		85				0/1		
		100				3/3		
306	TYRAMINE·HCL	95	I.P.	WATER		1/2	-	<95
307	SOD. FLUOROACETATE	0.1	I.P.	WATER		1/1	-	-
		0.3				0/1		

TOXICITY OF ANTIRADIATION CHEMICAL AGENTS IN THE DOG

WR#	NAME OF COMPOUND	Mg/Kg	ROUTE	VEHICLE	pH	NO.OF DEATHS / NO. INJ.	PREMEDICATION	APPROX.MAX TOLER.DOSE Mg/Kg
334	2-METHYLALLYLISO-THIURONIUM· CL	40 60 80 96	IV	WATER		0/1 1/4 1/1 1/1	ATROPINE 0.1	50
335	3-AMINO-1-PROPANETHIOL HCL	67 100	IV	WATER		1/1 1/1	-	<67
336	1-AMINO-2-PROPANETHIOL HCL	150 200	IV	WATER		0/2 2/2	ATROPINE 0.1	150
348	CYSTEINE	100-1000	ORAL	WATER	7.45	0/8	NONE	1000*
351	REDUCED GLUTATHIONE	1000	IV	P.S.S.		2/2	-	<1000
419B	CYSTEAMINE-N-ACETIC ACID·HCL	200 235 250 500	IV	WATER	7.4	0/1 0/1 0/1 1/1	-	250
539B	S-ETHYLISOTHIURONIUM· HBr	30 50 75 100 150 200	IV	WATER	7.4	0/1 1/1 0/1 0/1 1/1 1/1	-	100
572	2-AMINO-4THIAZOLYLMETHYL ISOTHIURONIUM·2HCL	200 250 300 350	IV	WATER	UNADJUSTED	0/1 0/1 0/1 1/2	-	300
613	3-β-AMINOETHYL-1,3-THIAZANE-2,4-DIONE·HCL	35 50 60 70 75 125 150 200 250 300	IV	M C	3.5-7.4	1/1+ 0/1 1/1+ 1/1+ 1/2 0/1 1/1 0/1 2/2 0/1		+Rapid (1-2Min) INJECTION 70 (SLOWLY)

TOXICITY OF ANTIRADIATION CHEMICAL AGENTS IN THE DOG

WR#	NAME OF COMPOUND	Mg/Kg	ROUTE	VEHICLE	pH	NO.OF DEATHS / NO. INJ.	PREMEDICATION Mg/Kg	APPROX.MAX TOLER.DOSE Mg/Kg
648	S-(3AMINO-N-METHYL) ISOTHIURONIUM·Br·HBr	75 80 90	IV	WATER		0/1 1/1 1/1	-	75
728	2-AMINOETHANETHIOL - 2-AMINO-ETHANE SULFONATE -2HCL	470 600 665	IV	WATER	7.45	0/1 1/1 1/1	ATROPINE 0.15	470
740	HYDROXYLAMINE	10 25 35 50	IV			0/1 0/1 0/1 1/1	ATROPINE 0.1	35
783	2-(S-THIOPSEUDOUREIDO)-ETHYLDITHIOCARBAMIC ACID	50 90 100 117 130 150 200	IV		7.2-7.4	1/1 0/2 3/5 1/1 1/1 2/2 1/1	-	90
		200 250 400 800	ORAL	WATER	7.2	0/4 0/1 0/1 0/1	-	800*
		400	ORAL (CAPSULES)	-	-	0/2	-	400*
904	RESERPINE	0.1 0.5	IV	WATER		0/1 0/2		0.5
		2.4	IM	WATER		1/1		
1161	2,4-DIHYDROXYETHYLPHENYL KETONE	125 200 400	IV	WATER	7.4	0/1 0/1 1/2	-	200
1606B	N-(1-METHYLHEPTYL)-AMINO-ETHANETHIO-SULFURIC ACID	15 20 25 30 37.5	IV	M.C.	7.45	0/2 0/1 0/1 0/1 1/1	-	30

TOXICITY OF ANTIRADIATION CHEMICAL AGENTS IN THE DOG

WR#	NAME OF COMPOUND	Mg/Kg	ROUTE	VEHICLE	pH	NO. OF DEATHS /NO. INJ.	PREMEDICATION Mg/Kg	APPROX.MAX TOLER.DOSE
1607		5	IV	HOT PROPYLENE		0/1	-	10*
		10		GLYCOL	UNADJ.	0/4		
	n-DECYLAMINOETHANE-THIOSULFURIC ACID	30	IV	$Ca(OH)_2$	12.0	0/1	-	30
		40				1/1		
		50				1/1		
		20	IV	NaOH	11.8	0/1	-	25
		25				0/1		
		30				1/3	DIBENZYLINE ORAL 10(24HR PRE)	
2078	n-HEPTYLAMINOETHANETHIOSULFURIC ACID	35	IV	NaOH	11.0	0/2	-	40
		42				0/1		
		50				1/1		
X19	COLCHICINE	0.15	IV	WATER		0/2	-	0.25
		0.25				0/2		
		0.40				1/2		
		0.50				2/2		

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TABLE V CONFIDENTIAL

TOXICITY OF COMBINATIONS OF ANTIRADIATION CHEMICAL AGENTS IN THE DOG

	----------Mg/Kg----------								
AR#	PAPP	POHØØ	MEA	CYSTEINE	Mg/Kg	ROUTE	NO.OF DEATHS NO.INJECTED	PREMEDICATION Mg/Kg	APPROX. MAX. TOLER. DOSE
40	5	10	100	300	20	IV	0/1	ATROPINE 0.2 METH.BLUE 1.5	20*
49A	-	-	-	300	150	IV	0/2	ATROPINE 0.1	150*
	5	10	-	300	200	IV	0/1		200*
	5 ORAL	10	-	-	500	ORAL	0/2	"	500*
62	5	10	100	300	50	IV	0/1	ATROPINE 0.2	50*
	-	-	100	300	100	IV	1/3	EQUANIL 70	-
	5	10	100	300	100	IP	0/1	METH.BLUE 1-2	-
81A	5	10	33	100	33	IV	0/1	ATROPINE 0.1	100
	5	10	67	200	67		0/1		
	5	10	100	300	100		0/2		
	5	10	100	300	120		2/2		
	5	10	100	300	175		1/1		
115A	5	10	100	300	20	IV	1/1	ATROPINE 0.1	<20
	5	10	100	300	25		2/3		
	5	10	100	300	30		1/1		
	5	10	100	300	50		1/1		
229	5	10	100	300	20	IV	1/1	ATROPINE 0.1	<20
247	-	-	100	300	50	IV	1/1	-	<50
299	-	-	80	--	80	IV	1/1	EQUANIL 60	<80
	-	-	75	300	100		1/1	-	<100
-	7.5	15 ORAL	-	-	-	ORAL	1/1	-	<7.5-15
307	-	-	100	300	.05	IV	0/2	-	.05*
334	5	10	100	300	60	IV	0/2	ATROPINE 0.1	60*
335	-	-	-	300	60	IV	1/1	-	<60
336	-	-	-	200	125	IV	0/1	-	125*
339B	-	-	100		28	IV	0/1	-	40
	-	-	100		42		0/1		
	-	-	100		60		1/1		
	-	-	100		70		0/1		
	-	-	100		85		1/1		
			50		50	IV	0/1	-	50
			50		75		1/1		

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TABLE VI

TOXICITY OF ADDITIONAL COMBINATIONS OF ANTIRADIATION CHEMICAL AGENTS IN THE DOG

AGENT		AGENT					
WR#	Mg/Kg	WR#	Mg/Kg	ROUTE	NO. DEAD / NO. INJ.	PREMEDICATION Mg/Kg	APPROX. MAX. TOLER. DOSE
48A	200	215A	40	IV	1/1	ATROPINE 0.1	-
62	110	306	45	IV	1/1	-	-
539B	50	1607	5	IV	0/1	-	50-5
	50		10		1/2		
	50		20		1/2		
539B	7.5	166B	100	IV	0/1	-	7.5-100
	7.5		150		1/1		
	5.0		100		0/2		
539B	50	1607	20	IV	0/1	DIBENZYLINE 10Mg/Kg IV 3 HRS PRE	75-20
	75		20		0/2		
	75		30		1/1		

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TABLE VII

ANTI-RADIATION CHEMICAL AGENTS. — RADIATION PROTECTION STUDIES: A. MEA AND COMBINATIONS OF MEA

WR#	NAME OF COMPOUND	DOSE OF AGENT Mg/Kg	ROUTE	TIME BEFORE RAD.	ADDITIONAL MEDICATION Mg/Kg	RAD DOSE (r)	NO. OF DOG PAIRS	DEATHS DUE TO CHEMICAL TOXICITY	TIME OF DEATH (DAYS POST RAD)		30 DAY SURVIVAL	
									CONTROLS	PROTECTED	CONTROLS	PROTECTED
	SINGLE AGENTS											
347	MEA	100	IV	1-6MIN	-	450	3	1/3	10,12,12	0	0/3	2/2
		150	IV	1MIN	CORTISONE ORAL 20-30	775	2	2/2	10,10	0,1	0/2	0/0
361	2-AMINOETHYL-THIOSULFURIC ACID	300	IV	1-9MIN	ATROPINE 0.1	450	6	2/6	14,17,21	0,0,6,11,12	3/6	1/4
		350	IV	2-3MIN	ATROPINE 0.1	450	6	4/6	11,17,17, 20,21	0,1,1,1,14	1/6	1/2
	COMBINATIONS											
347 348	MEA CYSTEINE	100-113 500-570	IV IV	1-3MIN 1-3MIN	- -	775	16	9/16	10,10,11, 11,11,12, 12,12,12, 12,12,12, 12,12,12,13	0,0,0,0,0, 0,0,0,1,12	0/16	6/7
164		50 150	IV IV	1-3MIN 1-3MIN	- -	400 400	5	0/5	11,14,14, 19	—	1/5	5/5

ANTI RADIATION CHEMICAL AGENTS. — RADIATION PROTECTION STUDIES: A. MEA AND COMBINATIONS OF MEA

NR#	NAME OF COMPOUND / COMBINATIONS	DOSE OF AGENT Mg/Kg	ROUTE	TIME BEFORE RAD.	ADDITIONAL MEDICATION Mg/Kg	RAD DOSE (r)	NO. OF DOG PAIRS	DEATHS DUE TO CHEMICAL TOXICITY	TIME OF DEATH (DAYS POST RAD) CONTROLS	TIME OF DEATH (DAYS POST RAD) PROTECTED	30 DAY SURVIVAL CONTROLS	30 DAY SURVIVAL PROTECTED
302	PAPP	5	IV	30-120 MIN	ATROPINE .1 METH BLUE 1-3	700-775	7	0/7	6,8,9,10 10,10,12, 13	17	1/7	6/7
347	MEA	100	IV	1-3MIN		1500	4	0/4	3,4,4,4	7,13,17,18	0/4	0/4
348	CYSTEINE	300	IV									
		5	ORAL	2.5HR	BONAMINE ORAL 2-3	700	2	0/2	10,12	12,21	0/2	0/2
		100	ORAL	30-40MIN								
		300	ORAL		"							
302	PAPP	5	IV	30-120 MIN	ATROPINE.1 METH BLUE 1-3	775	23	7/23	5,6,7,8, 8,9,9,10 11,11,11, 12,12,13 13,13,13 13,13,13 16	0,15,16,19, 20	0/23	18/22
1795	POHØØ(p-PHENYLPHENOL)	10	IV									
347	MEA	100	IV			1200	2	0/2	6,6	15	0/2	1/2
348	CYSTEINE	300	IV	1-3MIN (*12MIN)		1500	6	0/6	4,4,4, 4,5,	2*,8,11,13	0/5	2/6
		5	ORAL	2-2.5HRS		775	9	0/9	3,8,9,9 9,9,10 10,10,11	3,9,10	0/9	6/9
		10	ORAL	"	"							
		100	IV	1-4MIN	"							
		300	IV	"								
		5	ORAL	2HRS	BONAMINE	775	3	0/3	10,11,12	11,11,20	0/3	0/3
		10	"	"	25							
		100	"	1HR								
		300	ORAL	"								
		5	ORAL	32-35	ATROPINE 0.1	450	3	0/3	12,13,13,	8,18	0/3	1/3
		10	ORAL	MIN								
		200	ORAL	"								
		500	ORAL	"								
302	PAPP	5	IV	40-50	ATROPINE 0.1	450	6	0/6	12,12,15 16,18	—	0/6	6/6
1795	POHØØ	10	IV	MIN								
361	2-AMINOETHYLTHIO-	300	IV	40-50								

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ANTI-RADIATION CHEMICAL AGENTS. — RADIATION PROTECTION STUDIES: B. ANALOGUES OF MEA

WR#	NAME OF COMPOUND	DOSE OF AGENT Mg/Kg	ROUTE	TIME BEFORE RAD.	ADDITIONAL MEDICATION Mg/Kg	RAD DOSE (r)	NO. OF DOG PAIRS	DEATHS DUE TO CHEMICAL TOXICITY	TIME OF DEATH (DAYS POST RAD)		30 DAY SURVIVAL	
	SINGLE AGENTS								CONTROLS	PROTECTED	CONTROLS	PROTECTED
1607	n-DECYLAMINOETHANE-THIOSULFURIC ACID											
	HOT PROPYLENE GLYCOL VEHICLE	10	IV	20-30MIN	—	450	11	1/11	9,11,12 12,14,14, 15,16,16	0,8,11,12 17,17	0/9	5/10
	CALCIUM SALT pH 10.5	20	IV	5-9MIN	—	450	7	*2/7 *30Mg/Kg	13,15,17 17,17	0,0	0/5	5/5
	SODIUM SALT pH 11.2	30	IV	25-35MIN	—	450	7	5/7	12,13,13, 13,15	0,0,2,4, 6,13	0/5	1/2
		20	IV	30MIN	DIBENZYLENE ORAL PRE 24HR	450	6	0/6	11,13,15, 18,20,24	16	0/5	5/6
1606	n-METHYLHEPTYLAMINO-ETHANETHIOSULFURIC ACID	20	IV	2-2MIN	ATROPINE 0.1	450	6	0/6	13,13,13, 15,22	13,15,19,20 20,20	1/5	0/6
	COMBINATIONS											
302 1795 1607	PAPP POHØØ n-DECYLAMINOETHANE-THIOSULFURIC ACID (SODIUM SALT)	5 10 20	IV IV IV	45MIN 45MIN 4 -17MIN	—	450	6	0/6	7,10,11 12	—	1/5	6/6
302 1795 1607	PAPP POEØØ n-DECYLAMINOETHANE-THIOSULFURIC ACID (SODIUM SALT)	5 10 20	IV IV IV	30MIN 30MIN 25MIN		450	6	3/6	10,14,18, 18,22	1,3,4,11 12,22	1/6	0/3

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ANTI-RADIATION CHEMICAL AGENTS. — RADIATION PROTECTION STUDIES: B. ANALOGUES OF MEA

WR#	NAME OF COMPOUND / COMBINATIONS	DOSE OF AGENT Mg/Kg	ROUTE	TIME BEFORE RAD.	ADDITIONAL MEDICATION Mg/Kg	RADE DOSE (=)	NO. OF DOG PAIRS	DEATHS DUE TO CHEMICAL TOXICITY	TIME OF DEATH (DAYS POST RAD) CONTROLS	TIME OF DEATH (DAYS POST RAD) PROTECTED	30 DAY SURVIVAL CONTROLS	30 DAY SURVIVAL PROTECTED
302 1795 2073	PAPP POHØØ n-HEPTYLAMINOETHANE-THIOSULFURIC ACID (SODIUM SALT pH=11.0)	5 10 35	IV IV IV	45MIN " 30MIN	—	450	6	0/6	12,15,15 16,17,17	13,17,21	0/6	3/6
302 1795 166B	PAPP POHØØ 1-AMINO-2-PROPANE-THIOL	5 10 150	IV IV IV	45MIN 45MIN 2MIN	—	450	6	1/6	10,11,12, 13,16,17	0	0/6	5/5
302 1795 147 348	PAPP POHØØ 2-AMINOBUTANETHIOL CYSTEINE	5 10 75 300	IV IV IV IV	2HRS 2 HRS 2MIN 2MIN	—	475	4	0/4	8,10,11 11	10,14,15, 22	0/4	0/4
302 1795 166B 348	PAPP POHØØ 1-AMINO-2-PROPANE-THIOL CYSTEINE	5 10 150 300	IV IV IV IV	80MIN 80MIN 1 MIN 1MIN		475	3	0/3	9,9,11	—	0/3	2/3

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ANTI-RADIATION CHEMICAL AGENTS. — RADIATION PROTECTION STUDIES: C. ISOTHIURONIUM COMPOUNDS

WR#	NAME OF COMPOUND	DOSE TESTED	ROUTE	TIME BEFORE RAD.	ADDITIONAL MEDICATION	RAD DOSE (r)	NO. DOG PRS.	DEATHS DUE TO CHEMICAL TOXICITY	TIME OF DEATH (DAYS POST RAD)		# 30 DAY SURVIVAL	
	SINGLE AGENTS	Mg/Kg			Mg/Kg				CONTROLS	PROTECTED	CONTROLS	PROTECTED
298	2-AMINOETHYLISO-THIURONIUM Br·HBr	50	IV	2-5MIN	ATROPINE 0.1	450	6	6/6	9,9,10, 10,11,12	5,6,10,13 14,18	0/6	0/6
334	2METHYLALLYL ISOTHIURONIUM-CL	40	IV	35-90MIN	"	450	6	6/6	10,14,15 15,16,17	6,13,14,15 15,16	0/6	0/6
		60	IV	30-45MIN	"	450	6	3/ 6	8,13,16 17,26	0,0,2,11* *DRUG ONLY 3MIN PRE-RAD	0/6	2/3
		70	IV	2MIN	"	450	4	3/4	14,15,17 19	0,0,1,17	0/4	0/1
539	S-ETHYL ISOTHIURONIUM HBr	75	IV	3-9MIN	ATROPINE 0.1	450	6	5/6	11,11,11, 15,18,11	1	0/6	5/5
572	2-AMINO-4-THIAZOLYL METHYLISOTHIURONIUM HCL	300	IV	7-14MIN	—	450	2	2/2	13,17	19	0/2	1/2
		350	IV	4-14MIN	—	450	4	3/4	8,15,19	—	1/4	4/4
	COMBINATIONS											
302 1795	PAPP POHØØ	5 10	IV IV	90-120MIN "	ATROPINE .1 METH. BLUE 2	1500 775	3	2/3	3,3,4	0,0,13	0/3	0/1
293 347	AET CYSTEINE	100 500	IV IV	2MIN "	" "	775	4	3/4	12,12,12, 16	—	0/4	4/4
539 347	S-ETHYLISOTHIOURONIUM MEA	50 50	IV	5MIN	—	450	6	2/6	14,16,18	0,0	2/5	4/4
539 1607	S-ETHYL ISOTHIURONIUM n-DECYLAMINOETHANE-THIOSULFURIC ACID (DIBENZYLENE PRE-MED)	75 15-20 10	IV IV IV	6MIN 3-5MIN 5 HRS	—	450	6	4/6	13,14,16, 22	0,0,1,2,	1/5	2/2
302 298	PAPP AET	7 200	ORAL ORAL	2 HRS "	ATROPINE .1 BONAMINE 13	775	2	0/2	8,10	8,9	0/2	0/2

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ANTI-RADIATION CHEMICAL AGENTS. — RADIATION PROTECTION STUDIES: D. PHENOLS AND PHENONES

WR#	NAME OF COMPOUND	DOSE OF AGENT Mg/Kg	ROUTE	TIME BEFORE RAD.	ADDITIONAL MEDICATION Mg/Kg	RADE DOSE (r)	NO. DOGS PRS.	DEATHS DUE TO CHEMICAL TOXICITY	TIME OF DEATH (DAYS POST RAD) CONTROLS	TIME OF DEATH (DAYS POST RAD) PROTECTED	#30 DAY SURVIVAL CONTROLS	#30 DAY SURVIVAL PROTECTED
	SINGLE AGENTS											
302	PAPP	15	IV	?	ATROPINE.2	775	1	1/1	—	0	0/0	0/0
	COMBINATIONS											
40	2,4-DINITROPHENOL	20	IV	30MIN	EQUANIL	1500	2	2/2	8,8	0,0	0/2	0/0
302	PAPP	5	IV	2-4HRS	25-40							
1795	POHØØ	10	IV	2-4HRS	(ORAL)							
347	MEA	100	IV	2-3MIN	ATROPINE .1							
348	CYSTEINE	300	IV	2-3MIN	METH BLUE .2-.5							
134	4,4-DICHLOROBENZOPHENONE	200	I.P.	103-120 MIN	—	775	3	0/3	12,13,14	21	0/3	2/3
347	MEA	100	IV	1-3MIN								
348	CYSTEINE	300	IV	"								
134	4,4-DICHLOROBENZOPHENONE	200	ORAL	3-4HRS	—	775	3	0/3	9,12,12,	—	0/3	3/3
1795	POHØØ	10	"	"								
347	MEA	100	IV	1-3MIN		1200	1*	0/1	3	11	0/1	0/1
348	CYSTEINE	300	"	"			*ATROPINE					
X20	DIPHENYLAMINE	10	IV	75-100	ATROPINE.1	1500	3	0/3	3,4,4,	12,15,16	0/3	0/3
302	PAPP	5	IV	MIN	METH BLUE							
1795	POHØØ	10	IV	1-3MIN	2.5							
347	MEA	100	IV	"								
348	CYSTEINE	300	IV	1-3MIN								
302	PAPP	5	IV	55-75MIN	ATROPINE 0.2 METH	400	5	0/5	11,14,15,15 16	13,17,18	0/5	2/5
1795	POHØØ	10	IV	45-55MIN	BLUE 1-3	450	6	0/6	23	—	5/6*	6/6*
				45-60MIN		450	6	1/6	13,13,13, 13,13,15	2,23,24, 14	0/6	2/5
302	PAPP	5	ORAL	95-102	ATROPINE	1500	3	0/3	2,4,4,	6,8,11	0/3	0/3
1795	POHØØ	10	ORAL	MIN	.1							
347	MEA	100	IV	1-2MIN	METH BLUE							
348	CYSTEINE	300	IV	"	3							
1798	α-MERCAPTOACETANILIDE CARBAMATE	195	ORAL	95-102 MIN								

*FAULTY IRRADIATION

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ANTI-RADIATION CHEMICAL AGENTS. — RADIATION PROTECTION STUDIES: E. SEROTONIN AND COMBINATIONS WITH SEROTONIN

WR#	NAME OF COMPOUND	DOSE OF AGENT Mg/KG	ROUTE	TIME BEFORE RAD.	ADDITIONAL MEDICATION Mg/Kg	RAD DOSE (r)	NO. DEATHS DUE DOG TO CHEMICAL PRS.TOXICITY	TIME OF DEATH (DAYS POST RAD)		30 DAY SURVIVAL	
	SINGLE AGENT							CONTROLS	PROTECTED	CONTROLS	PROTECTED
62	SEROTONIN	10	IV	1-2MIN	ATROPINE 0.1	775	6 0/6	9,10,11,12,13 14	9,10,10,11 13,14	0/6	0/6
		15	IV	1-7MIN	(IN SOME	775	8 1/8	7,9,10,10,11, 11,12,13	0,10,12,13, 16,19,19,38	0/8	1/7
		25	IV	1-5MIN	CASES)	775	7 2/7	8,9,10,11,12 12,	0,0,9,11,11, 12,14	0/6	0/5
		50	IV	3-13MIN	"	775	11 6/11	11,11,12,12, 14,14	0,0,0,0,0,0, 10,11,12,12, 12	0/7	0/5
		100	IP	3-5MIN	"	775	3 0/3	10,13,13,	13,13,13,	0/3	0/3
		125	IP	1-2MIN	"	775	2 0/2	10,12	11,12,	0/2	0/2
	COMBINATIONS										
62	SEROTONIN	10	IV	2-3MIN	ATROPINE	1500	2 0/2	5,5	12,14	0/2	0/2
347	MEA	100	IV	"	.2						
348	CYSTEINE	300	IV	"							
302	PAPP	5	IV	2.5-3.5	ATROPINE.1	1500	5 0/5	5,5,5,5,5,	13,13,16,19	0/5	1/5
1795	POHØØ	10	IV	HOURS	EQUANIL						
347	MEA	100	IV	2-4MIN	40-100						
34	CYSTEINE	300	IV	"	METH.BLUE						
62	SEROTONIN	10	IV	10	1-2						

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ANTI-RADIATION CHEMICAL AGENTS. — RADIATION PROTECTION STUDIES: F. CARBAMATES

JR#	NAME OF COMPOUND	DOSE OF AGENT Mg/Kg	ROUTE	TIME BEFORE RAD.	ADDITIONAL MEDICATION Mg/Kg	RAD DOSE (r)	NO. DOGS PRS.	DEATHS DUE TO CHEMICAL TOXICITY	TIME OF DEATH (DAYS POST RAD)		30 DAY SURVIVAL	
									CONTROLS	PROTECTED	CONTROLS	PROTECTED
	SINGLE AGENTS											
48A	N-β-MERCAPTOETHYL DITHIOCARBAMIC ACID	500	ORAL	60MIN	ATROPINE 0.1	400	3	0/3	12,15	15,21	1/3	1/3
	COMBINATIONS											
48A	N-β-MERCAPTOETHYL-DITHIOCARBAMIC ACID	150	IV	1-2MIN	ATROPINE 0.1	400	4	0/4	15,17	—	2/4	4/4
348	CYSTEINE	300	IV	1-2MIN	"							
302	PAPP	5	ORAL	120MIN	ATROPINE 0.1	400	3	0/3	11,14,19	7	0/3	2/3
1795	POHØØ	10	ORAL	"								
48A	N-β-MERCAPTOETHYL-DITHIOCARBAMIC ACID	500	ORAL	60MIN	"							

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ANTI-RADIATION CHEMICAL AGENTS. — RADIATION PROTECTION STUDIES: G. COLCHICINE, COLCEMIDE

WR#	NAME OF COMPOUND	DOSE OF AGENT	ROUTE	TIME BEFORE	ADDITIONAL MEDICATION	RAD DOSE	NO. DOG	DEATHS DUE TO CHEMICAL	TIME OF DEATH (DAYS POST RAD)		30 DAY SURVIVAL	
	SINGLE AGENTS	Mg/Kg		RAD.	Mg/Kg	(r)	PRS.	TOXICITY	CONTROLS	PROTECTED	CONTROLS	PROTECTED
X19	COLCHICINE	0.15	IV	60-90MIN	—	450	2	0/2	11,21	8,16	0/2	0/2
170	COLCEMIDE	0.50	IV	24-48HR	—	775	4	0/4	—	3,10,12,12	NONE IRRAD	0/4
	COMBINATIONS											
X19	COLCHICINE	0.25	IV	60-90MIN	—	450	4	1/4	11,12,13,14	2	0/4	3/3
347	MEA	100	IV	2-4MIN	—							
170	COLCEMIDE	0.50	IV	2DAYS	ATROPINE.1	1500	4	0/4	3,3,3,3,	7,10,12	0/4	1/4
302	PAPP	5	IV	2-2.5								
1795	PCHØØ	10	IV	HOURS								
347	MEA	100	IV	3MIN								
348	CYSTEINE	300	IV	3MIN								

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ANTI-RADIATION CHEMICAL AGENTS. — RADIATION PROTECTION STUDIES: H. MISCELLANEOUS

WR#	NAME OF COMPOUND / COMBINATIONS	DOSE OF AGENT Mg/Kg	ROUTE	TIME BEFORE RAD.	ADDITIONAL MEDICATION Mg/Kg	RAD DOSE (r)	NO. DOG PRS.	DEATHS DUE CHEMICAL TOXICITY	TIME OF DEATH (DAYS POST RAD) CONTROLS	TIME OF DEATH (DAYS POST RAD) PROTECTED	30 DAY SURVIVAL CONTROLS	30 DAY SURVIVAL PROTECTED
302	PAPP	5	IV	75-90MIN	ATROPINE 0.1	1500	3	1/3	5,5,5,	1,13,20	0/3	0/2
1795	POHØØ	10	IV	"	MECLIZINE							
306	TYRAMINE	200	ORAL	75-85MIN	2.5-3.5							
347	MEA	100	IV	1-3MIN	METH BLUE							
348	CYSTEINE	300	IV	1-3MIN	4-5							
804	RESERPINE	0.5	IV	15-20MIN	MARSALID 50 ORAL	775	2	1/2	12,13	1,11	0/2	0/1
1796	5-AMINO-1,3,4, TRIAZOLE	1000	IV	45-90MIN	TOTP 100 (PRE 2DAYS)	1500	5	0/5	3,4,4,4,4,	7,11,13,17, 19	0/5	0/5
302	PAPP	5	IV	115-165MIN	ATROPINE.1							
1795	POHØØ	10	IV	"	METH BLUE							
347	MEA	100	IV	2-45MIN	2-3							
348	CYSTEINE	300	IV	2-45MIN	MEA 20M/Kg POST RAD							

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TABLE VIII

CHEMICAL PROTECTION AGAINST 10,000 to 18,000 r WHOLE BODY RADIATION IN THE DOG

PROTECTED DOGS

302	PAPP	5Mg/Kg	1-3 HOURS PRE RAD
1795	POHCC	10Mg/Kg	
347	MEA	100Mg/Kg	1-5 MINUTES PRE RAD
348	CYSTEINE	300Mg/Kg	

RAD DOSE	NO. OF DOG PAIRS	NO. OF DOGS EXHIBITING SEVERE CNS SIGNS		TIME OF DEATH	
		CONTROL	PROTECTED	CONTROL	PROTECTED
10,000	14	14	1	1,1,2,2,2,2,2,3,3,3,3,4,4,4,	0,2,3,3,3,3,3,4,4,4,4,4,4,4
12,000	14	11	4	0,0,2,2,2,2,2,2,2,2,3,3,3,4,4,	0*,1,1,1,1,2,3,3,3,3,3,3,9
14,000	1	1	0	2	3
15,000	1	1	-	3	1
16,000	1	1	-	2	2
18,000	1	1	-	1	0

* TOXICITY DEATH

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FIGURE I.

SURVIVAL TIME OF DOGS EXPOSED TO 450r
OF WHOLE BODY X-IRRADIATION

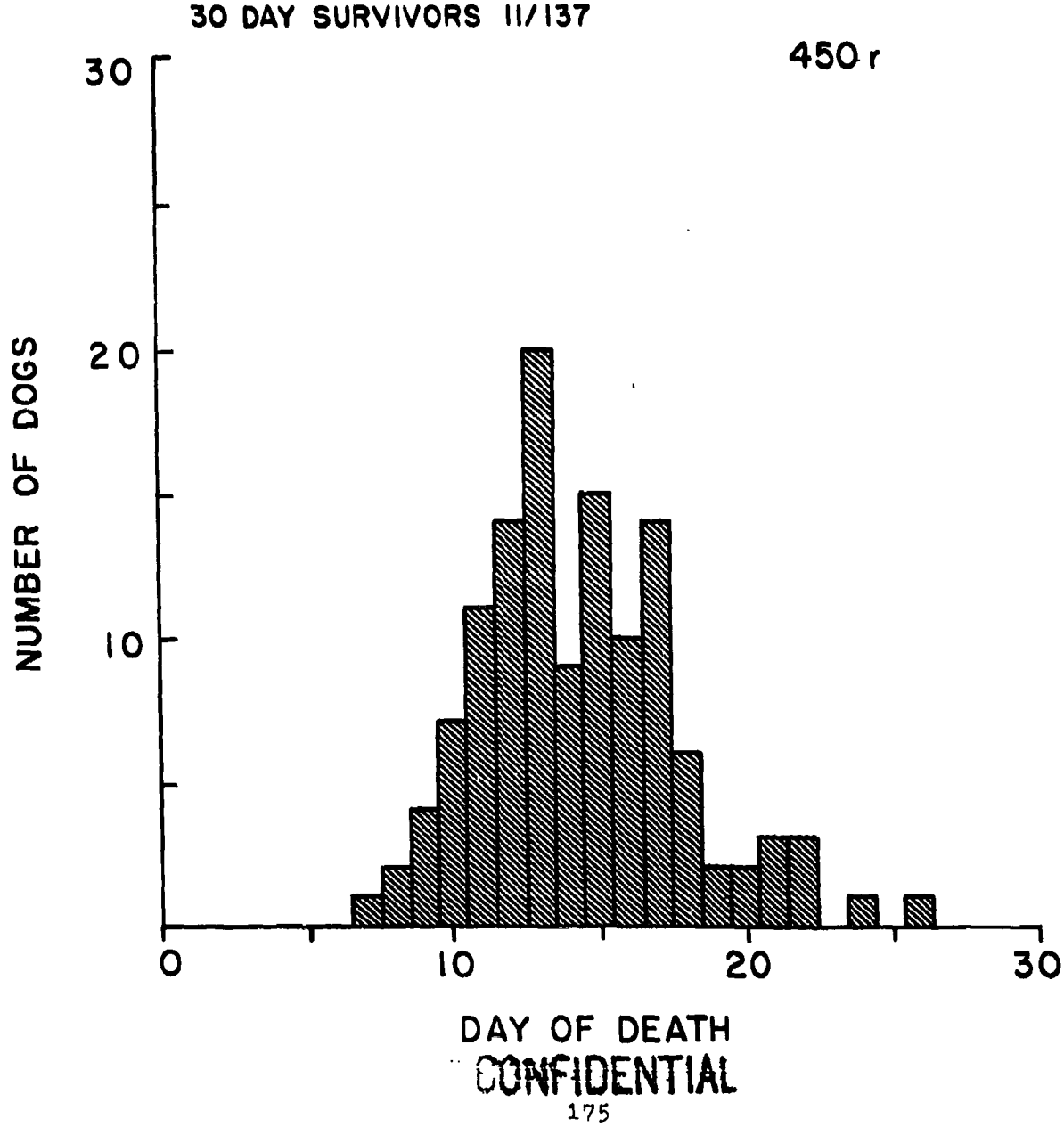
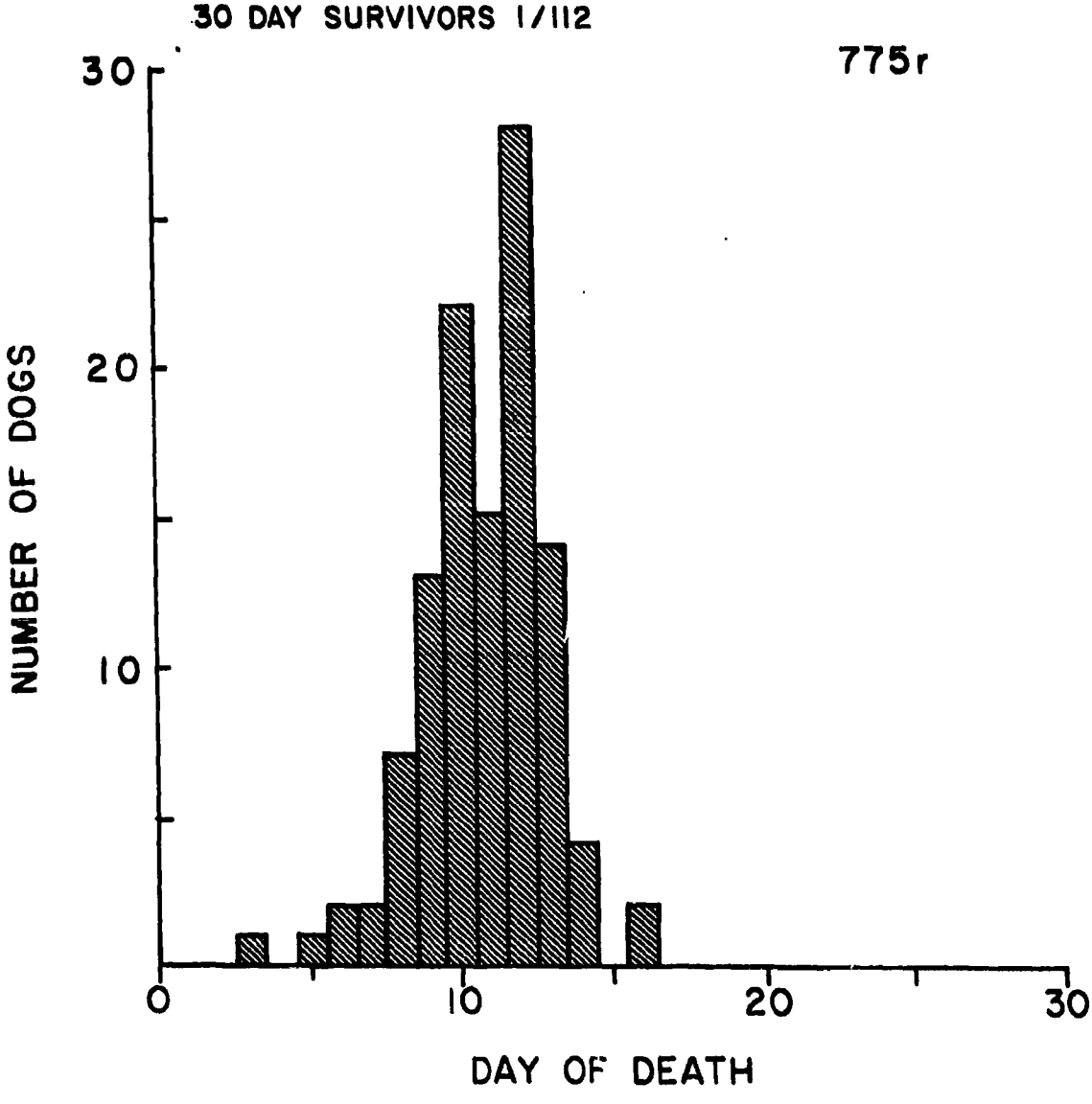


FIGURE 2.

SURVIVAL TIME OF DOGS EXPOSED TO 775r
OF WHOLE BODY X-IRRADIATION



Section K

FIGURE 3.

SURVIVAL TIME OF DOGS EXPOSED TO 1500r
OF WHOLE BODY X-IRRADIATION

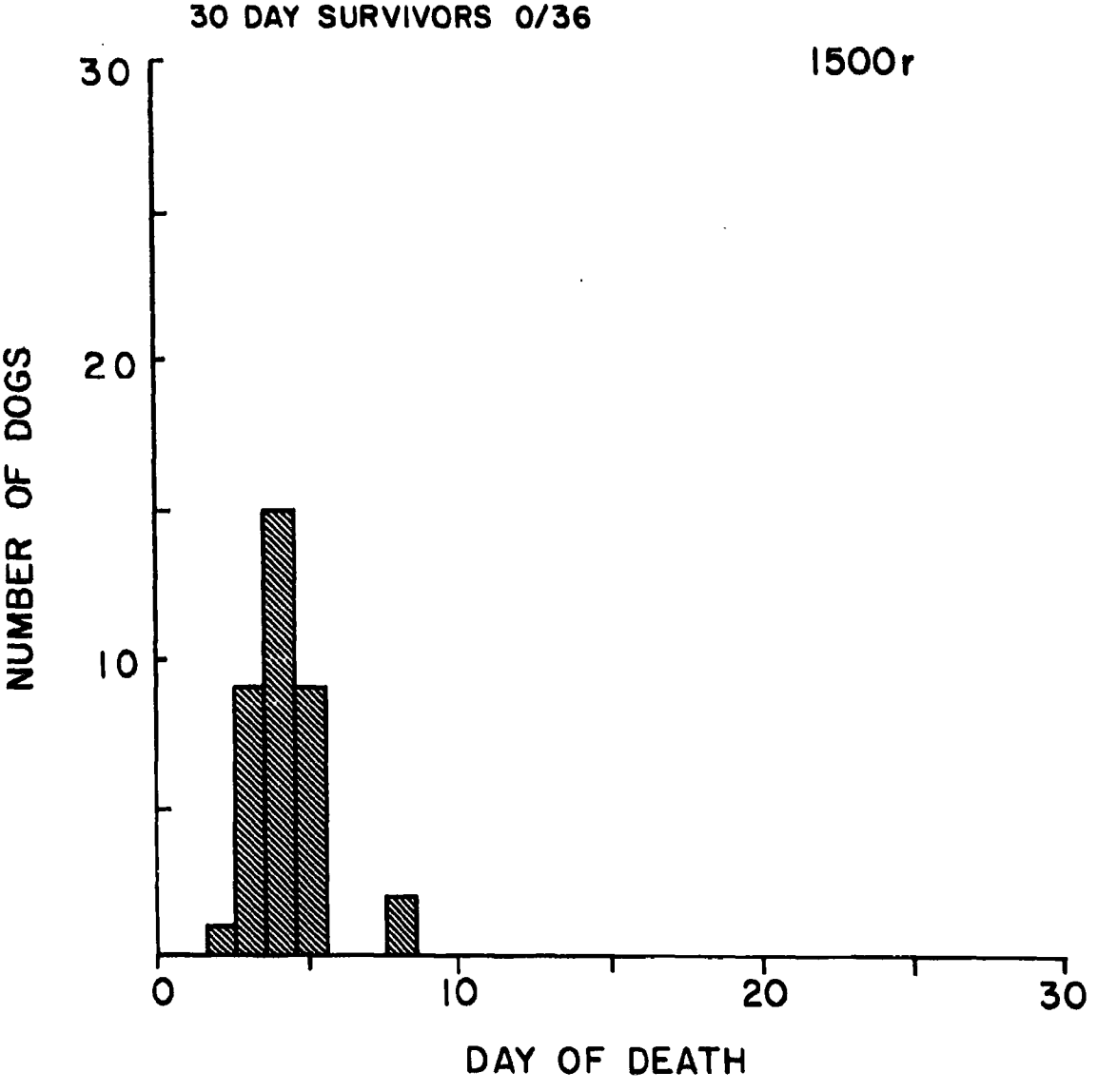


FIGURE 4.

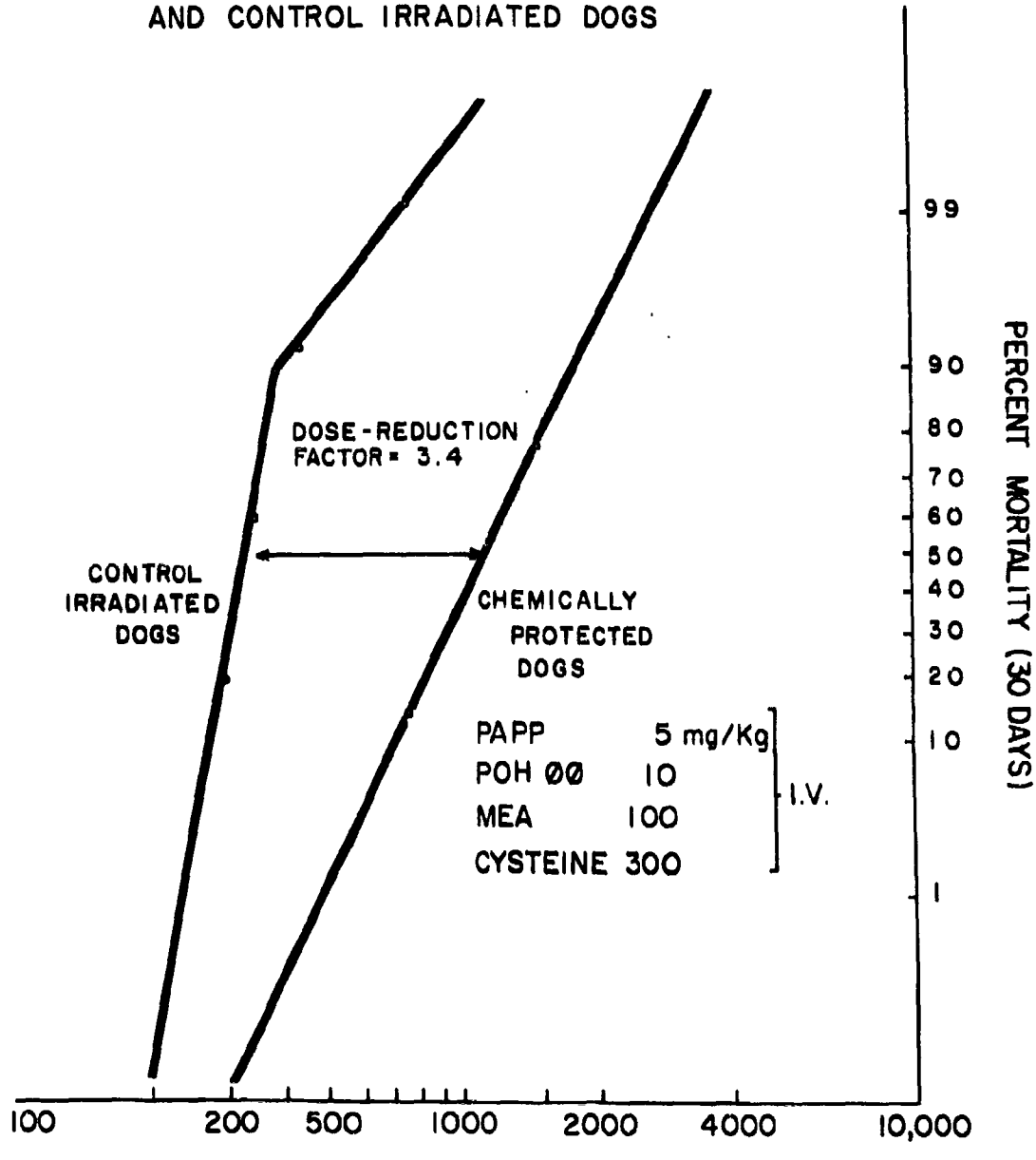
SURVIVAL TIME OF DOGS EXPOSED TO 10,000 TO 18,000r WHOLE BODY X-IRRADIATION

32 DOGS – NO SURVIVORS

10,000-18,000r

FIGURE 5.

DOSE-MORTALITY CURVES FOR CHEMICALLY PROTECTED AND CONTROL IRRADIATED DOGS



PRODUCTION OF OSTEOLATHYRISM BY SOME RADIOPROTECTIVE DRUGS

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Series of experiments were undertaken to study the lathyric activity of certain radioprotective drugs on the albino mouse. It is our aim, eventually, to relate chemical structures to lathyric activity, and possibly to uncover some mode of their action.

In this preliminary report, lathyrogenicity of certain radioprotective compounds will be discussed. This was determined by feeding experiments, in which compounds were added to the diet, and their ability to produce skeletal deformities noted.

When osteolathyrogenic agents, such as BAPN are administered to rats and mice, they appear to affect specifically the connective tissue (skeleton, blood vessels, and skin). Some investigators feel that the collagen fraction is affected directly; others feel that the chief effect is on the mucopolysaccharides of the ground substance. It has also been suggested that a common mode of action on the lathyrogens may be through the formation of chelates. Follis, in 1957, found some bone changes which were similar to osteolathyrism in rats on copper deficient diets.

*The technical assistance of Mr. L. Whitehead is appreciated.

Levene and his associates, using the chick embryo assay method, have reported on the lathyrogenicity of an extensive series of compounds, including derivatives (especially the N-substituted) of the aminonitriles, hydrazides, hydrazines and ureides. However, there are differences in the response between the chick embryo and the rat. MEA did not produce lathyrism in the chick embryo but did in the rat and the mouse. Isonicotinyl hydrazide is lathyrogenic in the chick embryo but not in the rat. Dasler, Ramamurti and others have showed that MEA was lathyrogenic in rats, and produces lesions less severe than those produced by BAPN.

Bachhuber and others testing various aminonitriles and related substances found that only BAPN and aminoacetonitrile were osteolathyrogenic. Their studies indicated that the ability of an organic nitrile to produce lathyrism was influenced by the presence of a reactive amino group. Substitution of a methyl or acetyl into the amino group or a substitution of an hydroxy for the amino group in BAPN results in a loss of biologic activity. The secondary amine corresponding to BAPN (bis-B-(cyano-ethyl)-amine is only mildly osteolathyrogenic but markedly neurolathyrogenic.

Dasler has also shown that semicarbazide HCl when fed to weanling rats produced lesions similar to osteolathyrism. Aortic damage seems to develop more slowly and to be less severe than

that shown in BAPN treatment. He also found that when L-cysteine, L-glutamine, and casein were added to diets of rats fed BAPN, they offered partial protection by delaying the development of the lesions, the severity, however, was not altered.

Experiments currently being conducted in our laboratory deal with some structurally related compounds and their ability to form osteolathyrism.

Experimental Bagg-Swiss, female mice, 5-6 weeks of age, weighing about 20 to 22 grams were used in all experiments, except mice fed MEA . HCl. The latter were weanlings, 4 weeks old. The basal diet was a specific pathogen free rat and mouse baked biscuit obtained from the G. L. Baking Company, and finely ground. Experimental diets were made up by the addition of varying amounts of the test compounds to suitable quantities of the ground food. In all cases, the drug, either in powder or liquid form was found together with the diet in a mortar, and then transferred to a Waring blender to ensure thorough mixing. The liquid drugs or those in powder form which had to be dissolved in a special vehicle before being added to diet, were treated similarly, but were spread in a baking dish and allowed to dry thoroughly at room temperature before being used.

Mice were set up in groups of ten and housed jointly. Comparable age controls were fed same basal diet of ground food.

Diets were fed ad libitum. Mice were weighed daily, food consumption per day was ascertained. X-ray films were taken at time intervals for observations of developing skeletal deformities. All animals were autopsied at death, and tissues and skeletons saved for pathology.

In order to recognize the symptoms associated with osteolathyrism, we set up an experiment using BAPN as our test compound. (BAPN produces severe skeletal lesions as well as aortic aneurisms.) Groups of mice were fed diets containing 1%, 2%, and 3% BAPN respectively. All mice on the 3% diet steadily lost weight and by 23 days were all dead. They ate very little, less than 1g/d/mouse. Because of the severe weight loss, true BAPN intoxication was obscured. Mice on the 2% diet lost weight, but managed to survive long enough to display the lathyric symptoms. At 30 days, the mice were lethargic, developed a waddling gait with some lameness, and bladder distention, and exostoses of the ventral and lateral aspects of the mandibles could be palpated. Exostoses also appeared on the long bones near the sites of muscle attachment. (Bones on autopsy were abnormally red, especially about the joints.) By 44 days, 8 mice had died and the 2 survivors were sacrificed. No pathologic abnormalities of organs were noted, other than slight petechial hemorrhages in the skin and around the heart. Mice on the 1% diet followed the same pattern, but the loss of weight

was slow. As the prominent exostoses worsened, the mice had difficulty in walking and eating. 50% of the mice survived over 45 days, and were then sacrificed. Severe exostoses were found on the long bones, ribs, spine and mandibles.

Diets containing some radioprotective drugs were then set up. Our results are summarized in the attached table and in detail below:

WR # 347 - MEA · HCl:

1% Effect on weanling mice Definitely lathyrogenic
1. Waddling gait at 34 days
2. Stunted growth. At 94 days on diet, the 6 survivors have a mean weight of 20 grams, while their age controls have a weight of 30 grams.
3. Spinal curvatures apparent along with a crooking of the tails. (This has been reported on rats fed semicarbazide.)
4. Exostoses, which were not palpable, showed up on x-ray film at 34 days, along with deformities of the dorsolumbar spine. Lesions are less severe than those displayed with BAPN, and progress slowly as plates taken at 94 days reveal. (These findings confirm Dasler's observation in rats. However he did not comment on the crooking of the tails.)

WR # 361 - MEA Bunte: 5-6 week old mice Lathyrogenic

3% Symptoms similar to MEA · HCl
1. Waddling gait at 15 days, but less noticeable than in BAPN treatment.
2. Weight loss most severe
3. Spinal curvatures noted, and slight lesions appear noticeable only on x-ray film. Crooking of tails absent.

4. Lesions progress slowly, but spinal deformities appear to be worsening by 66 days. (3 survivors)

1% Mice appear quite normal. Only a slight loss in weight in the first week, with a gradual increase in weight. At 72 days, mice still show no lathyric symptoms.

WR # 1607 - N-decyl Bunte: 5-6 week old mice Non-lathyrogenic

.25%

1. Gradual loss of weight. 1/10 dead at 20 days. Mice consume 3-4g food/day/mouse, but weight stays below normal.
2. Anemia noted, mice quite sick. 50% dead by 34 days, and by 70 days all were dead.
3. Gross pathology showed no lesions, but some mucosal hemorrhages in gut.

.20%

1. Gradual loss of weight, but at 3 weeks mice began to gain slowly. However, at 56 days, the mean weight of the 9 survivors is 23.5g compared to their controls whose weight is 33g. (1/10 dead at 20 days)
2. No deformities noted.

WR # 228 - N-decylaminoethanethiol: 5-6 week old mice Non-lathyrogenic

.5% Very toxic. All mice dead in a week.

.25%

1. Gradual weight loss. Mice gain slowly after 2 weeks.
2. At 30 days, no abnormalities noted.

WR # 698 - 2-(2-mercaptoethyl)aminoethanol: 5-6 week old mice Non-lathyrogenic

3% Very toxic, all mice dead by 18 days.

2%

1. Severe weight loss. Bladder incontinence.

2. No palpable lesions at 42 days.
3. No abnormal pathology other than free blood in gut.

1% Mice normal, after 8 weeks on diet.

WR # 2347 - 3-(2-mercaptoethyl)amino-1,2,4-butanetriol: Severely lathyrogenic

5%

1. Loss of weight gradual for first 2 weeks. Slow gain. Food consumption 3-4g/d/mouse.
2. At 7-10 days, waddling gait and bowleggedness noted. Hair ruffed. 1/10 dead at 8 days.
3. Palpable exostoses noted at 36 days (as severe as in BAPN treatment).
4. 50% dead by 40 days.

2% All normal, except for an initial drop in weight, then normal weight gain, after 49 days on diet.

WR # 1616 - 2-hydroxymethyl-2-(2-mercaptoethyl)amino-1,3, propanediol Severely lathyrogenic

5% Very toxic

1. Weight loss severe. At 10 days, waddling gait, and puffiness of the face. Eye infections prevalent. Diarrhea and bladder incontinence.
2. Severe lesions palpable at 33 days. All dead at 37 days.

2.5%

1. Slight weight loss at start, very little gain. 50% dead at 9 weeks.
2. Severe exostoses.

WR # 2578 - S-2(aminoethylamino)ethyl phosphorothioic acid: Slightly lathyrogenic

2.5% Very toxic. Severe loss of weight. No outward symptoms. At 51 days, 2 survivors show slight lesions. (53 days all dead)

WR # 638 - Monosodim-S-(B-aminoethyl)monothiophosphate: Slightly lathyrogenic

2.5% Gradual to severe loss of weight. Diarrhea and bladder incontinence. Mice extremely sick. 1/10 dead at 51 days, grossly showed no lesions, but film taken on some survivors reveal the beginning of exostoses.

DISCUSSION:

These agents were tested for lathyrogenic activity when administered in the diet in high concentrations - much higher concentrations than would be anticipated if these agents were to be administered to man. In view of the reports previously cited on the lathyrogenic activity of mercaptoethylamine, we felt we should attempt to confirm these findings and also extend the series to other antiradiation agents under test. This report constitutes the first confirmation of the lathyrogenic activity of mercaptoethylamine (originally reported by Waldemar Dasler) and also constitutes the first study demonstrating the ability of these agents to produce lathyrism in mice. Due to differences in availability of the agents and of the mice, the age at which these agents were administered to the test animals varies. Mercaptoethylamine was given to weanling mice--a relatively sensitive age with respect to development of osteolathyrism. Beta-aminopropionitrile was given to mice of two age groups. One group, age 5 to 6 weeks

and the other group 6 months. There did not appear to be any differences in the susceptibility to lathyrism of these two age groups.

The lathyrogenic activity of the compounds reported here may be roughly divided into two groups: Beta-aminopropionitrile and the alcohol derivatives of mercaptoethylamine produce severe osteolathyrism with pronounced exostoses. Mercaptoethylamine, the Bunte and thiophosphates produce relatively small lesions.

The two decyl derivatives which were evaluated for lathyrogenic activity did not produce lathyrism although they did produce some chronic toxicity as evidenced by lethality. We do not know the mechanism of the lethality. The absorption of these compounds from the gastrointestinal tract is poor, if in fact it occurs at all. We therefore feel there is a possibility that the toxicity resulting from the administration of relatively large amounts of these decyl derivatives may be due to some local gastrointestinal effect. On the other hand, Lasier has reported to us by private communication that n-ethyl aminoethyl mercaptan is not effective in producing lathyrism. If this report can be substantiated then the failure of these long chain derivatives to produce lathyrism may be associated with the fact that they are simple secondary alkyl amino derivatives rather than the fact that they were not absorbed by mouth.

SUMMARY:

Mercaptoethylamine and derivatives are capable of producing lathyrism when administered in the diet in high concentration.

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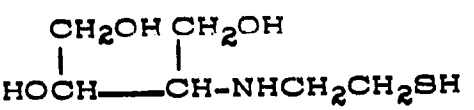
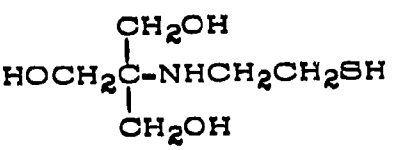
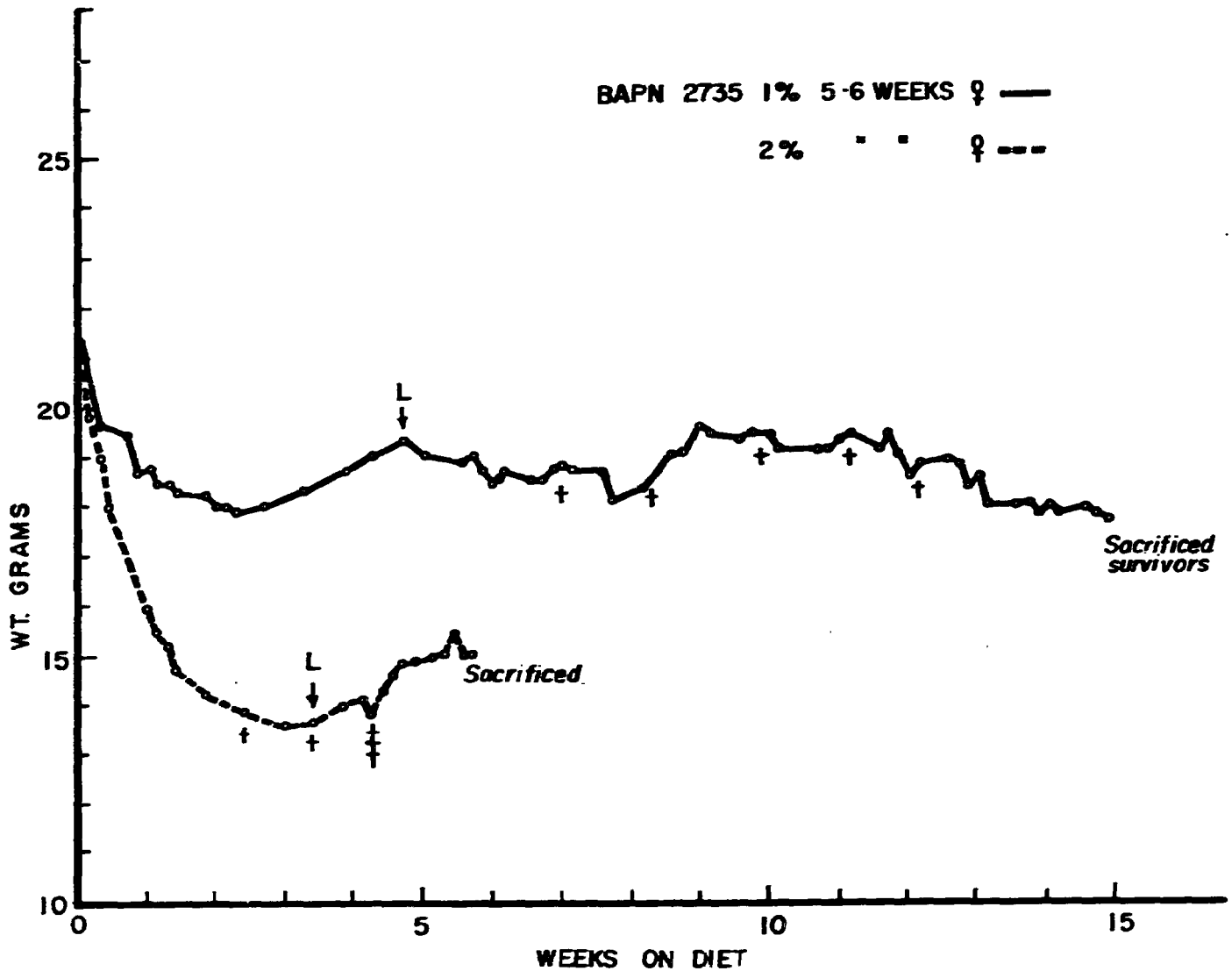
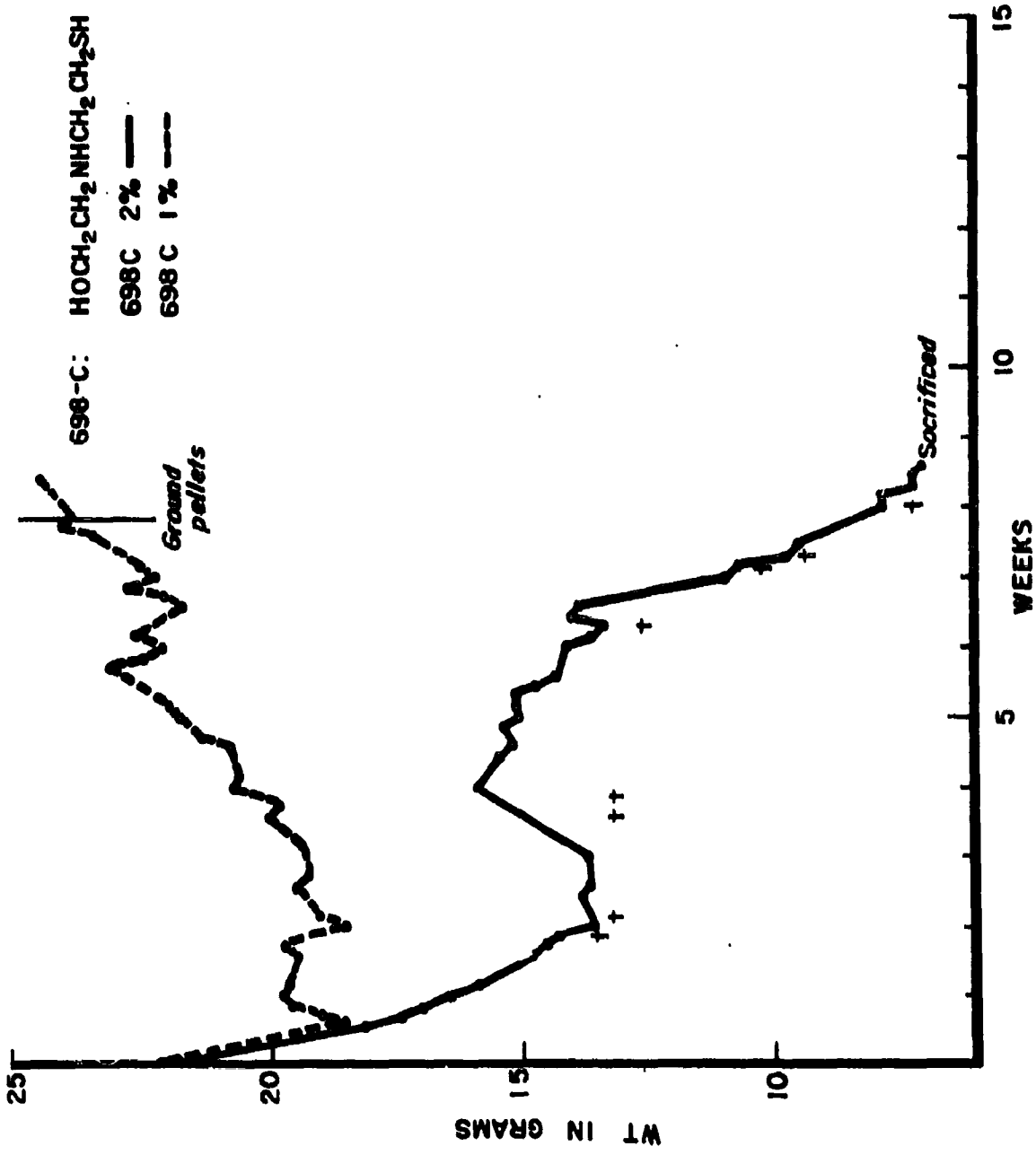
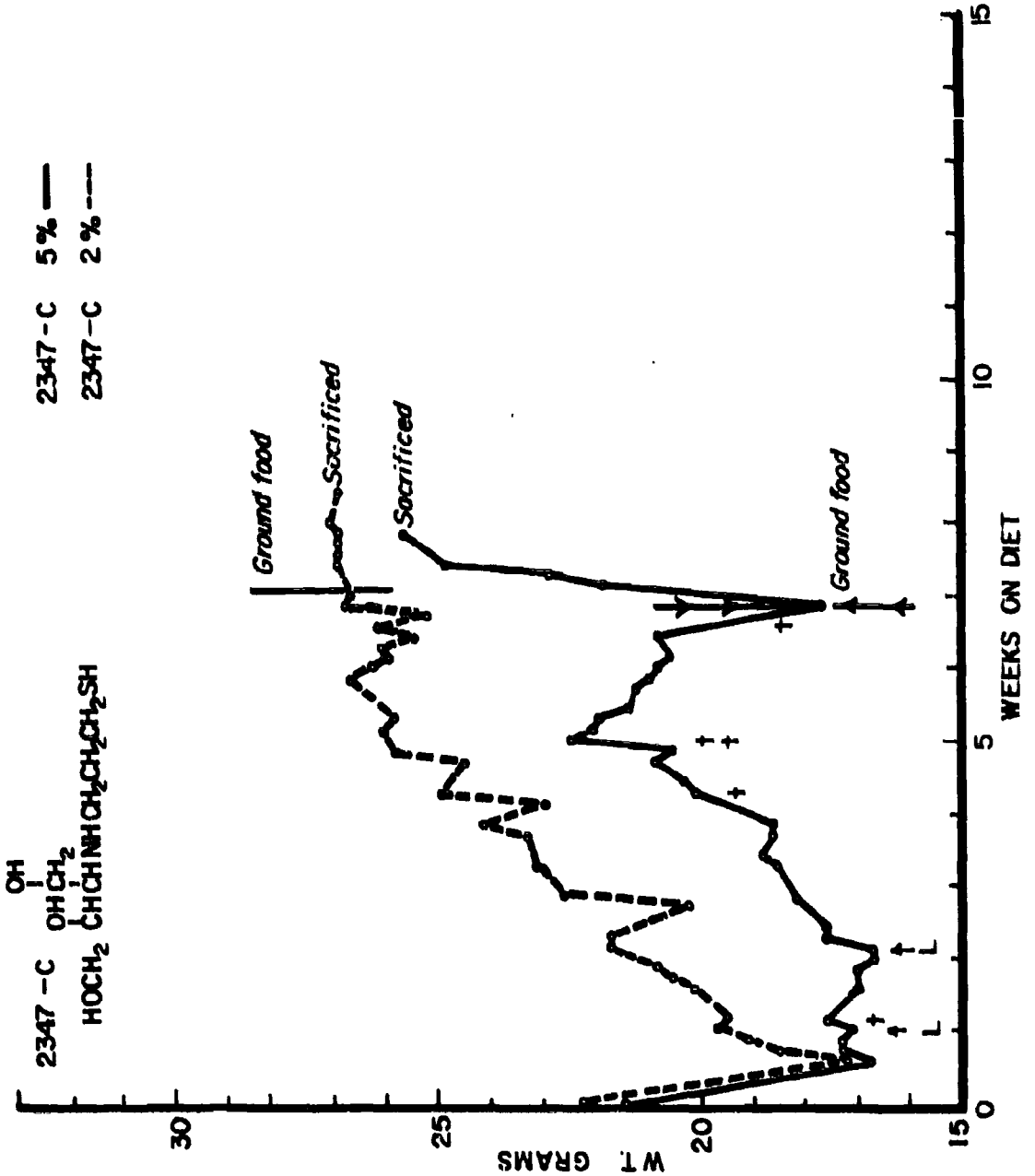
LATHYROGENIC ACTIVITY

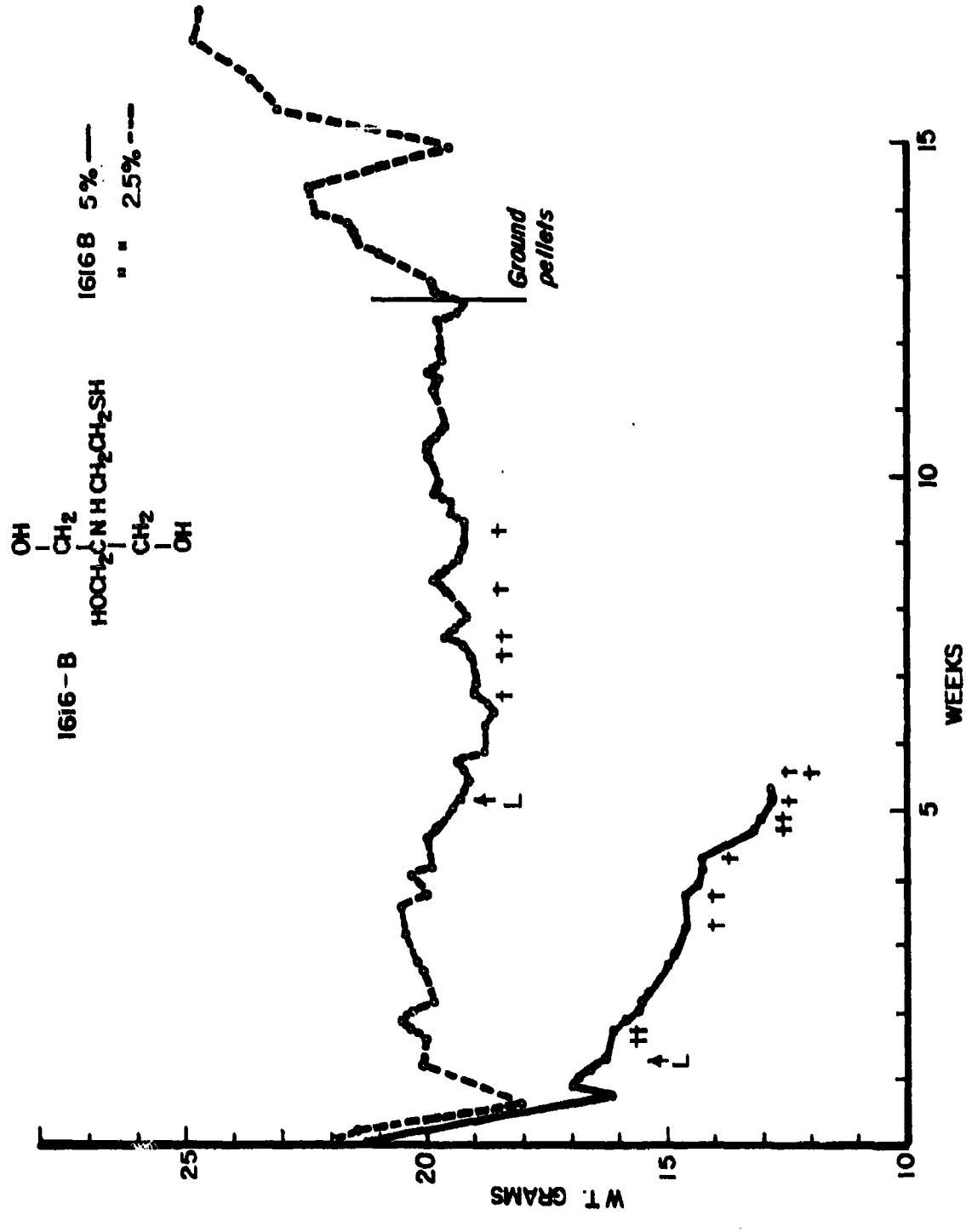
WALTER REED:

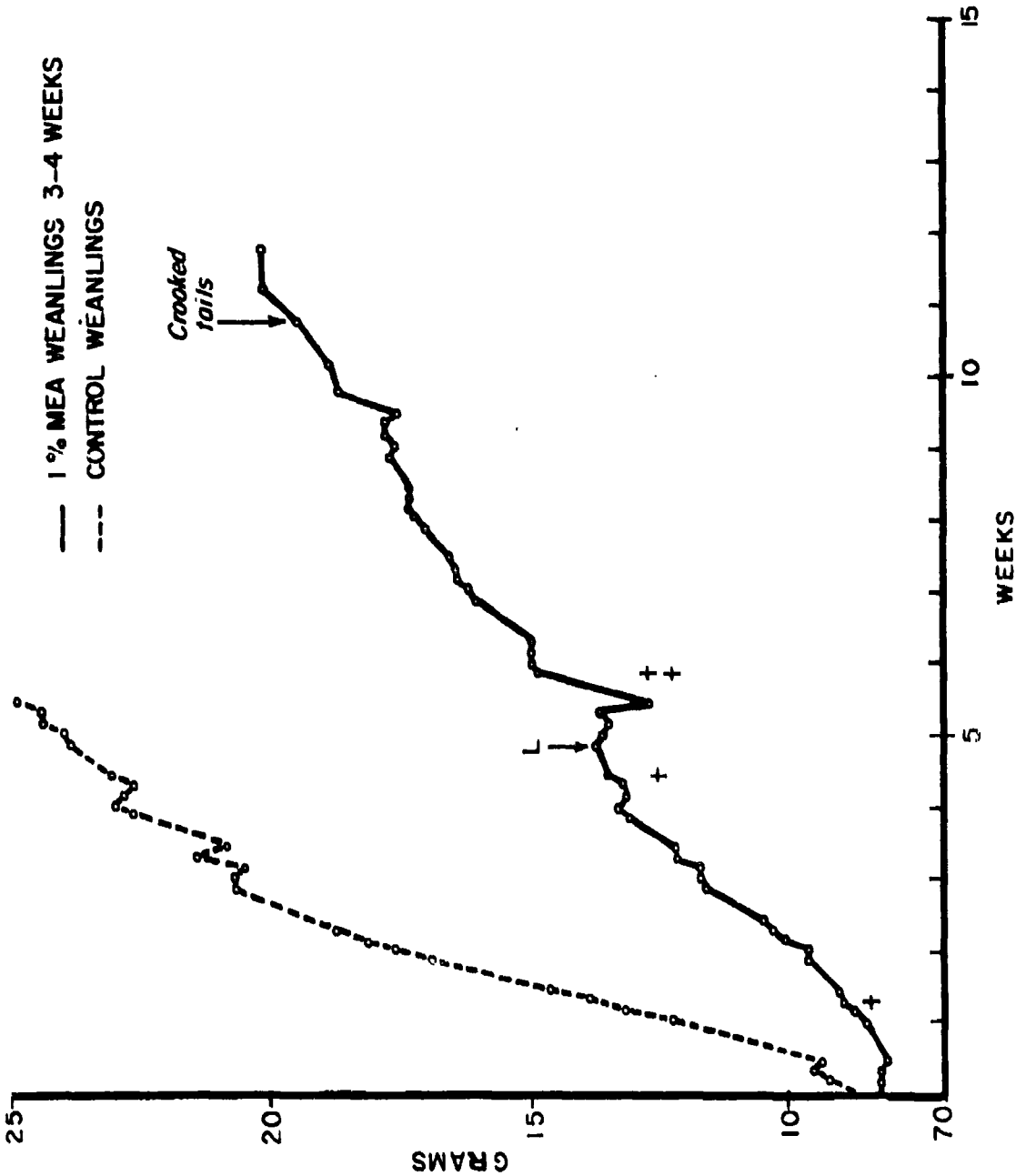
$HOCH_2CH_2NHCH_2CH_2SH$	-
	+++
	+++
$H_2NCH_2CH_2SSO_3H$	++
$HS-CH_2CH_2NH_2$	++
$CH_3(CH_2)_9NH_2CH_2CH_2SSO_3H$	-
$CH_3(CH_2)_9NH_2CH_2CH_2SH$	-
$H_2N-CH_2-CH_2NHCH_2CH_2SPO_3H_2$	+
$H_2NCH_2CH_2S-PO_3H_2$	+

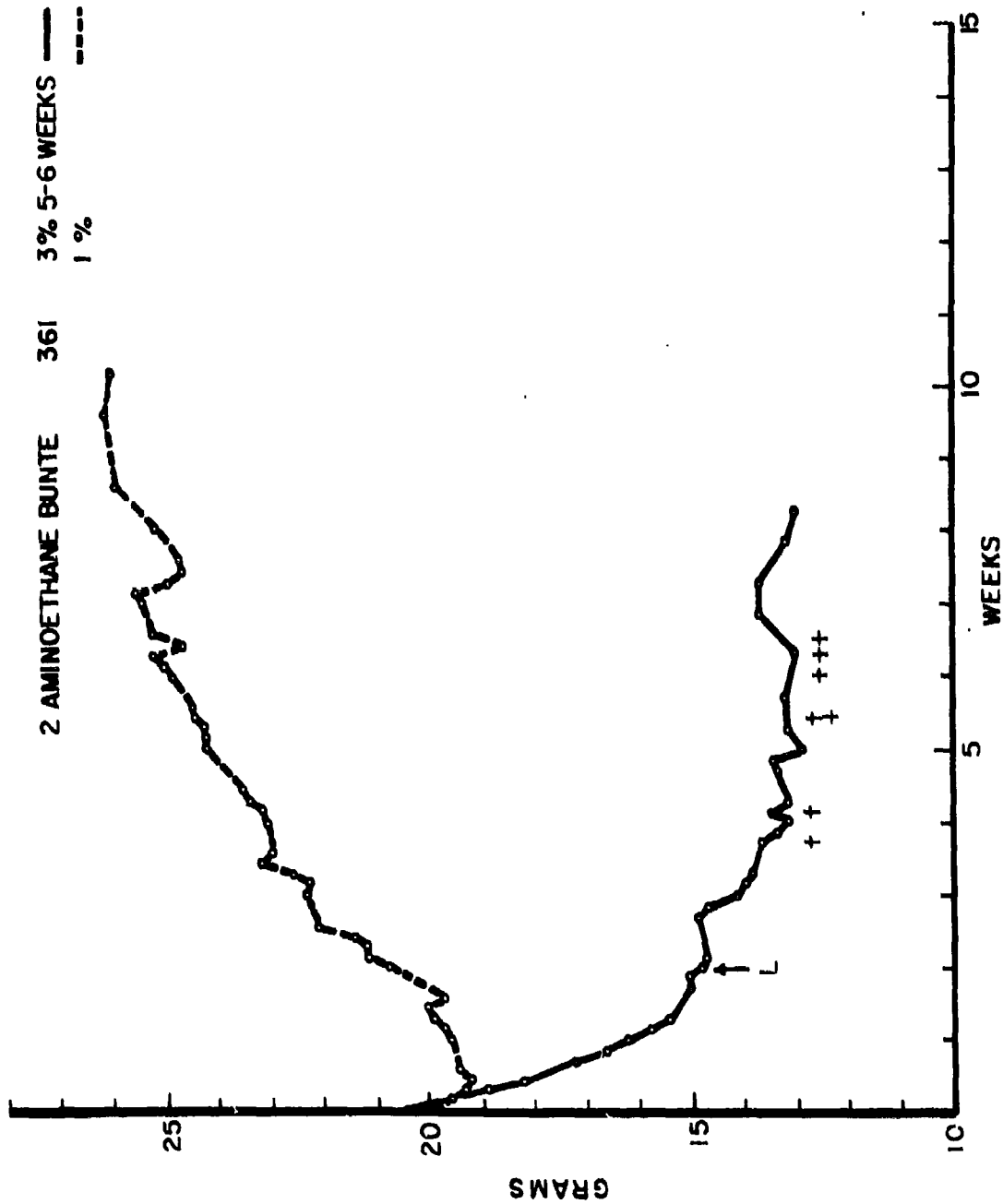


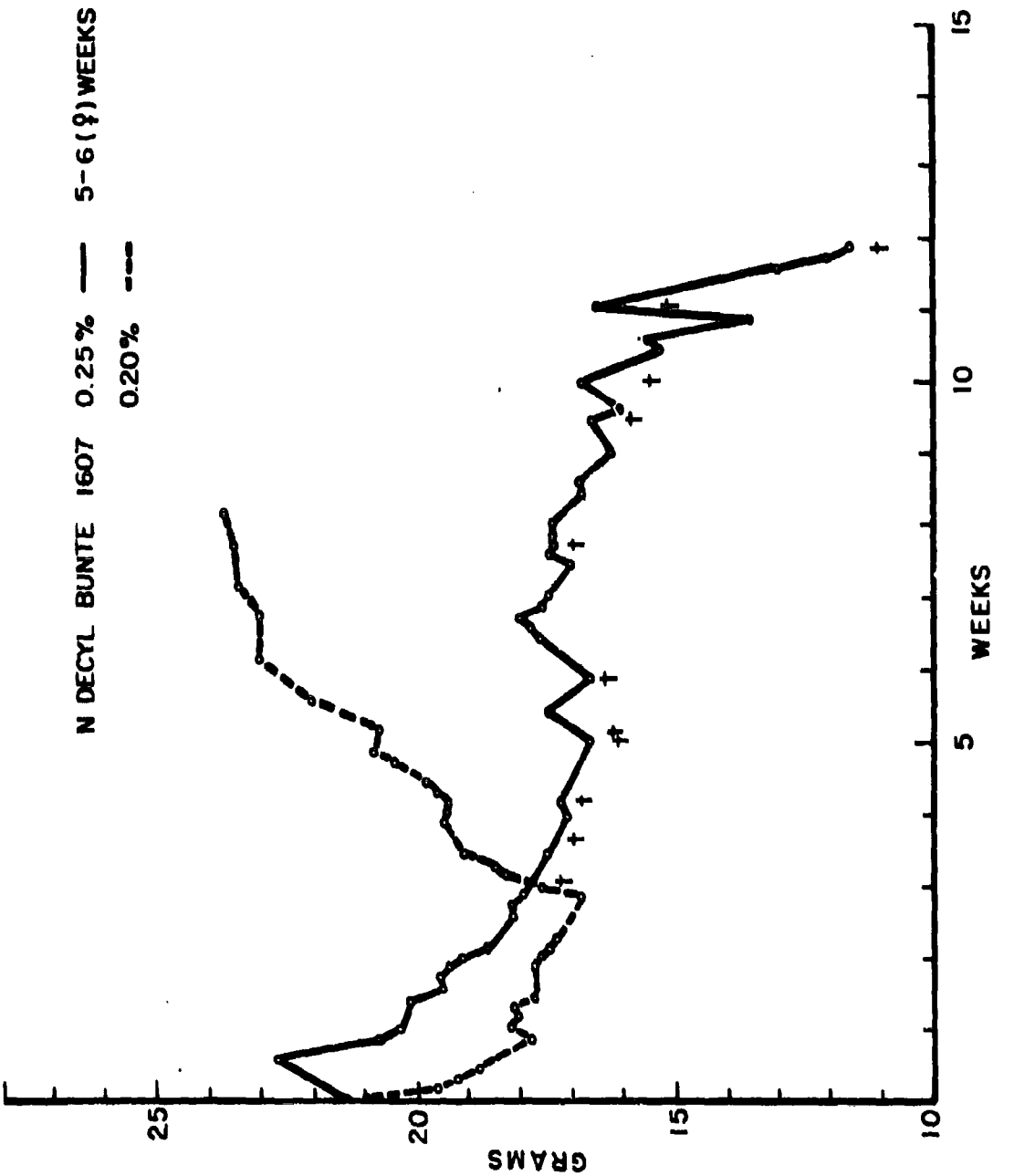

698-C: $HOCH_2CH_2NHCH_2CH_2SH$
698C 2% ——
698C 1% – –
Ground pellets
Sacrificed
WT IN GRAMS
WEEKS
25
20
15
10
5
10
15

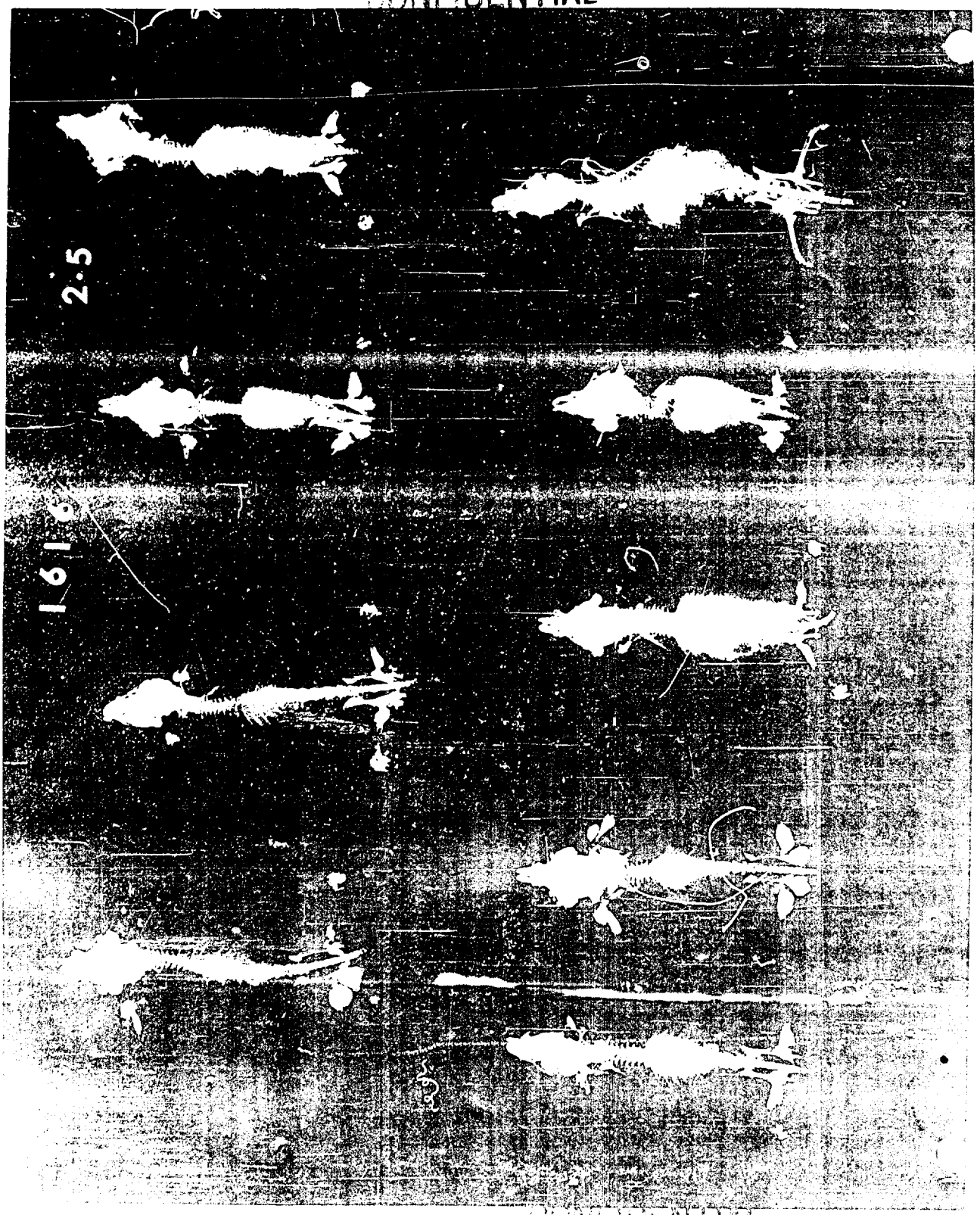

2347-C 5% —
2347-C 2% ---
2347-C OH OHCH2 HOCH2 CHCHNHCH2CH2CH2SH
Ground food
Sacrificed
Sacrificed
Ground food
WT GRAMS
WEEKS ON DIET
30
25
20
15
0
5
10
15


1616 5%—
" " 2.5%---
Ground pellets
1616-B
OH
CH2
HOCH2C NHCH2CH2SH
CH2
OH
WT. GRAMS
25
20
15
10
5
10
15
WEEKS




2 AMINOETHANE BUNTE 361
3% 5-6 WEEKS
1 %
L
+ +
+ + +
25
20
15
10
GRAMS
5
10
15
WEEKS


N DECYL BUNTE 1607 0.25% — 5-6(♀) WEEKS
0.20% ---
GRAMS
25
20
15
10
5
WEEKS
10
15



Section M

SECONDARY ALKYL ACID AND ACID DERIVATIVES OF MEA

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Washington, D. C. 20012

INTRODUCTION

Secondary alkyl amino derivatives of mercaptoethylamine, its corresponding Bunte salt and sometimes the corresponding disulfides have interesting properties as antiradiation agents if the alkyl chain is polyfunctional. Accordingly, a series of agents was made in which the alkyl chain carried an acidic group an ester or an amide. These compounds might be expected to differ appreciably in their properties from the other secondary alkyl derivatives with respect to their localization, penetration, distribution and uptake. Polyfunctional secondary alkyl amino antiradiation compounds made to date have emphasized amines, hydroxyl, and mercaptan groups. One acidic compound, cysteamine N-acetic acid, was tried in 1961 and found to be uninteresting. A convenient explanation can be made that acidic groups on an antiradiation compound will tend to keep the molecule out of the highly acid environment of DNA with the result that such groups might decrease the effectiveness of the agent. However, a number of examples of derivatives of standard anti-

radiation agents were made with N-alkyl substituents containing acidic or amide groups. While the number of compounds with such acidic groups are few, they do represent an active class which might be expected to have different pharmacological activity.

MATERIALS AND METHODS

All studies were done on Walter Reed Bagg Swiss female mice of 6 weeks of age weighing 23 g. These mice are raised under closed colony conditions and all are within three days of the same age. All mice are raised on D&G sterilized chow. The drinking water contains ten parts per million clorox. All mice used in the experiments are randomized prior to utilization.

The pharmacologic response of the agents in the mice is determined by observing them over a period of several hours following the administration of the agent. These observations are recorded directly on the sheet illustrated in Figure 1. Each drug level used is tested in 5 mice. Deaths are recorded as acute, i.e. within the hour, during the first day, after 24 hours, 48 hours, 72 hours and ten days. All survivors at the end of ten days are subjected to gross post mortem examination and reported on the sheet illustrated in Figure 2.

All doses of agents administered are calculated as the free base. These materials are weighed out approximately 15 minutes prior to use, are dissolved in water or 0.2% saline solution of methyl

cellulose and 0.4% tween. Adjustment is made, if pH has an acidity of 5.5 or less, or a pH of 8.0 or above. All animals treated with antiradiation agents are exposed to radiation along with randomized controls injected with vehicle. These groups are then housed in wire bottom cages so that there are equal numbers of treated and untreated mice in each cage. Treated animals are marked with picric acid solution. Mice were exposed either to lethal radiation or to sublethal radiation. Mice were exposed to lethal radiation in groups of 10-15 at each drug level, with 10 controls. Sublethal radiation are in groups of 20 plus 20 controls. A single survivor against lethal radiation in the treated group represents significant protection. Significant protection at sublethal radiation is determined by differences in the total number of survivors with 95% confidence.

An index of the potential interest ("Index of Potential Interest = IPI") of antiradiation agents has been developed. The method of calculation is shown in Figure 3. As one can see from inspection of the formula, a great deal of emphasis is placed upon the safety of an agent. The lower the tested dose is with respect to the approximate LD_{50} for a given survival, the higher the index. The largest multiplier in this system is 2 which is comparable to a dose reduction factor of 2 which these agents are usually assumed to have. This index, therefore, has

the advantage of emphasizing safety. In the tables on the anti-radiation data the millimole per kilogram figure refers to the top tested dose. The index also becomes greater with agents which demonstrate significant protection against radiation injury at 1/4 and 1/8 of the maximum tolerated doses. Compounds which therefore show a high index on the screening procedure are then tested at lower levels in order to determine their interest.

OVERALL RESULTS

The overall results of this series of compounds are shown in Tables 1 and 2. The carboxylic acid derivatives, WR 419 and 2346 must be given in very large doses to effect any protection against ionizing radiation injury. The indices on these compounds are not very interesting. The sulfonic acid derivatives are not available as exact analogs to the carboxylic compounds but instead contain one or more methylene groups in comparison to the carboxylic derivatives. These compounds are also uninteresting in protecting mice against radiation injury although they do in fact demonstrate some protection. All of these compounds are lacking in convulsive properties (i.e. depressant) when given at the maximum tolerated level. WR 2380 was not available in sufficient quantity to permit testing of radiation protection effects at a level higher than 1000 mg/kg nor was enough material available to evaluate the approximate LD_{50} at levels higher than 1600 mg/kg.

The two carboxylic acid esters were not effective against radiation injury in spite of the fact that WR 2427 is an exact analog of 2346 and was given at a comparable millimolar level. It also demonstrated depression. There was not a sufficient quantity of the agent available to permit testing at higher levels than the ones reported.

WR 2529 is the amide corresponding to 2346 and 2471. This compound is surprisingly effective in protecting mice against radiation injury inasmuch as it demonstrates good protection at one quarter of the maximum tolerated dose. The index level of 10 reflects this correspondingly good protection. The compound has some convulsant as well as depressant properties but was a depressant at all the levels tested.

Table 2 includes all acidic groups or derivatives of alkyl acidic side groups having covered sulfur functions. There are two categories of covered sulfur functions, Buntes and disulfides. WR 2651, the disulfide corresponding to 2650, demonstrates protective action and toxicology comparable to 2650. The Bunte carboxylic acid analogs are not available. WR 2379, the butyl sulfonic acid disulfide derivative, corresponds exactly to 2380 in the mercaptan series and 2453 in the Bunte series in all respects including a similar rodent pharmacology and lack of protective action. WR 2093, the methyl ester of the Bunte salt may be compared to 2471 and 2346 and has

no protective action. WR 2093 corresponds to the ethyl ester 2471 in the mercaptan series. The two esters appear to be similar in terms of their pharmacologic response. WR 1527, a pyrrolidone, is not of interest and is quite toxic. It is also a convulsant. The toxicity of this compound and its convulsive properties might be predicted from its tertiary amide structure.

DISCUSSION

The most interesting finding is the good protective activity of 2529. The discovery that an amide could have good protective action constitutes the discovery of a new functional group which offers the way for obvious exploitation since amides are well-known in medicinal chemistry. It is possible to synthesize multiple amide variations to obtain different pharmacologic side effects. The compound is apparently not a convulsant at the levels tested and therefore convulsions will probably not be a problem in large animals. The compound is water soluble so that it should be absorbed by mouth. The amide clearly ranks the carboxylic acid in terms of interest inasmuch as we have an exact comparison in 2346. The sulfonic acid derivatives are also active but obviously the two carbon derivatives should be made in order to have an exact comparison. The discovery of activity in the carboxylic acid amides opens the way for development of sulfonamides related to 2650 and its derivatives. Esters are clearly shown to be non-interesting at

least if the carbon chain is short. Likewise, WR 1105 demonstrates the continued lack of interest in two-arm structures. Tertiary amines continue to be uninteresting as demonstrated by 1527. Another conclusion of interest is that in this series of compounds the disulfides appear to be as effective as mercaptans. This equal protective action of disulfides is unusual and has been found only in the guanadine series in mice. The Bunte appears as effective as the corresponding mercaptan and disulfide suggesting that Bunte variations on the above compounds also can lead to active compounds. Our conclusion is that a major pathway has been opened to water soluble non-convulsant antiradiation agents. The amide or sulfonamide side chain will probably have a relatively weak ability to release histamine.

SUMMARY

A new class of antiradiation agents has been discovered which merits further development.

RODENT CLINICAL MANIFESTATIONS						DATE	TECHNICIAN					
AGENT							WR NO					
SYMPTOMS	MG/KG					SYMPTOMS	MG/KG					
1. ALERT						22. DEPRESSION						
RESPIRATION						23. ANALGESIA						
2. RAPID						24. ATAXIA						
3. SLOW						25. NARCOSIS						
4. LABORED						26. PROSTRATION						
5. ARRHYTHMIC						27. HYPOTHERMIA						
OCULAR						28. HYPERTHERMIA						
6. LACRIMATION						29. SALIVATION						
7. SWOLLEN LIDS						30. MOUTH DRY						
8. PTOSIS						31. DEFECATION						
9. MIOSIS						32. URINATION						
10. MYDRIASIS						33. PILOERECTION						
REFLEXES						34. VASOCONSTRICTION						
11. CORNEAL						35. VASODILATION						
12. PINNAL						36. CYANOSIS						
13. RIGHTING						BEHAVIOR UNUSUAL						
14. TAIL						37. STRAUB TAIL						
15. MYOTACTIC						38. VOCALIZATION						
16. HYPERSENSITIVE						39. CIRCLING						
TREMORS						40. HYPERACTIVE						
17. LOCALIZED						41. STRETCHING						
18. GENERAL						DEATH						
19. INTERMITTANT						42. - 1 HR						
CONVULSIONS						43. + 1 HR						
20. CLONIC						OTHER						
21. TONIC												

MORTALITY RECORD

MICE	DOSE (MG/KG)	ACUTE	24 HR	48 HR	72 HR	10 DAYS	PATHOLOGY

GROSS PATHOLOGY INDEX

1. MOUSE WEIGHT LOSS	13. HEMORRHAGIC LIVER	25. ADHESIONS OF ABDOMEN
2. MOUSE DEHYDRATED	14. PALE KIDNEYS	26. ADHESIONS OF STOMACH
3. MOUSE ORGANS SMALL IN SIZE	15. HEMORRHAGIC KIDNEYS	27. ADHESIONS OF LIVER
4. NVL	16. NEPHRITIS	28. ADHESIONS OF GUT
5. WHITE FILM ON LIVER	17. WHITE FILM ON SPLEEN	29. ADHESIONS OF KIDNEYS
6. ENLARGED LIVER	18. LARGE SPLEEN	30. BLOODY ASCITES
7. THIN LIVER	19. SMALL SPLEEN	31. MILKY ASCITES
8. SMALL LIVER	20. DARK SPLEEN	32. CLEAR, WATERY ASCITES
9. FATTY LIVER	21. PALE SPLEEN	33. DRUG DEPOSITS ON SPLEEN
10. ATROPHIC LIVER	22. MOTTLED SPLEEN	34. DRUG DEPOSITS ON LIVER
11. PALE LIVER	23. HYPOPLASTIC SPLEEN	35. DRUG DEPOSITS ON KIDNEYS
12. MOTTLED LIVER	24. HYPERPLASTIC SPLEEN	36. DRUG DEPOSITS IN GUT MESENTERY
37. DRUG DEPOSITS AT INJ. SITE (S.C.)		

REMARKS

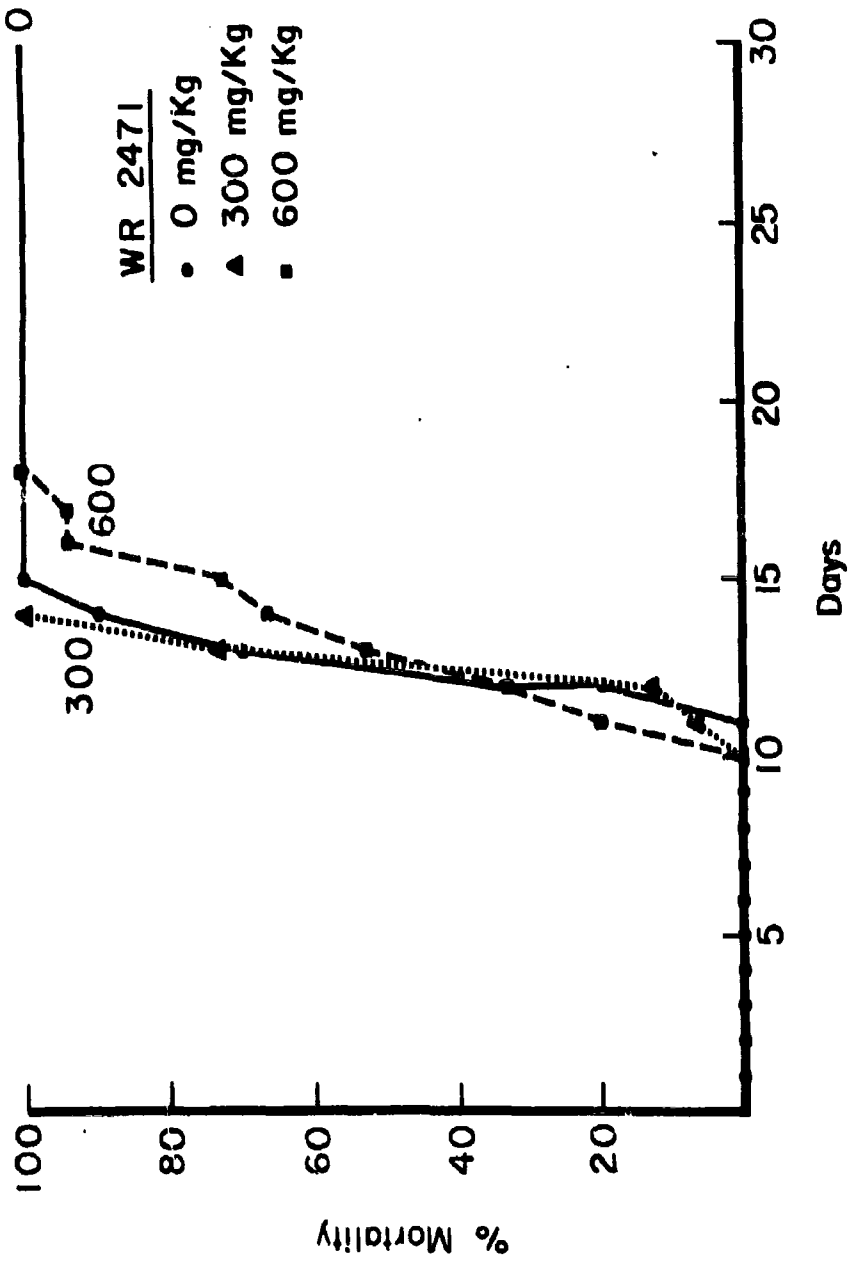
Section M
Figure 3

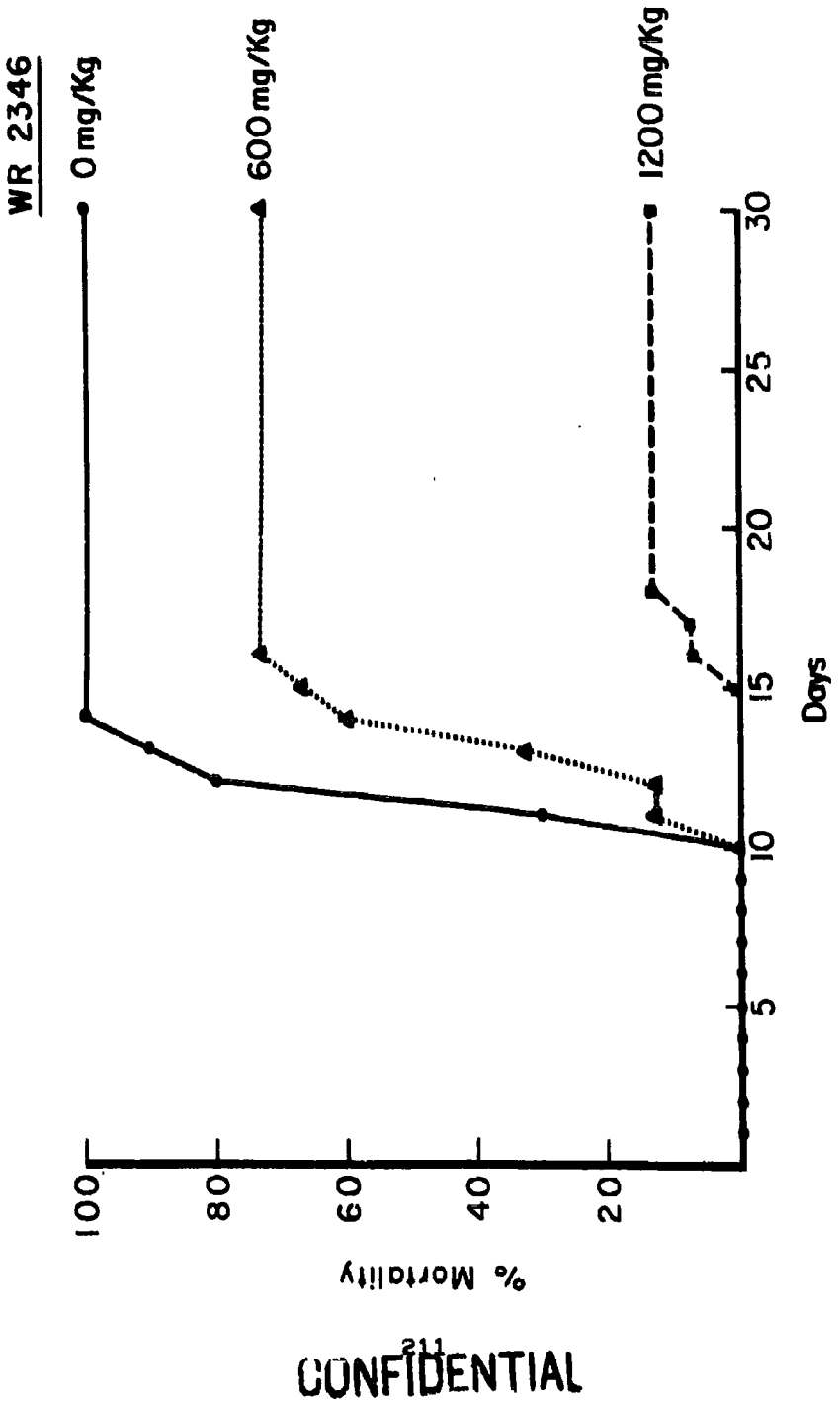
$$\text{Index} = \frac{\text{Approx } LD_{50}}{\text{Tested Dose}} \times \text{Factor}$$

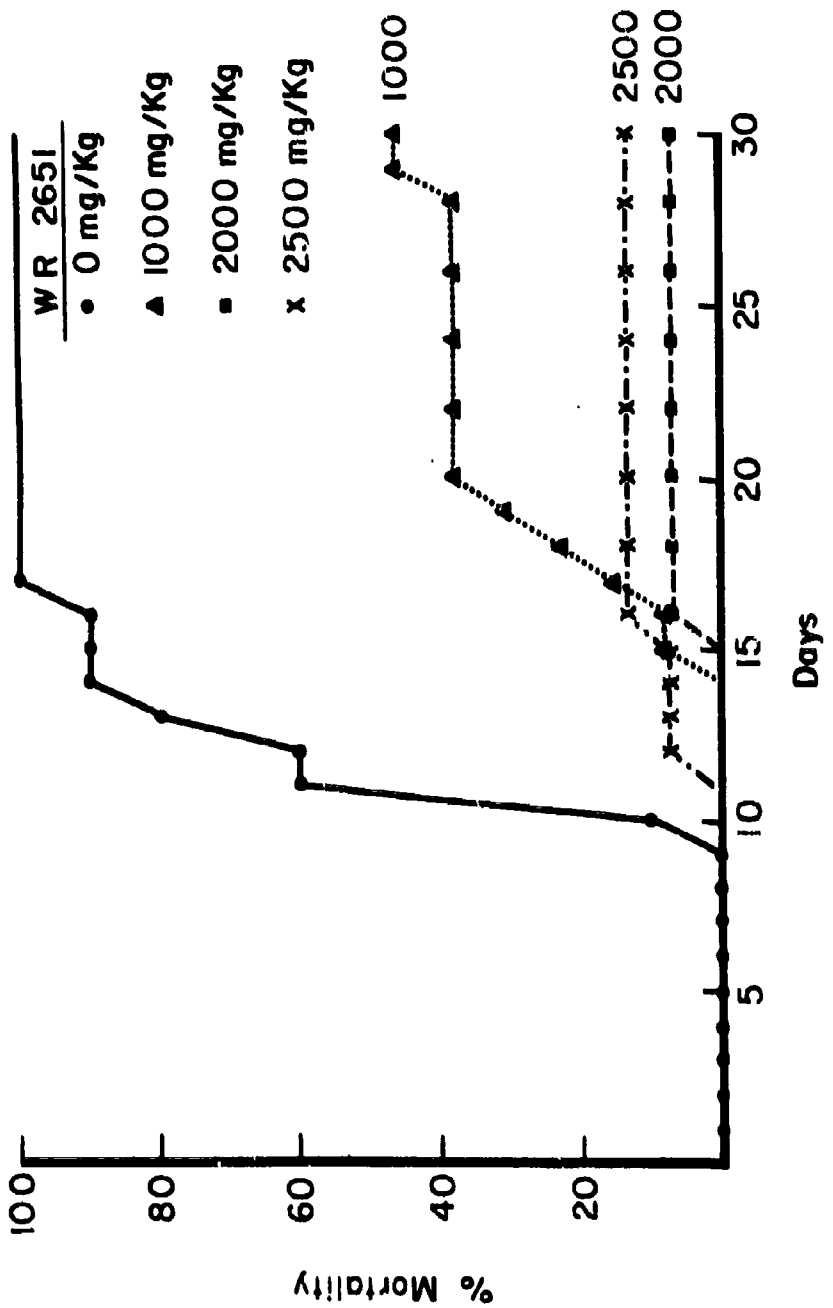
Factor = 1 if agent effective only at mid lethal radiation

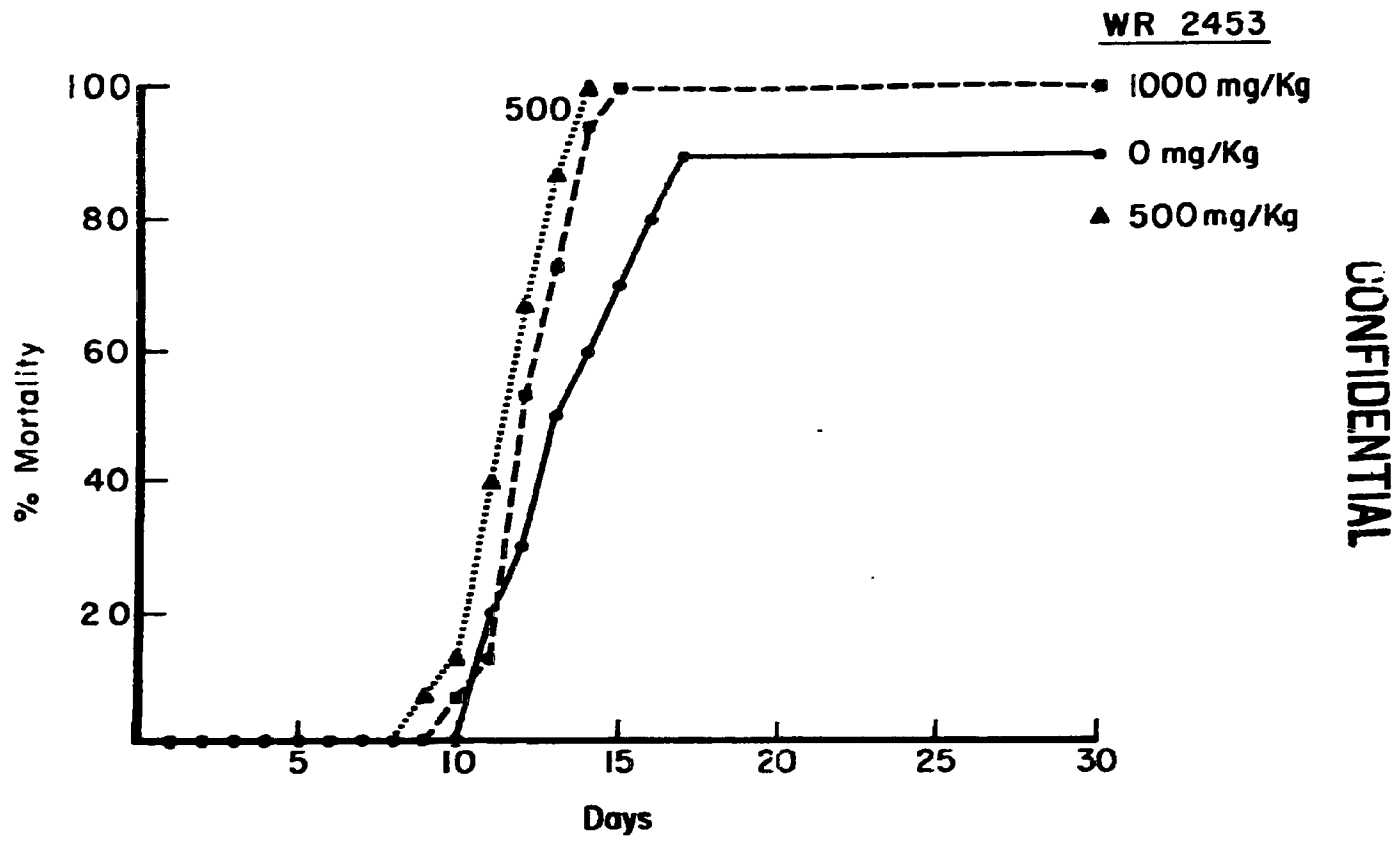
= 1.X X = % Survival at lethal radiation

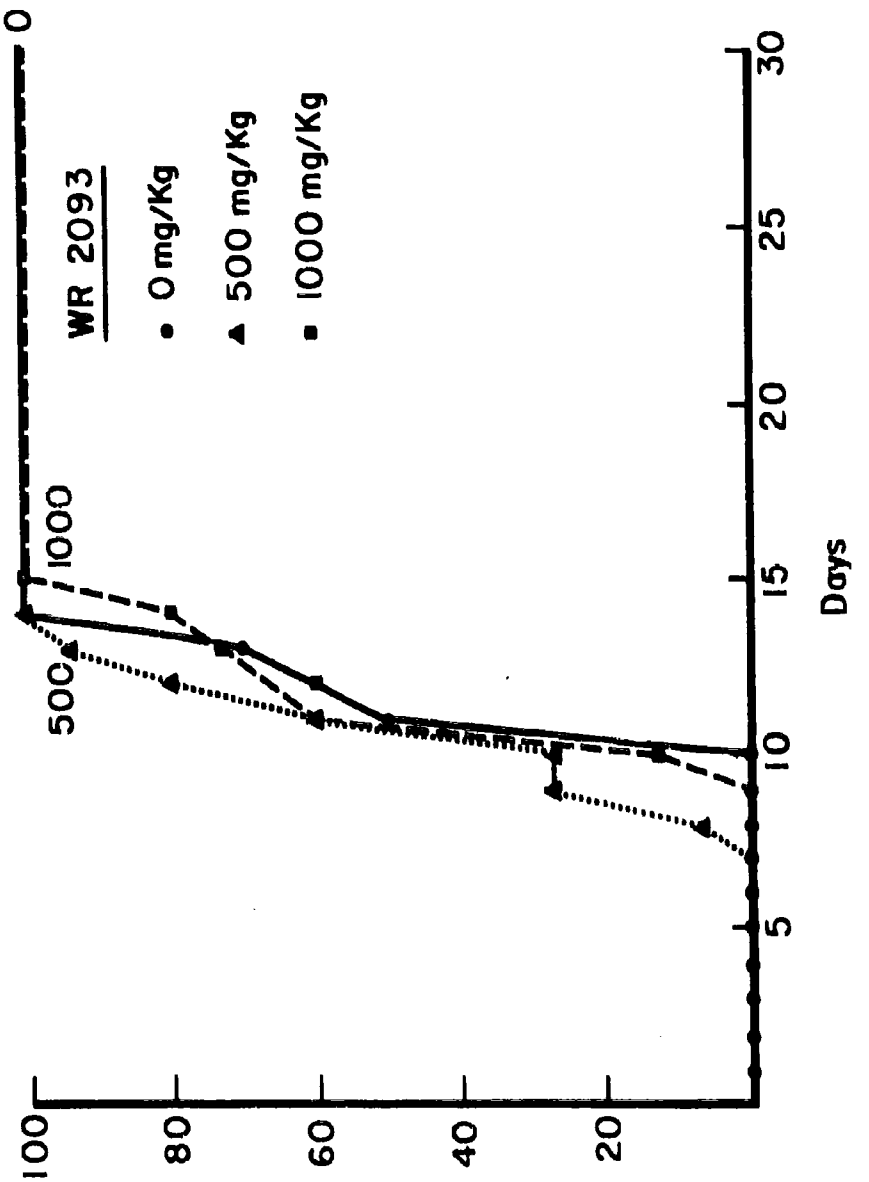
For Example:			Factor
	10%	Survival	= 1.10
	20%	"	= 1.20
	80%	"	= 1.80
	99%	"	= 1.99
	100%	"	= 2.0

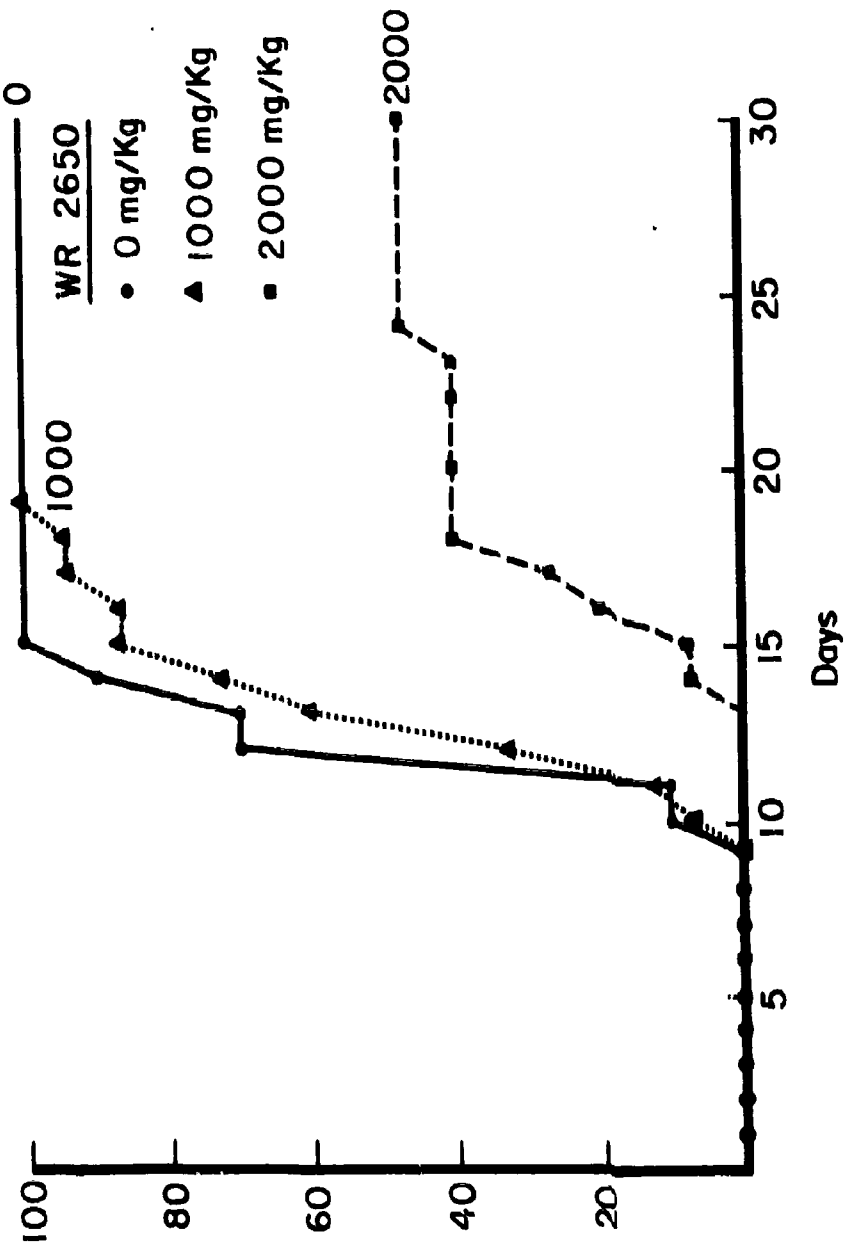

WR 2471
• 0 mg/Kg
▲ 300 mg/Kg
■ 600 mg/Kg
600
300
0
5
10
15
20
25
30
Days
100
80
60
40
20
% Mortality

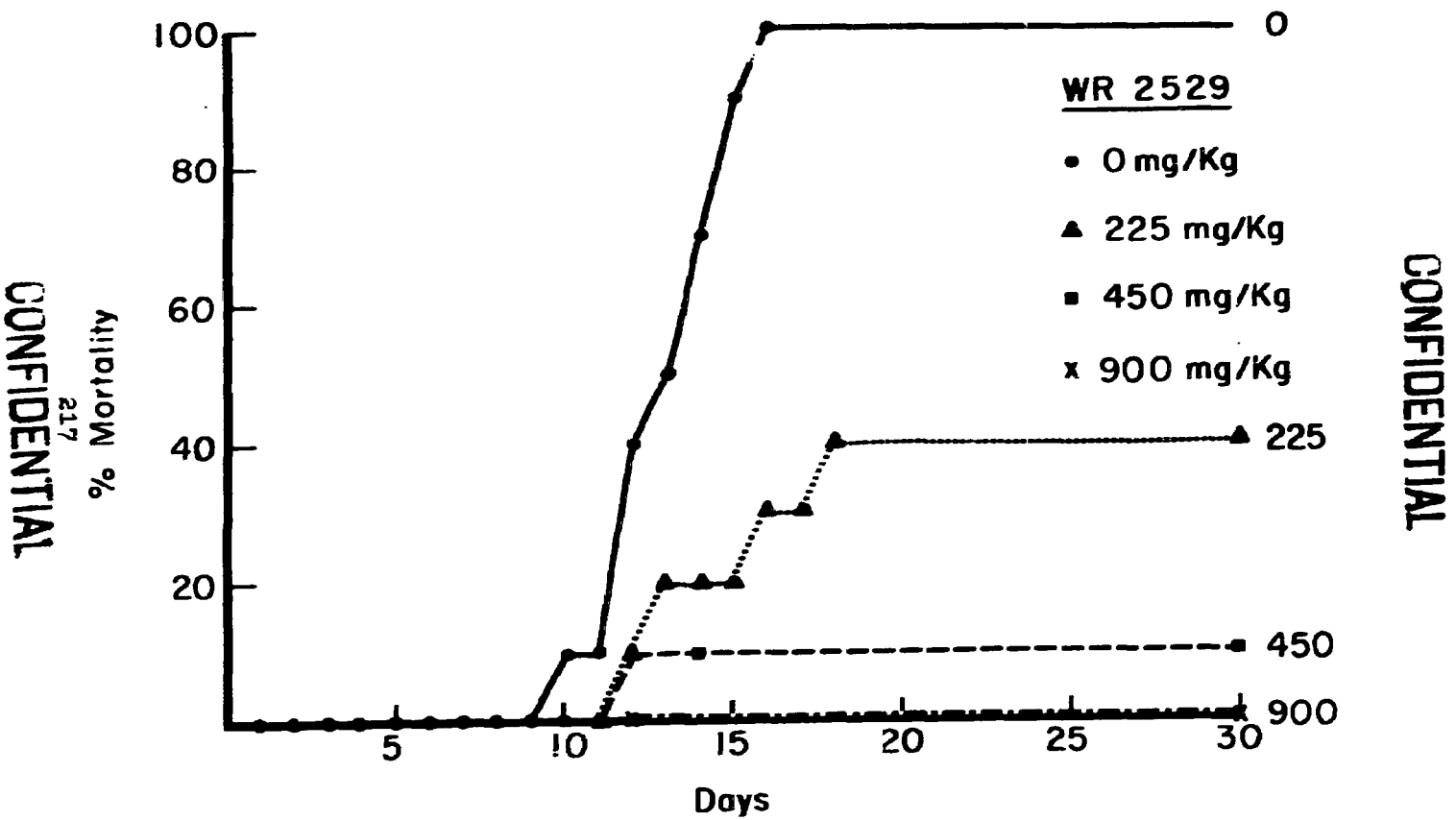

WR 2346
0 mg/Kg
600 mg/Kg
1200 mg/Kg
% Mortality
100
80
60
40
20
5
10
15
20
25
30
Days


WR 2651
• 0 mg/Kg
▲ 1000 mg/Kg
■ 2000 mg/Kg
x 2500 mg/Kg
1000
2500
2000
% Mortality
100
80
60
40
20
5
10
15
20
25
30
Days




WR 2093
• 0 mg/Kg
▲ 500 mg/Kg
■ 1000 mg/Kg
0
1000
500
100
80
60
40
20
% Mortality
5
10
15
20
25
30
Days


WR 2650
• 0 mg/Kg
▲ 1000 mg/Kg
■ 2000 mg/Kg
0
1000
2000
100
80
60
40
20
% Mortality
5
10
15
20
25
30
Days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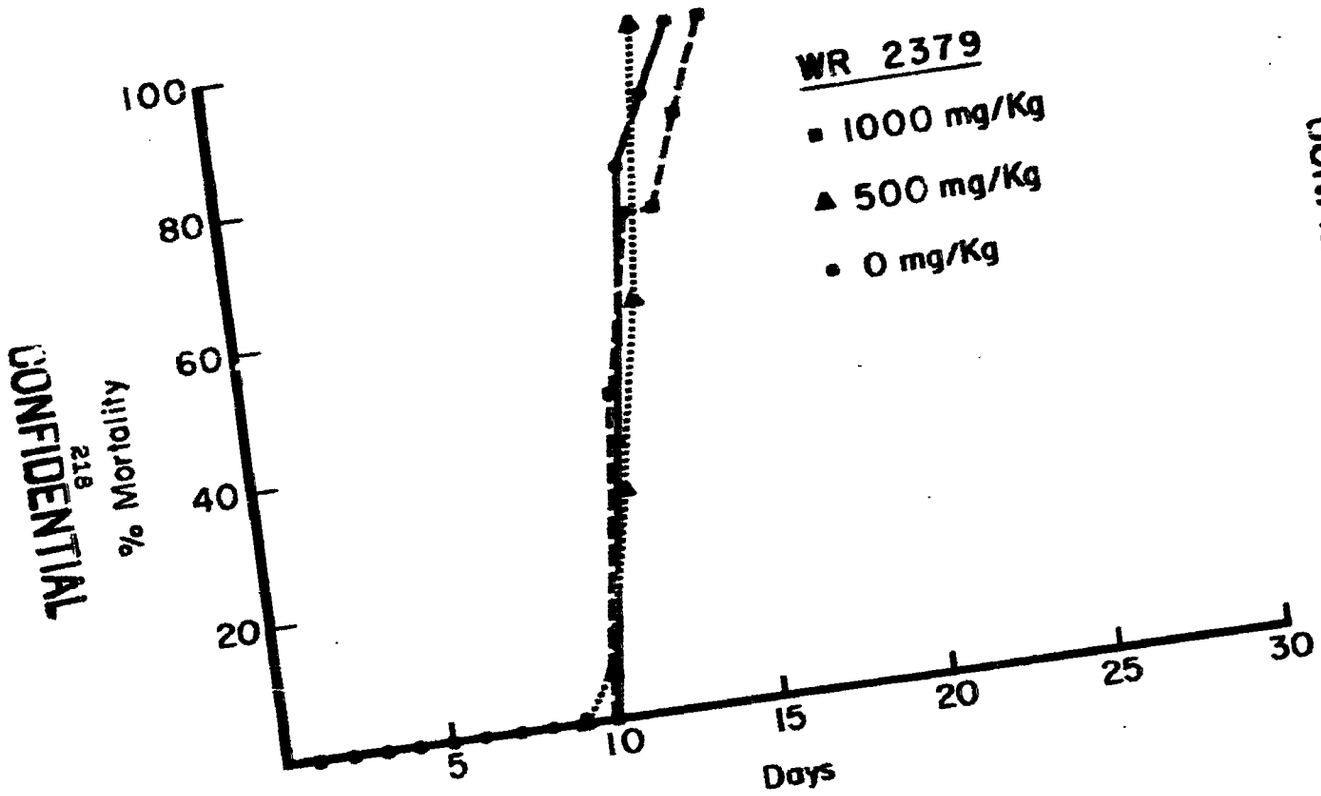
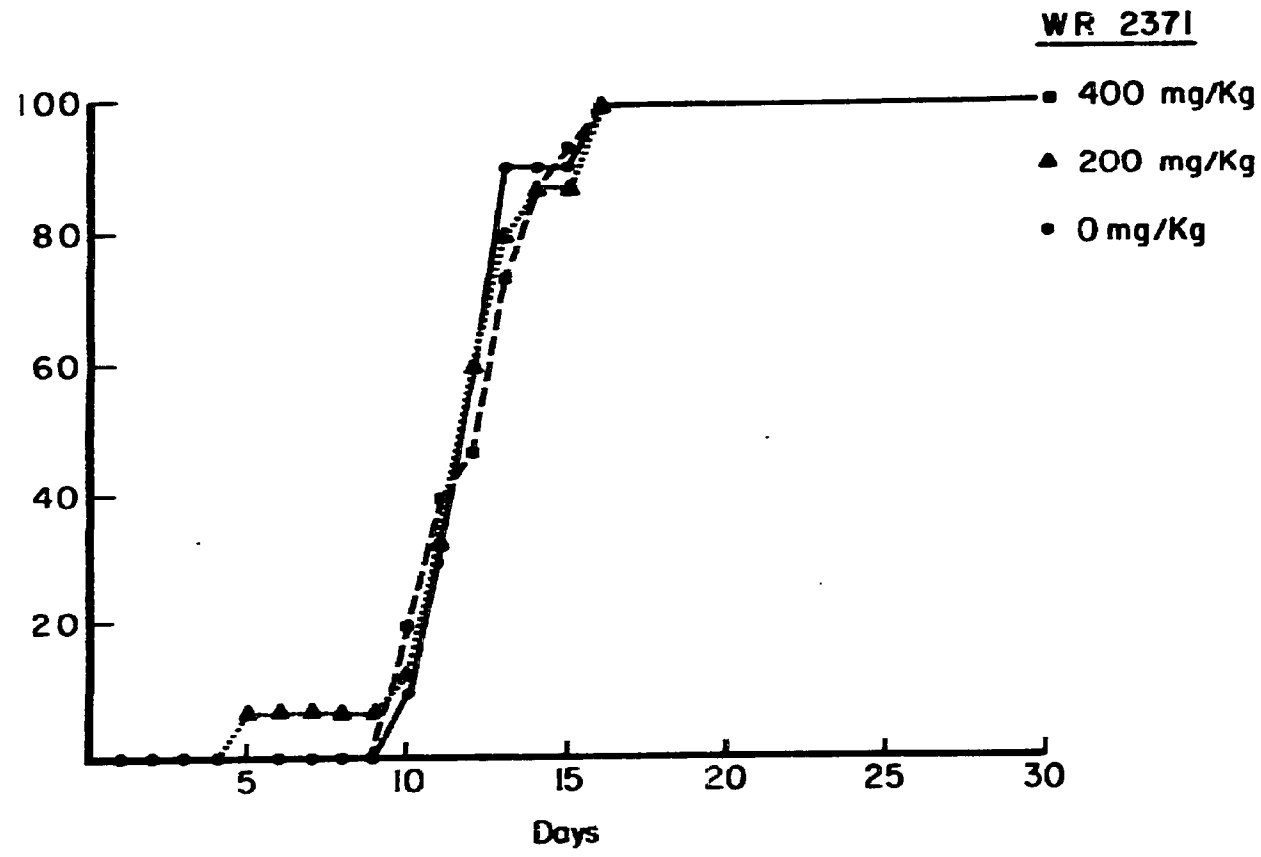
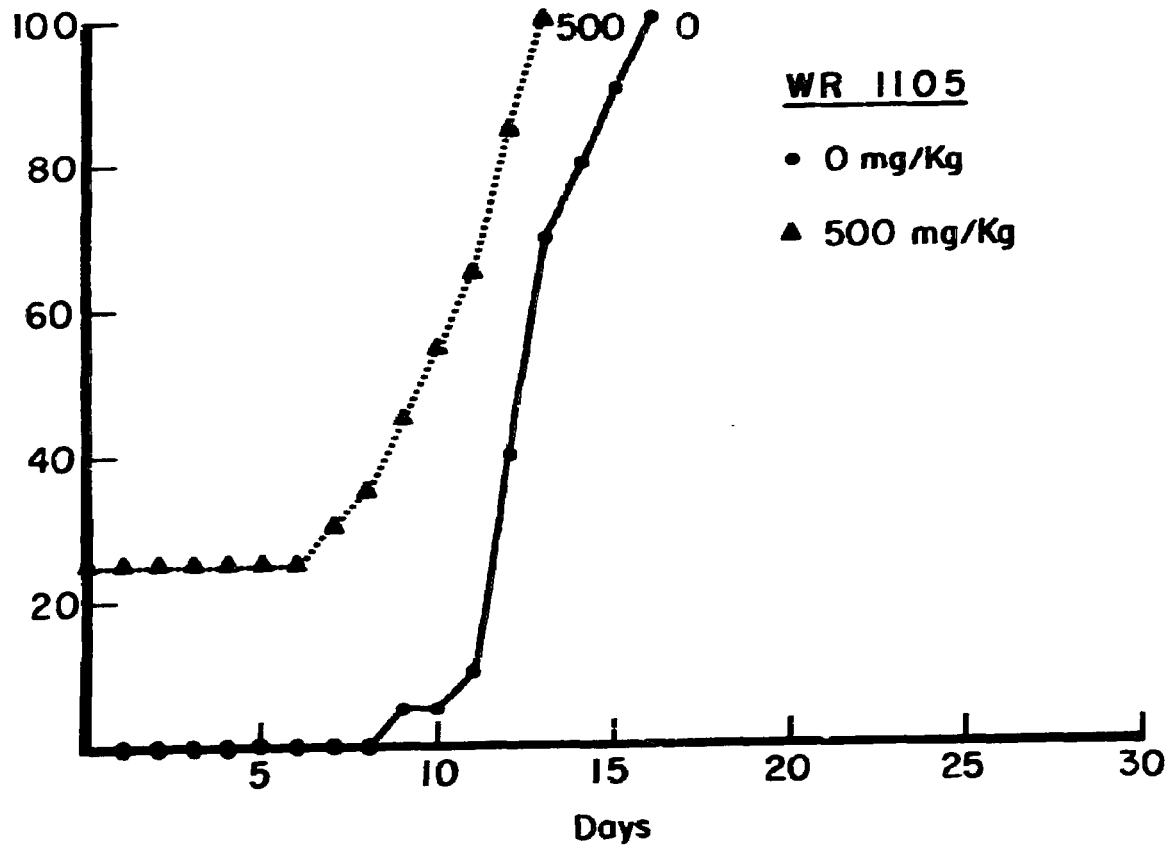
% Mortality
100
80
60
40
20
5
10
15
20
25
30
Days
WR 2529
• 0 mg/Kg
▲ 225 mg/Kg
■ 450 mg/Kg
x 900 mg/Kg
0
225
450
900
CONFIDENTIAL







Section M
Table 1

$HSCH_2CH_2NHR$

R-	WR No.	mM/Kg	Approx LD_{50}	Tested	% Survival	Index
$-CH_2COOH$ (Depression)	419	7.4	2000+	1000	55	3.1
-CH CH COOH	2346	8.1	1200+	1200	87	1.8
				600	27	2.5
				300	5	4.0
$-CH_2CH_2SSO_3H$ (Depression)	1105	1.7	600	500	Sig	1.2
$-(CH_2)_3 \cdot SO_3H$ (Depression)	2650	10	3500	2000	53	2.6
				1500	53	3.5
				1000	0	0
				750	7	4.7
$-(CH_2)_4SO_3H$ (No sx)	2380	4.5	1600+	1000	0	0
$-(H_2CH_2C\overset{O}{\overset{\|}{=}}C-OC_2H_5$ (Depression)	2471	3.5	1000+	600	0	0
$-CH_2CH_2C\overset{O}{\overset{\|}{=}}C-NH_2$ (Convulsion-depression)	2529	5.5	1800	900	100	2.9
		2.75		450	90	5.9
		1.35		225	70	10.0
		1.05		175	0	0

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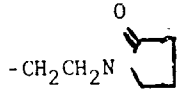
Section M
Table 2

$[RNHCH_2CH_2S-]_2$

R-	WR No.	mM/Kg	Approx LD_{50}	Tested	% Survival	Index
$-(CH_2)_3SO_3H$ (Depression)	2651	12.5 10 5	2000+ [presumably ~ 3500]	2500 2000 1000 500	87 93 54 0	[2.5 3.3 5.3 0]
$-(CH_2)_4SO_3H$ (Depression)	2379	3.1	2000+ [presumably ~ 3500]	1000	0	0

$RNHCH_2CH_2SSO_3H$

$-(CH_2)_4SO_3H$ (No sx)	2453	3.4	2000+	1000	0	0
$-CH_2CH_2C(=O)-OCH_3$ (Depression)	2093	4.1	1200+	1000	0	0
 (Convulsion)	1527	0.17	150	50	0	0