A STUDY OF THE INTERACTION OF CO-INSULT TREATMENTS WITH METHYLMERCURIC CHLORIDE AND X-IRRADIATION AND DEMONSTRATION OF A PEROXIDE INDUCED PROTECTIVE MECHANISM

DISSERTATION

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By

James M. Earhart, B.A., M.S.
Denton, Texas
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The initial purpose of this work was to investigate the interaction of methylmercuric chloride (MMC) and X-irradiation given as a co-insult upon the rat blood-brain barrier (BBB). The indicators used to determine BBB alterations were mortality and the in vivo tissue uptake of radioactive sulfate administered as $^{35}$S-sodium sulfate. The results of the interaction studies indicated a neutralization of effects when MMC and X-irradiation were given together. X-irradiation as a single insult generally caused an increase in sulfate uptake by the brain regions monitored, whereas MMC treatment generally resulted in decreased sulfate uptake. The neutralization patterns following co-insult treatments were somewhat varied in the different brain regions, exhibiting cancellation of effects in some cases and overriding by one insult in other cases.

The neutralization of effects observed in the mortality study suggested that X-irradiation treatment reduced the MMC induced death rate. It was reasoned that a less severe
insult, mimicking the effects of X-irradiation, might stimulate the protective mechanism, while allowing the animals to live long enough for further evaluation of the mechanism. Based upon the above observations and upon evidence from the literature that peroxidation is responsible for the secondary effects of X-irradiation, a hydrogen peroxide pretreatment regime was devised in which animals received five 1 ml doses of hydrogen peroxide at 24 hour intervals. Forty-eight hours after the last dose of peroxide, a lethal dose of MMC was administered. This regime significantly reduced death resulting from MMC intoxication in male and female rats of varying ages.

The latter part of the work was involved in investigating the peroxide induced protective mechanism (PIPM). A post-treatment of MMC intoxicated rats with hydrogen peroxide offered no therapeutic value. This finding along with the fact that hydrogen peroxide pretreated rats did not receive MMC until 48 hours after the last dose of peroxide, suggested that anti-MMC protection is not obtained by direct interaction of MMC and hydrogen peroxide. That the PIPM could be saturated was indicated by the results obtained when hydrogen peroxide pretreated rats were subjected to increasing doses of MMC.

A system of peroxisome-like organelles demonstrated in the literature and observed in this study may be a functional
component of the BBB and the responsive mechanism which protects against large doses of MMC after pretreatment with hydrogen peroxide. The P-L organelles have been shown to be rich in sulfhydryl groups and peroxidase and to proliferate after large acute doses of X-irradiation. In the current study a 32% increase in the P-L organelles was observed in a sample area of the nucleus arcuatus after treatment with hydrogen peroxide.

From the data obtained by this work and in the literature, it is hypothesized that the P-L organelle system of the perivascular glia serves as a trap for MMC, preventing MMC from reaching the neurons. The system appears to proliferate in response to increased peroxides in the body fluids, thereby increasing tolerance to larger doses of MMC.
### TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>LIST OF TABLES</td>
<td>vi</td>
</tr>
<tr>
<td>LIST OF ILLUSTRATIONS</td>
<td>vii</td>
</tr>
<tr>
<td>INTRODUCTION AND REVIEW OF THE LITERATURE</td>
<td>1</td>
</tr>
<tr>
<td>MATERIALS AND METHODS</td>
<td>14</td>
</tr>
<tr>
<td>Maintenance of Animals</td>
<td>14</td>
</tr>
<tr>
<td>X-Radiation Treatment</td>
<td>14</td>
</tr>
<tr>
<td>Gamma Radiation Treatment</td>
<td>15</td>
</tr>
<tr>
<td>Methylmercuric Chloride Treatment</td>
<td>16</td>
</tr>
<tr>
<td>Rat Mortality Following Single and Co-Insults with MMC and X-Radiation</td>
<td>16</td>
</tr>
<tr>
<td>Rationale for Choice of Insult Doses</td>
<td>18</td>
</tr>
<tr>
<td>Experiment 1</td>
<td>20</td>
</tr>
<tr>
<td>Experiment 2</td>
<td>20</td>
</tr>
<tr>
<td>Effects of Single and Co-Insults of MMC and X-Radiation Upon the Uptake of Sulfur-35 Sodium Sulfate by Various Brain Areas</td>
<td>20</td>
</tr>
<tr>
<td>The Effect of Hydrogen Peroxide Pretreatment on MMC-Induced Mortality</td>
<td>20</td>
</tr>
<tr>
<td>The Effect of Hydrogen Peroxide Post-Treatment on MMC-Induced Mortality</td>
<td>25</td>
</tr>
<tr>
<td>Response of Peroxide and Non-Peroxide Pretreated 90-Day-Old Female Rats to Graded Doses of MMC</td>
<td>25</td>
</tr>
<tr>
<td>Histological Analyses of the Peroxisome-Like Organelle System of the Nucleus Arcuatus After Treatment with 800 R Whole Body Gamma Radiation, 10 KR Head X-Radiation and Hydrogen Peroxide Pretreatment</td>
<td>27</td>
</tr>
<tr>
<td>RESULTS</td>
<td>29</td>
</tr>
<tr>
<td>Part I. Rat Mortality Following Single and Co-Insults with MMC and X-Irradiation</td>
<td>29</td>
</tr>
</tbody>
</table>
TABLE OF CONTENTS CONTINUED

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experiment 1</td>
<td>29</td>
</tr>
<tr>
<td>Experiment 2</td>
<td>31</td>
</tr>
</tbody>
</table>

Part II. Effects of Single and Co-Insults of MMC and X-Radiation Upon the Uptake of Sulfur-35 Sodium Sulfate by Various Brain Areas | 31 |
| Summary of Single Insult Effects | 39 |
| Summary of Co-Insult Effects | 40 |

Part III. The Effects of Hydrogen Peroxide on MMC Induced Mortality | 41 |
| A. Hydrogen Peroxide Pretreatment | 41 |

| Ninety-Day-Old Female Rats | 41 |
| Experiments 1 and 2 | 41 |
| One Hundred Eighty-Day-Old Females | 47 |
| Experiment 3 | 47 |
| Experiment 4 | 48 |
| Experiment 5 | 48 |
| Ninety-Day-Old Male Rats | 48 |
| Experiment 6 | 48 |

| B. Response of 90-Day-Old Female Rats to Graded Doses of MMC | 49 |
| C. The Effect of Hydrogen Peroxide Post-Treatment of MMC Induced Mortality | 50 |

Part IV. Histological Analysis of Gomori Positive Cells in the Periventricular Glia of the Nucleus Arcuatus | 50 |

DISCUSSION | 54 |

| Rat Mortality Following Single and Co-Insults with MMC and X-Irradiation | 54 |
| Effects of Single and Co-Insults of MMC and X-Irradiation Upon the Uptake of Sulfur-35 Sodium Sulfate by Various Brain Areas | 55 |
| The Effect of Hydrogen Peroxide Pretreatment on MMC-Induced Mortality | 58 |
TABLE OF CONTENTS CONTINUED

<table>
<thead>
<tr>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Response of Peroxide and Non-Peroxide Pretreated 90-Day-Old Female Rats to Graded Doses of MMC</td>
<td>64</td>
</tr>
<tr>
<td>Histological Analysis of the P-L Organelle System of the Nucleus Arcuatus After Treatment with 800 R Whole-Body Gamma Radiation, 10 KR Head X-Radiation and Hydrogen Peroxide Pretreatment</td>
<td>66</td>
</tr>
<tr>
<td>Summary of Data Supporting the P-L Organelle System (Srebro, 1972) as the PIPM</td>
<td>68</td>
</tr>
<tr>
<td>Hypothetical Functioning of the PIPM</td>
<td>70</td>
</tr>
<tr>
<td>SUMMARY</td>
<td>73</td>
</tr>
<tr>
<td>LITERATURE CITED</td>
<td>76</td>
</tr>
</tbody>
</table>
### LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Description of control and hydrogen peroxide pretreated rats receiving lethal doses of methylmercuric chloride (MMC)</td>
<td>26</td>
</tr>
<tr>
<td>2.</td>
<td>Comparison of percent of blood concentration (PBC) values obtained by treating rats with varying doses of single and co-insults of methylmercuric chloride and X-irradiation to the head</td>
<td>33</td>
</tr>
<tr>
<td>3.</td>
<td>Comparison of $^{35}$S-sodium sulfate uptake by ten body tissues in rats treated with single and co-insults of methylmercuric chloride (MMC) and X-irradiation</td>
<td>38</td>
</tr>
<tr>
<td>4.</td>
<td>Percentage survival of hydrogen peroxide pretreated and non-hydrogen peroxide pretreated 90-day-old female rats after graded doses of MMC. Each group consisted of 15 animals</td>
<td>50</td>
</tr>
<tr>
<td>5.</td>
<td>Mean number of gomori-positive glial cells in 0.01337 mm$^3$ of the nucleus arcuatus of the rat brain. The number of animals in each group is given in parenthesis.</td>
<td>53</td>
</tr>
</tbody>
</table>
## LIST OF ILLUSTRATIONS

<table>
<thead>
<tr>
<th>Figure</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Schematic representation of the cellular components of the blood-brain barrier (BBB). Modified from Holman (1972)</td>
<td>8</td>
</tr>
<tr>
<td>2. Experimental design for studying rat mortality following single and co-insults with MMC and X-radiation</td>
<td>17</td>
</tr>
<tr>
<td>3. Experimental design for studying the effects of single and co-insults of MMC and X-radiation upon the uptake of sulfur-35 sodium sulfate by various brain areas</td>
<td>21</td>
</tr>
<tr>
<td>4. Mortality response of 90-day-old male rats following single and co-insults with methyl mercury chloride (MMC) and X-irradiation</td>
<td>30</td>
</tr>
<tr>
<td>5. Mortality response of 120-day-old female rats following single and co-insults with methylmercuric chloride (MMC) and X-irradiation</td>
<td>32</td>
</tr>
<tr>
<td>6. Comparative profiles of in vivo $^{35}$S-sodium sulfate uptake by rat brain regions in response to single and co-insults of X-irradiation and methylmercuric chloride (MMC)</td>
<td>35</td>
</tr>
<tr>
<td>7. Comparative profiles of in vivo $^{35}$S-sodium sulfate uptake by rat brain regions in response to single and co-insults of X-irradiation and methylmercuric chloride (MMC)</td>
<td>36</td>
</tr>
<tr>
<td>8. Comparative profiles of in vivo $^{35}$S-sodium sulfate uptake by rat skeletal muscle and liver in response to single and co-insults of X-irradiation and methylmercuric chloride (MMC)</td>
<td>37</td>
</tr>
<tr>
<td>9. Comparison of accumulated mortality (%) in hydrogen peroxide pretreated and non-pretreated 90-day-old female rats after a lethal does of methylmercuric chloride (10 mg/kg body weight)</td>
<td>42</td>
</tr>
</tbody>
</table>
LIST OF ILLUSTRATIONS CONTINUED

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>10.</td>
<td>Comparison of accumulated mortality (%) in hydrogen peroxide pretreated and non-pretreated, 90-day-old female rats after a lethal dose of methylmercuric chloride (10 mg/kg body weight).</td>
<td>43</td>
</tr>
<tr>
<td>11.</td>
<td>Combined data comparing the accumulated mortality (%) in hydrogen peroxide pretreated and non-pretreated, 90-day-old female rats after a lethal dose of methylmercuric chloride (10 mg/kg body weight).</td>
<td>44</td>
</tr>
<tr>
<td>12.</td>
<td>Comparison of accumulated mortality (%) in hydrogen peroxide pretreated and non-pretreated mature male rats after a lethal dose of methylmercuric chloride (3 mg/animal).</td>
<td>45</td>
</tr>
<tr>
<td>13.</td>
<td>Comparison of accumulated mortality (%) in hydrogen peroxide pretreated and non-pretreated 90-day-old male rats after a lethal dose of methylmercuric chloride (10 mg/kg body weight).</td>
<td>46</td>
</tr>
<tr>
<td>14.</td>
<td>Response of hydrogen peroxide pretreated and non-pretreated 90-day-old female rats to graded doses of methylmercuric chloride (MMC). MMC doeses in mg/kg body weight.</td>
<td>51</td>
</tr>
<tr>
<td>15.</td>
<td>A regression line analysis of the response to graded doses of methylmercuric chloride exhibited by hydrogen peroxide pretreated and non-pretreated 90-day-old female rats.</td>
<td>52</td>
</tr>
</tbody>
</table>
INTRODUCTION AND REVIEW OF THE LITERATURE

Paralleling modern technological progress has been the introduction of many new environmental factors, some of which have been shown to be injurious to man and the other species. Some of the deleterious factors represent relatively recent syntheses. The chlorinated hydrocarbons, which have been of no little concern to the environmentalist and the general public, are a case in point. However, naturally occurring phenomena, such as the impinging of ionizing radiation upon the earth's crust or the presence of toxic elements such as the heavy metals, may represent hazards equal to those created by the introduction of synthetic materials into the environment. We may assume that these naturally occurring environmental factors are a part of the evolutionary background of man and the other presently living species. The major problem with these environmental pressures is not their presence, but the alteration in their local concentrations and the redistribution in their geographic locations by the activities of one species, namely man (Selikoff, 1971; Vostal and Clarkson, 1973).

It is certainly conceivable that a natural phenomenon, e.g. erosion, can redistribute a material in such a way that the material becomes a harmful contaminant for a particular species or for the members of an entire food chain. Sediments
downstream from ore deposits have been shown to contain from 0.1 to 200 ppm inorganic mercury (Wallace et al., 1971). Microorganisms, living in the sediments of lakes and streams, have been found to be capable of converting the bound inorganic mercury into methylmercury and dimethylmercury. These forms of mercury are rapidly absorbed and retained by fish (Jensen and Jernelov, 1969; Wood et al., 1968). Certain types of fresh water and marine fish represent the chief source of mercury in the human diet (Vostal and Clarkson, 1973). Man may someday be able to exert control over these events; at the present time, however, it seems more appropriate that he direct his attention to the alterations in natural phenomena which have been fostered by his activities. Hopefully, awareness and understanding of environmental problems may produce attitudinal changes which will, in turn, result in behavioral changes. It appears that only by changing his habits, can man favorably influence the effluent produced by his existence.

Many studies may be found concerning each of the environmental factors which man has recognized as a menace to his welfare. Seemingly, fewer studies have been made concerning the interaction between two or more of these factors. It seems appropriate that such studies be made, since each environmental problem may be the result of several interacting forces. The purpose of this study is to explore the
the nature of the interaction between two environmental factors as they influence biological activity. More specifically, the combined effects of methylmercuric chloride (MMC) and X-irradiation upon the rat blood brain barrier (BBB) will be considered.

Mercury, as a toxic agent, has been known for many centuries (D'Itri, 1972). More recently, triggered by such events as the Minamata incident (Kurland, 1960; D'Itri, 1972; Kojima and Fujita, 1973), methylmercury poisoning in Iraq (Rustam and Hamdi, 1974), and other individual and mass poisonings by mercurial compounds (Wallace et al., 1971), a keen interest has developed in mercury and its various compounds.

Mercury is found in the environment in its elemental form and compounded with other substances to form both organic and inorganic compounds. Mercury, in all of its forms, may have toxic effects upon living systems when administered in sufficiently large amounts. Mercurial poisonings have been found to occur: in treatment with diuretics (De Graff and Nadler, 1942; Russek and Zohman, 1949); through careless industrial use of mercury (Lee, 1968); after the consumption of food contaminated by fungicides (Pierce, et al., 1972); from inhalation of mercury vapor (Swaiman and Flagler, 1971); after accidents involving thermometers (Rachman, 1974); and as a result of other contamination (Wallace, et al., 1971; D'Itri, 1972).
Organic mercurials show greater toxicity toward the central nervous system than do other forms of mercury. This is particularly true of short chain alkyls like the various methylmercury compounds (Vostal and Clarkson, 1973). Intoxication by these mercurials is characterized by neurological symptoms such as sensory disturbances, ataxia, concentric constriction of visual fields, and loss of hearing (Friberg and Vostal, 1972). A number of methylmercury compounds exist based upon the anion moiety possessed. The anion is important in imparting various physical properties to the compounds, such as solubility and volatility (Klein et al., 1972). The anion is reported to be of minor consequence in determining toxicity, however, since the compound is rapidly dissociated (Ulfvarson, 1969a). Although Garcia et al. (1974) have shown that some carbon-mercury bond breakage does occur, it has been demonstrated that in much of the in vivo methylmercury, the carbon-mercury bond remains intact (Norseth and Clarkson, 1970). The intact CH₃-Hg⁺ is considered of primary importance in producing toxicity. Because of the extreme toxicity of methylmercury and its importance as a contaminant in the environment, it has been chosen for this study.

According to Butler (1972), the lesions induced are related to the class of mercury compound. The greater neurotoxicity of methylmercury compounds is probably
associated with their abilities to penetrate the exclusive BBB and to form bonds with brain protein (D'Itri, 1972; Yoshino et al., 1966; Hughes, 1954).

There is considerable variation in the ability of various mercurials to penetrate the BBB. The inorganic mercuric form \((\text{Hg}^{+2})\) is particularly restricted, possibly due to its high capability of forming thiol bonds with blood cell proteins (Hughes, 1954). The primary target organ for this compound is the kidney, and it apparently has little effect upon the nervous system (Butler, 1972). Although the mercuric form does not easily penetrate the BBB, it has been shown to alter the structural integrity of membranes believed to be associated with that barrier (Joo, 1969).

Methylmercury and organic mercurials in general pass rather easily through membranes. Hughes (1954) attributed the ease with which methylmercury halides penetrate cell membranes to their greater fat solubility, being one-hundred times as soluble in lipid as in water. Garcia et al. (1974) showed that when rats were fed MMC, plasma fat contained a much larger percentage of labeled MMC than did plasma protein.

Steinwall and Olsson (1969) found that the presence of organic methylmercuric dicyandiamide, as well as inorganic \(\text{HgCl}_2\), brought about a change in BBB permeability, increasing the influx of the fluorescent Evens blue-protein complex, while reducing the penetration of another indicator, \(\text{Se}^{75}\)-selenomethionine. Although methylmercury compounds penetrate
the BBB, their flow into certain brain areas has been found to be at a somewhat slower rate than their flow into other body organs (Ulfvarson 1969b and 1969c; Aberg et al. 1969; Swensson and Ulfvarson, 1967). For example, a dose of 40 ng/g tissue of methylmercuric hydroxide showed an accumulation of 13 ng/g in the rat cerebrum at day 3 post-injection and 32 ng/g at day 24 when the last mercury determination was made (Ulfvarson, 1969a). Mercury concentrations in blood, liver, kidney, and muscle were initially high at day 3, but diminished rapidly through day 24 post-injection. At the higher doses of 400 and 4000 ng/g, the organs showed a similar pattern as with the lowest dose, but the cerebral concentration peaked more rapidly. A peak was observed at day 13 for the 400 ng/g dose, whereas a peak for the largest dose was observed at day 6. The cerebellum likewise showed a delay in reaching maximum concentration of mercury. These observations also indicate that the movement of methylmercury from the brain occurs at a slower rate than its movement from other organs normally involved. This slower turn-over rate causes a rather long exposure of brain tissues to mercury. This retention of mercury by the brain may be due to bonds formed with brain proteins. In chemical fractionation studies, Yoshino et al. (1966) noted that nearly all brain mercury in dogs treated with methylmercury thioacetamide was found in the protein fractions. It seems reasonable
that the longer exposure period contributes to the central nervous system effects seen at rather low mercury concentrations (Pierce et al., 1972).

The other insult to be studied in connection with methylmercury is X-irradiation. Since the advent of the "atomic age", numerous studies have been made concerning the biological effects of various types of ionizing radiation, as evidenced by the large body of scientific literature which has accumulated concerning this subject. Several fairly recent reviews concerning the response of the nervous system to ionizing radiation serve to substantiate this point (Haley and Snider, 1962; Kimeldorph and Hunt, 1965; Altman and Gerbey, 1970).

There are numerous investigations which have attempted to confirm the presence and/or to elucidate the activity of the BBB (Wislocki and Leduc, 1952; Dempsey and Wislocki, 1955; Breeman and Clemente, 1954; Joo, 1969; Nair and Roth, 1964, to name a few). Schettler and Shealey (1970) state that there is considerable evidence to indicate that the BBB is composed of the walls of brain capillaries and associated glial cells, and that the barrier is not merely a peculiarity of brain metabolism as suggested by some investigators (Dobbing, 1961). There is evidence that, anatomically, the BBB consists of the capillary endothelium, the basement membrane and the glia as is shown in Fig. 1.
Fig. 1. Schematic representation of the cellular components of the blood-brain barrier (BBB). Modified from Holman (1972) A = closed tight junction. B = gap junction. BM = basement membrane. EC = endothelial cell of capillary. GFP = glial foot process.
Tight junctions connect endothelial cells of the brain and there are few pinocytotic vesicles. Therefore, the passage of molecules via these routes, which is common in other organs, is disallowed. Holman (1972) concludes that "passage through the endothelial cell is the only way to get across this barrier, limiting passage to substances that are highly soluble and substances with specific active transport mechanisms within the cell membrane". The implication is that the most effective anatomical component of the BBB is the capillary endothelium. Lipid soluble substances, such as ethyl alcohol, oxygen and carbon dioxide, move readily through the barrier and equilibrate rapidly. Hydrophillic substances are excluded unless their access is accommodated by a carrier mechanism. Such mechanisms have been shown to be carrier mediated limited capacity transport systems. Some are energy dependent as in the active transport of amino acids. Glucose transport, however, does not appear to be energy dependent (Holman, 1972).

Nair and Roth (1964) using radiiodinated serum albumin (RISA) as an indicator found that 10 KR X-irradiation to the heads of rats caused only subtle changes in the permeability of the BBB to that particular indicator. However RISA is a large protein molecule and may not be a good indicator of the effects of ionizing radiation upon the permeability of a membrane system. In the study,
the investigators also found that the same dosage of X-irradiation caused an increase in the uptake of S\textsuperscript{35}-sodium sulfate at 48 hours post-irradiation. Although the hypothalamus showed the greatest increase in permeability, increased uptake occurred in other brain areas including the cerebrum and cerebellum.

In another study, using 3 KR X-irradiation to the heads of cats as a co-insult with ultrasonic sound, an increased penetration of the indicators, trypan blue and \textsuperscript{203}Hg was found (Schettler and Shealy, 1970). In the same study, a single insult of 2 to 3 KR failed to alter barrier permeability to the two indicators. Smaller chronically administered doses have likewise failed to increase permeability of the BBB. Scott (1967) showed that whole body exposures to \textsuperscript{60}Co irradiation fields of 20 R, 10 R, 5 R and 2 R per day for 90 days has no influence upon the uptake of silver nitrate by rat brains. The anticonvulsant action of the normally slowly penetrating drug, acetazolamide, was increased by 10 KR X-irradiation delivered to the heads of rats. The effect of the drug was maximal at 48 hours post-radiation (Nair et al., 1964). Penetration of the drug \textsuperscript{35}S-acetazolamide was found to be increased in the cerebral cortex, hypothalamus, hippocampus and cerebellar cortex.

The literature suggests that BBB permeability to certain substances may be altered by the action of ionizing radiation,
depending upon the molecular size and other properties of the indicator, its attachment to plasma protein, the metabolism of brain tissue, and the dosage of radiation delivered (Schettler and Shealey, 1970). The implication is that a large, acute dose of radiation is required in order to achieve discernable alterations in BBB permeability.

Investigations have shown that "organelles" with a strong affinity for Gomori's chrome alum-haematoxylin occur in the glial cells associated with the BBB. Tests, using 2, 2'-dihydroxy-6, 6'-dinaphthyl disulphide (DDD reagent) developed by Barnett and Seligman (1954) have indicated that these organelles have a much higher concentration of thiol and disulfide groups than the surrounding cytoplasm (Srebro and Cichocki, 1971). The same authors found that these organelles are strongly positive for peroxidase, probably having as their main function the decomposition of organic peroxides. It is further hypothesized that the glial cells in which these peroxisome-like organelles (P-L organelles) are found form a trap for various bloodborne toxic substances including the heavy metals (Srebro et al., 1972). These observations and hypotheses agree to some extent with the electron microscopic studies of Wislocki and Leduc (1952) and Dempsey and Wislocki (1955) in which they located silver deposits in the walls of cerebral capillaries and in the basement membrane between the capillary wall and the glia
after oral administration of silver nitrate. Also, these observations support the report by Yoshino et al. (1966) that there is a latent period between the time when methylmercury thioacetamide reaches its peak concentration in the brain and the onset of observable nervous symptoms. It has been shown that the P-L organelles increase considerably after whole body X-irradiation in rats (Srebro, 1969, 1970; Srebro et al., 1972). These investigations suggest that the P-L organelle proliferation is a compensatory reaction in order to decompose the increased peroxides produced by ionizing radiation. Srebro et al. (1970) have also observed that the periventricular glia tend to be localized perivascularly: a strategic location for a protective role as a component of the BBB.

Various regions of the brain concentrate mercury independently of one another. In this sense the brain regions respond in a way similar to the responses of other body organs (Steinwall and Olsson, 1969; Yoshino et al., 1966; Glomski and Brody, 1971). In general, however, greater concentrations are reported in cerebellum and cerebrum, with considerable variation among various regions of the cerebrum.

The initial purpose of this study was to investigate the interaction of MMC and X-irradiation upon the rat BBB, using mortality and sulfate uptake as indicators of barrier alterations. Results of the mortality experiments suggested an
X-irradiation stimulated BBB mechanism operating against MMC toxicity. The latter portion of this study was concerned with investigating further this protective mechanism.
MATERIALS AND METHODS

Maintenance of Animals

Male and female Sprague-Dawley or Sprague-Dawley derived albino rats were used in this study. Animals were removed from the Texas Woman's University rat colony to quarters designated for each particular experiment. The rats were maintained in clean wire mesh cages at a temperature of 22°C and a day-night cycle of 13-11 hours. A constant supply of standard laboratory chow and water were available to each animal.

X-Radiation Treatment

X-radiation was delivered to the heads of unanesthetized animals by a General Electric Maximar 250 KVP X-Ray Unit at a target distance of 170 mm and a dose rate of 264.5 R per minute. Softer emissions were filtered by 0.5 mm copper and 1 mm aluminum filters. Dosage was measured in air at about mid-brain level with Victoreen ionization chambers. The length of treatment was determined by dividing the total desired dosage by the dose rate.

A restraining device, consisting of a quarter inch plywood base, a yoke formed by two 4 cm lengths of eighth inch doweling, a cotton fabric jacket, and masking tape, immobilized the animal and prevented its head from being pulled back.
under the 5.4 cm thick lead shielding which covered the remainder of the body. The rat was slipped into the jacket and taped securely to the base. The dowel rods were inserted into openings on the base on each side of the animal's neck, forming a yoke which was narrower than the width of either the rat's jaws or pectoral girdle. This restraining device restricted movement in all directions, yet was flexible enough to allow normal respiratory movements. Observation of control animals following the restraining period indicated no apparent ill effects.

The radiation field within which the rats' heads were placed measured 120 mm by 154 mm. Sham-irradiated control animals were held in the same type of restrainer and for the same period of time as animals receiving radiation.

Gamma Radiation Treatment

Whole body gamma radiation was delivered to unanesthetized animals by a U. S. Nuclear Corporation GR-9 gamma irradiator. The gamma dosage used in this study was 800 R, delivered at 58.1 R per minute. Rats to be irradiated were introduced into the machine in a cylindrical cardboard container having a length of 118 mm and a diameter of 84 mm. Control animals were placed in the same type of restrainer for a comparable period of time.
Methylmercuric Chloride Treatment

Rats in Exp. 1 of the mortality study received MMC dissolved in physiological saline solution. Because a precipitate was observed in the MMC-saline solution, subsequent stock solutions of MMC were made using distilled water. The dissolution of MMC was accomplished much more readily in water than in saline.

Stock solutions containing 2.5 or 3 mg of MMC/ml were mixed and doses in most cases were calculated on a body weight basis. In one mortality and in one H₂O₂ pretreatment experiment MMC was given on a per animal basis. All MMC doses were given by intraperitoneal injection. Control animals received a corresponding volume of saline.

Rat Mortality Following Single and Co-Insults with MMC and X-Radiation

Two experiments were conducted in this part of the study. The first experiment involved 80 male rats, while the second experiment included 80 female rats. In both cases, the animals were randomly assorted into four groups, each containing 20 animals. Fig. 2 outlines the basic experimental design used in both experiments. The four groups were designated A, B, C and D. Group A animals were sham-irradiated. Immediately following sham-irradiation each animal received an intraperitoneal injection of physiological saline solution. Group B animals were irradiated with 10 KR
METHYL MERCURIC CHLORIDE INJECTED INTRAPERITONEALLY

0 mg/kg of Body Weight 2.75 mg/Animal or 10 mg/kg of Body Weight

<table>
<thead>
<tr>
<th>0 KR</th>
<th>Group A 20 Animals</th>
<th>Group B 20 Animals</th>
</tr>
</thead>
<tbody>
<tr>
<td>X-RADIATION TO HEAD</td>
<td>Group C 20 Animals</td>
<td>Group D 20 Animals</td>
</tr>
<tr>
<td>10 KR</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Fig. 2. Experimental design for studying rat mortality following single and co-insults with MMC and X-radiation.
to the head followed by an intraperitoneal injection of saline. Group C animals were sham-irradiated, after which each animal was administered MMC. Group D animals received 10 KR head irradiation followed by MMC.

Since the X-ray device could accommodate only four animals at a time, it was convenient to carry out the experiment in ten blocks, each containing 8 animals. A block of 8 animals was formed by taking 2 animals each from group A, B, C and D. Immediately after irradiation or sham-irradiation the animals were released from the restrainers and injected intraperitoneally with either MMC or saline. After treatment animals were recaged and observed daily for a 30 day period. The time of death for each animal was recorded to the nearest day following treatment.

Rationale for Choice of Insult Doses

These experiments were designed to determine if the mortality response of rats to combined lethal insults of MMC and X-irradiation is different from mortality responses to the individual insults. Since the brain is particularly vulnerable to MMC toxicity (Chang et al., 1972), head irradiation was chosen in an attempt to focus X-irradiation upon the brain.

The choice of dosing for each insult was based upon several reports from the literature. Yoshino et al. (1966) found that 3-6 mg/100 g body weight of methylmercury
thioacetamide resulted in an increase of mercury in the dog brain. They noted particularly high levels in the calcarine area where the most noticeable histological changes took place. Suzuki (1969) found that rats which received doses larger than 4.6 mg/100 g body weight died before neurological symptoms appeared while rats receiving less than 3.2 mg/100 g body weight showed no neurological symptoms up to 13 days post-injection. As was pointed out earlier, Ulfvarson (1969b and 1969c) considered 40 mg/kg body weight of methylmercury hydroxide to be an LD$_{50}$ dose for adult rats, allowing animals to live long enough to accumulate mercury in the organs. Based upon these reports, unpublished results with methylmercuric chloride (Hoskins, 1973), and preliminary experiments with rats, 10 mg/kg of rat body weight or 2.75 mg/animal was given by intraperitoneal injection in about 0.5 ml of distilled water.

Although the brain appears to be relatively radioresistant in the light of criteria involving morphological damage (Keyeux, 1974), large doses of X-irradiation have shown rather striking effects upon the BBB. A large insult of 10 KR to the head has been shown to cause an increase in the permeability of the BBB (Nair and Roth, 1964; Nair, et al., 1964). Based upon these observations a large acute dose of 10 KR X-radiation was administered to the head.
Experiment 1

Eighty 90-day-old male rats were included in the first experiment. The animals ranged in weight from 300.5 g to 378.5 g, having a mean weight of 338.4 g and a standard deviation of ±19.3 g. The animals were given either 0 KR or 10 KR to the head, followed immediately by 0 mg of MMC in 1.1 ml of saline or 2.75 mg of MMC in 1.1 ml of solution.

Experiment 2

Eighty 120-day-old female rats were included in the second experiment. They ranged in weight from 233 g to 285 g with a mean weight of 252.9 g and a standard deviation of ±13.7 g. The animals received the same radiation treatment as was given in the first experiment. Immediately following irradiation animals received either saline or MMC on a 10 mg/kg body weight basis. The MMC was given intraperitoneally from a stock solution of 3 mg/ml. After treatment the animals were observed for a 30 day observation period. The date of death was recorded for each animal.

Effects of Single and Co-Insults of MMC and X-Radiation Upon the Uptake of Sulfur-35 Sodium Sulfate by Various Brain Areas

Ninety 90-day-old male rats were used in the experiment. The mean weight was 270.4 g with a standard deviation of ±16.4 g and a range from 230 g to 324 g.

The experimental design consisted of 9 groups containing 10 animals per group as shown in Fig. 3. Each group
**METHYL MERCURIC CHLORIDE INJECTED INTRAPERITONEALLY**

<table>
<thead>
<tr>
<th>Body Weight</th>
<th>0 mg/kg</th>
<th>4.03 mg/kg</th>
<th>8.06 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 KR Animals</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>X-RADIATION TO HEAD 5 KR Animals</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>10 KR Animals</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
</tbody>
</table>

Fig. 3. Experimental design for studying the effects of single and co-insults of MMC and X-radiation upon the uptake of sulfur-35 sodium sulfate by various brain areas.
received a different combination of doses of MMC and X-rays. The MMC doses were either 0, 4.03 or 8.06 mg/kg of body weight. The X-ray doses were either 0, 5 KR or 10 KR delivered to the head. The MMC dose rate was reduced from the 10 mg/kg rate used previously in the mortality study to insure that the animals lived through the 48 hour experimental period. All animals were anesthetized and killed by immersion in liquid nitrogen 48 hours after irradiation. Five minutes prior to sacrifice, each animal was injected in the femoral vein with sulfur-35 sodium sulfate. The dosage was based upon the findings of Nair and Roth (1964) that this per capita dosage resulted in measurable increases of that indicator in the rat brain at 48 hours post-irradiation.

Tissue samples of caudate nucleus, cerebral cortex, thalamus, hypothalamus, hippocampus, inferior colliculus, medulla, cerebellum, skeletal muscle and liver were taken for liquid scintillation counting and dry weight determination. Frontal sections of the frozen rat brain were sliced at intervals designed to expose each brain area to be sampled. The sections were laid flat on the dissection plate of a dry-ice box and desired samples were carved from the sections with a scalpel. Regions from which brain samples were taken were located by using a stereotaxic atlas (Konig and Klippel, 1970).
The mean dry weight of tissue samples was 6.7 mg with a standard deviation of ±4.5 mg and a range of 0.5 mg to 44.6 mg. The rather wide variation in sample size resulted in part from the relative availability of sample materials in various tissues. For example, the rat thalamus and hypothalamus are rather small regions. Samples taken from these areas tend to be much smaller than samples from larger areas such as the cerebral cortex, medulla, or liver. Greatest variation in sampling occurred during initial dissections. As dissection experience was gained, the sample weights became more nearly uniform.

One hundred microliter whole-blood samples were also taken for liquid scintillation counting. Tissue samples were prepared for counting after Mahlin and Lofberg (1970) and counting was done on a Beckman Model LS-200 Scintillation Counter. Radioactivity was determined for each sample and the results were expressed as percent of the amount of radioactivity contained in the blood (Nair, 1960; Nair and Roth, 1964). The percent of blood concentration values (PBC values) computed for each sample made comparisons of sulfate uptake by various tissues possible.

The Effect of Hydrogen Peroxide Pretreatment on MMC-Induced Mortality

It was reasoned that a less severe treatment mimicking the effects of X-radiation might produce a depression in
MMC-induced mortality similar to that observed when MMC was administered immediately after radiation. By trial and error it was determined that 1 to 2 ml of 1.5% hydrogen peroxide could be administered intraperitoneally without causing lasting, observable, detrimental effects. In the series of experiments which followed, animals were divided into two groups. The experimental group received five doses of hydrogen peroxide at 24 hour intervals. The individual dose ranged from 1 to 1.8 ml of hydrogen peroxide, depending upon animal weight. Each animal in the control group received physiological saline instead of hydrogen peroxide. Forty-eight hours after the last dose of either peroxide or saline each animal was injected intraperitoneally with a dose of MMC capable of killing 50 to 85% of the animals. In all cases except one, the MMC dosage given was 10 mg/kg of body weight. Since 10 mg/kg of body weight was found to produce 100% mortality in the mature male rats of experiment 4, the dosage of MMC was reduced in experiment 5. Also, the dosage of 1.5% hydrogen peroxide was increased.

Different age groups of both sexes were used in this part of the study. Experiments 1 and 2 involved 90-day-old females, experiment 3 included 180-day-old females, experiments 4 and 5 included mature adult males and experiment 6 included 90-day-old males. After treatment the animals were observed for a 30 day period. The date of death was
recorded for each animal. Experiments 1 through 6 are outlined in Table 1.

The Effect of Hydrogen Peroxide Post-Treatment on MMC-Induced Mortality

Since the H₂O₂ pretreatment regime had provided protection against MMC toxicity, a preliminary experiment was performed to determine if post-treatment with the chemical might have some effect. Nine animals ranging in weight from 236 g to 428 g and averaging 368 g were divided into two groups. Group A contained 5 animals and group B contained 4 animals. Each animal was injected at time 0 with MMC at a dose rate of 10 mg/kg of body weight.

Following treatment with MMC each rat in group A received three doses of 1.5% H₂O₂: 1.2 ml seventeen hours later, 1 ml twenty-three hours later, and 1.2 ml forty-seven hours later. Animals in group B received corresponding doses of saline.

Response of Peroxide and Non-Peroxide Pretreated 90-Day-Old Female Rats to Graded Doses of MMC

Ninety female rats, ranging from 189 to 249 g and averaging 212.2 g with a standard deviation of 11.3 g, were randomly divided into 3 groups. Each group contained 30 animals. Half the rats in each group were subjected to the hydrogen peroxide pretreatment regime, while the other half received sham treatment with saline. The three groups
**TABLE 1. Description of control and hydrogen peroxide pretreated rats receiving lethal doses of methylmercuric chloride (MMC).**

<table>
<thead>
<tr>
<th>Expt. No.</th>
<th>Age of Rat</th>
<th>Sex of Rat</th>
<th>No. of Rats</th>
<th>Range of Body Weights</th>
<th>Body Weight Mean ± Standard Deviation (grams)</th>
<th>MMC Dosage</th>
<th>M1 of 1.5% H$_2$O$_2$/Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>90 days</td>
<td>F</td>
<td>20</td>
<td>236-287</td>
<td>255.0 ± 13.0</td>
<td>10mg/kg</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>90 days</td>
<td>F</td>
<td>30</td>
<td>209-256</td>
<td>231.4 ± 13.7</td>
<td>10mg/kg</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>180 days</td>
<td>F</td>
<td>12</td>
<td>226-266</td>
<td>241.6 ± 11.7</td>
<td>10mg/kg</td>
<td>1</td>
</tr>
<tr>
<td>4</td>
<td>Mature</td>
<td>M</td>
<td>16</td>
<td>372-424</td>
<td>392.8 ± 13.8</td>
<td>10mg/kg</td>
<td>1</td>
</tr>
<tr>
<td>5</td>
<td>Mature</td>
<td>M</td>
<td>24</td>
<td>332-494</td>
<td>421.8 ± 51.8</td>
<td>3mg/rat</td>
<td>1.4-1.8</td>
</tr>
<tr>
<td>6</td>
<td>90 days</td>
<td>M</td>
<td>100</td>
<td>250-301</td>
<td>279.7 ± 13.9</td>
<td>10mg/kg</td>
<td>1</td>
</tr>
</tbody>
</table>
were subjected to graded dose levels of MMC. One group received 10 mg/kg of body weight, a second group received 12.5 mg/kg of body weight and a third group received 15 mg/kg of body weight. The rats were observed for 30 days and deaths occurring in each group were tabulated.

**Histological Analyses of the Peroxisome-Like Organelle System of the Nucleus Arcuatus After Treatment with 800 R Whole Body Gamma Radiation, 10 KR Head X-Radiation and Hydrogen Peroxide Pretreatment**

Twenty 120-day-old female rats were used in this portion of the study. The mean weight of the group was 223.8 g with a standard deviation of ±18.4 g. The weights ranged from 217 g to 283 g. The animals were divided into four groups, each containing 5 rats. Group A animals served as controls, receiving sham-irradiation treatment and saline. Animals in Group B were treated with 800 R whole body gamma irradiation. Group C animals received 10 KR X-irradiation to the head, while group D animals received the hydrogen peroxide pretreatment regime described above. Tissues from one animal in both group B and group C animals were rendered unusable during histological processing. The results for these two groups is, therefore, based upon four animals rather than five.

Animals treated with 800 R and 10 KR were killed by day 7 following treatment. Peroxide pretreated animals were killed forty-eight hours after the final injection of hydrogen peroxide.
Animals were killed by decapitation with a Harvard Guillotine. Brains were removed and placed in Bouins' fixative for at least twenty-four hours. Brains were trimmed and blocks of tissue containing the nucleus arcuatus were embedded in paraffin for sectioning. Serial sections, 7 microns thick, were cut on an A. O. Spencer "820" microtome. The sections were mounted on 3 x 1 inch glass slides of 0.96-1.06 mm thickness and stained using Bargmann's modification of the chrome-alum haematoxylin-phloxin technique of Gomori (Pearse, 1960). Cells containing granular material positive for the chrome hematoxylin were counted in the nucleus arcuatus near the attachment of the infundibulum. Positive cells were counted in a 0.191 mm$^2$ area on each of ten serial sections from the nucleus arcuatus of each animal. Counts were made on a Reichert, Nr. 315330 Vis-o-pan projection microscope. Differences between groups were tested for significance by use of Student's "t" Test.
RESULTS

Part I. Rat Mortality Following Single and Co-Insults with MMC and X-Irradiation

Experiment 1

The results of the first experiment are represented graphically in Fig. 4. The initial kill of co-insulted animals at day 1 is greater than the kill observed in either single insulted group. However, after the initial mortality of co-insulted animals, no others died until day 7 following treatment. The other 80% of this group died between days 7 and 9 following treatment, approximating the mortality curve exhibited by the animals receiving only X-ray treatment. Approximately 80% of the co-insult induced mortality, therefore, appears to be a result of X-radiation effects.

On the second day following treatment the accumulative percentage mortality of the MMC treated animals was 20% and equivalent to the accumulative percentage mortality of the co-insulted animals. At day 3 post-treatment the accumulative mortality of the MMC treated animals surpassed that of the co-insulted animals and remained greater until day 7 after which time radiation kill of the co-insulted animals began. Following the initial kill mortality appears to be
Fig. 4. Mortality response of 90-day-old male rats following single and co-insults with methylmercuric chloride (MMC) and X-irradiation.
depressed in the co-insulted animals until the onset of death resulting from radiation injury. Although the difference in the curves suggests an operative protective mechanism, statistical tests by chi square fail to show the difference to be significant.

Experiment 2

The results of the second experiment are represented graphically in Fig. 5. This experiment followed generally the same pattern as experiment 1. However, as in the first experiment, statistical significance was not achieved.

Initial mortality at day 1 was greater in the MMC treated animals than in co-insulted animals. With the exception of day 3, the accumulative mortality of the MMC treated animals remained greater until day 9, at which time apparent radiation death caused the mortality of the co-insulted animals to surpass the mortality of the animals treated with MMC.

The X-irradiated animals exhibited a mortality curve very similar in shape to that observed in the first experiment. None of the animals in the control group died during the experimental period.

Part II. Effects of Single and Co-Insults of MMC and X-Radiation Upon the Uptake of Sulfur-35 Sodium Sulfate by Various Brain Areas

Percent of Blood Concentration (PBC) values for each type of sampled tissue are tabulated in Table 2. An
Fig. 5. Mortality response of 120-day-old female rats following single and co-insults with methylmercuric chloride (MMC) and X-irradiation.
TABLE 2. Comparison of percent of blood concentration (PBC) values obtained by treating rats with varying doses of single and co-insults of methylmercuric chloride and x-irradiation to the head.

<table>
<thead>
<tr>
<th>Anatomical Area of Tissue</th>
<th>Control</th>
<th>5 kR</th>
<th>10 kR</th>
<th>4.03mg MMC/kg</th>
<th>8.06mg MMC/kg</th>
<th>5 kR + 4.03mg MMC/kg</th>
<th>8.06mg MMC/kg</th>
<th>10 kR + 4.03mg MMC/kg</th>
<th>8.06mg MMC/kg</th>
<th>10 kR +</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cerebral Cortex</td>
<td>20.8</td>
<td>35.0</td>
<td>30.6</td>
<td>29.7</td>
<td>17.1</td>
<td>30.0</td>
<td>27.6</td>
<td>30.8</td>
<td>29.4</td>
<td></td>
</tr>
<tr>
<td>Caudate Nucleus</td>
<td>21.7</td>
<td>27.9</td>
<td>36.7</td>
<td>22.4</td>
<td>19.1</td>
<td>24.7</td>
<td>23.0</td>
<td>26.5</td>
<td>25.5</td>
<td></td>
</tr>
<tr>
<td>Thalamus</td>
<td>14.2</td>
<td>25.8</td>
<td>30.0</td>
<td>19.9</td>
<td>17.0</td>
<td>21.2</td>
<td>17.7</td>
<td>19.7</td>
<td>22.3</td>
<td></td>
</tr>
<tr>
<td>Hypothalamus</td>
<td>28.5</td>
<td>29.9</td>
<td>39.2</td>
<td>25.1</td>
<td>18.2</td>
<td>25.9</td>
<td>25.7</td>
<td>41.4</td>
<td>33.5</td>
<td></td>
</tr>
<tr>
<td>Hippocampus</td>
<td>29.4</td>
<td>28.3</td>
<td>32.2</td>
<td>23.0</td>
<td>20.2</td>
<td>31.6</td>
<td>30.1</td>
<td>31.7</td>
<td>31.0</td>
<td></td>
</tr>
<tr>
<td>Inferior Colliculus</td>
<td>32.8</td>
<td>39.0</td>
<td>39.1</td>
<td>35.4</td>
<td>32.2</td>
<td>39.4</td>
<td>32.1</td>
<td>35.7</td>
<td>33.5</td>
<td></td>
</tr>
<tr>
<td>Medulla</td>
<td>20.8</td>
<td>23.3</td>
<td>25.3</td>
<td>20.9</td>
<td>23.2</td>
<td>22.5</td>
<td>24.2</td>
<td>26.1</td>
<td>21.7</td>
<td></td>
</tr>
<tr>
<td>Cerebellum</td>
<td>42.1</td>
<td>45.3</td>
<td>32.8</td>
<td>39.2</td>
<td>26.5</td>
<td>45.8</td>
<td>36.0</td>
<td>33.2</td>
<td>44.8</td>
<td></td>
</tr>
<tr>
<td>Skeletal Muscle</td>
<td>89.6</td>
<td>75.0</td>
<td>54.5</td>
<td>76.2</td>
<td>80.4</td>
<td>55.2</td>
<td>60.4</td>
<td>53.6</td>
<td>50.1</td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>263.0</td>
<td>259.7</td>
<td>246.0</td>
<td>240.6</td>
<td>182.0</td>
<td>249.4</td>
<td>265.5</td>
<td>218.4</td>
<td>222.0</td>
<td></td>
</tr>
</tbody>
</table>
analysis of variance was run for the PBC values of each tissue-type along with an F test for significant differences. When significant differences between PBC values were indicated by the F test, Duncan's Multiple Range Test was performed to determine the groups between which significant differences occurred. The relationship between treatments may be more easily visualized by observing the bar graphs representing the PBC values of each tissue-type. These are given in Figs. 6 through 8.

Analysis of the various brain regions revealed several patterns of sulfate uptake following the administration of single and co-insults of MMC and X-radiation. When differences were found to exist between the single and combined insults, the co-insult effects tended to neutralize each other. In some cases the effects of the insults were cancelled. In most cases, however, one insult tended to override the effect of the other insult. The various patterns of neutralization are summarized below.

A summary of the effects of the single and co-insult combinations is given in Table 3. Increases or decreases in sulfate uptake by various tissues were categorized according to whether they were statistically significant changes or whether they were not statistically significant changes. The latter were arbitrarily defined as changes equal to or greater than 10% of control values, although not statistically significant.
Fig. 6. Comparative profiles of in vivo $^{35}$S-sodium sulfate uptake by rat brain regions in response to single and co-insults of X-irradiation and methylmercuric chloride (MMC). MMC dosage given in mg/kg body weight. PBC = percent of blood concentration.
Fig. 7. Comparative profiles of in vivo $^{35}$S-sodium sulfate uptake by rat brain regions in response to single and co-
insults of X-irradiation and methylmercuric chloride (MMC). MMC dosage given in mg/kg body weight. PBC = percent of blood concentration.
Fig. 8. Comparative profiles of in vivo $^{35}$S-sodium sulfate uptake by rat skeletal muscle and liver in response to single and co-insults of X-irradiation and methylmercuric chloride (MMC). MMC dosage given in mg/kg body weight. PBC = percent of blood concentration.
TABLE 3. Comparison of 35S-sodium sulfate uptake by ten body tissues in rats treated with single and co-insults of methylmercuric chloride (MMC) and x-irradiation.

<table>
<thead>
<tr>
<th>Treatment: X-Irradiation(KR) +MMC (mg/kg)</th>
<th>Increased Uptake of 35S-Sulfate</th>
<th>Uptake of 35S-Sulfate Same as Control</th>
<th>Decreased Uptake of 35S-Sulfate</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 + 0</td>
<td>CN, CC, T, HT</td>
<td>HI, L</td>
<td>CE</td>
</tr>
<tr>
<td>5 + 0</td>
<td>CC, T</td>
<td>HT, HI, M, CE, L</td>
<td>S</td>
</tr>
<tr>
<td>0 + 8.06</td>
<td>T, M</td>
<td>I</td>
<td>CN, CC, S</td>
</tr>
<tr>
<td>0 + 4.03</td>
<td>CC</td>
<td>CN, CE, I, L, M</td>
<td>HT, CE, L, HI</td>
</tr>
<tr>
<td>10 + 8.06</td>
<td>T, CC</td>
<td>I, M, HI</td>
<td>L</td>
</tr>
<tr>
<td>10 + 4.03</td>
<td>HT, CC</td>
<td>HI, I</td>
<td>CE, L</td>
</tr>
<tr>
<td>5 + 4.03</td>
<td>T, CC</td>
<td>HI, HE, M, L</td>
<td>S</td>
</tr>
</tbody>
</table>

Abbreviations: CN - caudate nucleus, CE - cerebellum, CC - cerebral cortex HI - hippocampus, HT - hypothalamus, I - inferior colliculus, M - medulla, T - thalamus, L - liver, and S - skeletal muscle. Sig. differences are significant at p < 0.05 (Duncan's Range Test). Not sig. differences represent changes > 10% of control values, although not statistically significant.
Summary of Single Insult Effects

As indicated by Table 3, the X-radiation insult has a strong tendency to cause increased uptake of sulfate by the brain. Ten KR X-irradiation, for example, causes uptake in all brain regions monitored, except the hippocampus and the cerebellum. The uptake of sulfate by the hippocampus after treatment with 10 KR was the same as control, while the cerebellum showed only a numerical decrease in uptake. These observations agree generally with those of Nair and Roth (1964), whose basic technique for comparing tissue uptake of sulfate was used in this study. As is indicated by the work of Nair and Roth (1964), the current study shows a decrease in sulfate uptake by skeletal muscle tissue after treatment with X-radiation.

Treatment with 5 KR X-irradiation produced a similar but less pronounced pattern as that obtained with 10 KR X-irradiation.

MMC tends to have an effect opposite to that of X-irradiation upon the uptake of sulfate by brain tissues. MMC generally causes a decrease in sulfate uptake. The only statistically significant increase in sulfate uptake was indicated for the cerebral cortex after treatment with the smaller dose of MMC. This is unexplained, especially in view of the fact that the larger dosage of MMC causes a numerical decrease in sulfate uptake by the cerebral cortex.
Summary of Co-Insult Effects

The pattern most often seen after co-insult treatment is a neutralization of effects. In the hypothalamus after co-insult treatment with 10 KR and 8.06 mg MMC/Kg of body weight, a statistically clear case of cancellation of effects is seen. The single insults cause uptake changes in opposite directions; 10 KR X-irradiation resulting in a significant increase in sulfate uptake and the larger dose of MMC resulting in a significantly reduced uptake of sulfate. The co-insult treatment, however, produces no effect which is significantly different from the control value. Similar, although not significant patterns, are seen in the medulla after treatment with the largest co-insult doses of MMC and X-radiation and in the caudate nucleus after co-insult treatment with the lower radiation and the higher MMC doses.

X-irradiation tends to override the effect of MMC in a number of tissues. A case in point is the hippocampus in which sulfate uptake is significantly decreased by the larger dose of MMC, while the sulfate uptake after co-insult treatment with 10 KR and the larger dose of MMC is no different from the control or the 10 KR single insult treatment. Similar patterns may be seen in the cerebellum, hypothalamus, and medulla.
In other tissues MMC tends to override the effect of X-irradiation. A treatment of rat heads with 10 KR causes a significant increase in sulfate uptake by the caudate nucleus, while the larger dose of MMC causes a numerical decrease in uptake not significantly different from the control value. The co-insult involving 10 KR and the larger dose of MMC causes a numerical increase in sulfate uptake which does not differ significantly from the control value. Similar observations are made concerning the thalamus, inferior colliculus and cerebellum.

Part III. The Effects of Hydrogen Peroxide on MMC Induced Mortality

A. Hydrogen Peroxide Pretreatment

The following experiments are grouped by age and sex for a comparison of results. Statistical significance is measured by chi square. The data obtained by these experiments are graphed in Figs. 9 through 13.

Ninety-Day Old-Female Rats

Experiments 1 and 2

In experiment 1, 60% of the control animals (saline pretreated) died during the observation period, all deaths in this group occurring by day 17 after treatment with MMC (Fig. 9). Out of the ten rats receiving the hydrogen peroxide, one died during day 12 of the post-treatment
Fig. 9. Comparison of accumulated mortality (%) in hydrogen peroxide pretreated and non-pretreated, 90-day-old female rats after a lethal dose of methylmercuric chloride (10 mg/kg body weight).
Fig. 10. Comparison of accumulated mortality (%) in hydrogen peroxide pretreated and non-pretreated, 90-day-old female rats after a lethal dose of methylmercuric chloride (10 mg/kg body weight).
Fig. 11. Combined data comparing the accumulated mortality (%) in hydrogen peroxide pretreated and non-pretreated, 90-day-old female rats after a lethal dose of methylmercuric chloride (10 mg/kg body weight).
Accumulative Mortality

\[ \text{Accumulative Mortality} \]

\[ 60 \]

\[ 50 \]

\[ 40 \]

\[ 30 \]

\[ 20 \]

\[ 10 \]

\[ 0 \]

\[ \triangle - \text{Saline Pretreated} \]

\[ \text{○ - Hydrogen Peroxide Pretreated} \]

Days

0 2 4 6 8 10 12 14 16 18 20 28 30

Fig. 12. Comparison of accumulated mortality (%) in hydrogen peroxide pretreated and non-pretreated mature male rats after a lethal dose of methylmercuric chloride (3 mg/animal).
Fig. 13. Comparison of accumulated mortality (%) in hydrogen peroxide pretreated and non-pretreated 90-day-old male rats after a lethal dose of methylmercuric chloride (10 mg/kg body weight).
period. Although 60% is rather striking compared to 10%, the small sample size resulted in significant differences from days 7 through 11 only.

A second experiment, involving a larger sample size, showed significant differences from day 5 through the remainder of the experimental period. Eight (66%) of the 15 control animals died during the observation period, whereas only one (7%) of the peroxide pretreated animals died (Fig. 10). When the results of the two experiments are combined they give a clear picture of the protective activity of the hydrogen peroxide pretreatment (Fig. 11). All differences from day 5 to the end of the observation period are significant. Cumulative difference for the experimental period is significant at the 0.01 level.

One Hundred Eighty-Day-Old Females

Experiment 3

The mortality pattern was comparable to that exhibited by the 90-day-old females. Fifty percent of the saline pretreated animals died by day 7, after which no further deaths occurred. Since there were only 6 animals per group, the data were not statistically significant.
Large Mature Male Rats

Experiment 4

All of these adult male rats died during day 1, less than 24 hours after treatment with 10 mg MMC/kg of body weight. This experiment indicates that the per capita dose of MMC must be reduced in larger males in order to obtain a mortality pattern similar to that observed in smaller animals.

Experiment 5

As Fig. 12 indicates, 8 (67%) control animals died during the first 24 hour period, whereas only one hydrogen peroxide pretreated animal died during the 30 day observation period. No other animals died during the observation period. The difference is significant at the 0.01 level.

Ninety-Day-Old Male Rats

Experiment 6

As graphed values indicate, 84% (42) of the control animals died during the observation period, compared to 4% (2) of the rats pretreated with hydrogen peroxide (Fig. 13). All differences are significant. The cumulative difference for the experimental period is significant at the 0.001 level.

The results of the above experiment suggest rather strongly that the hydrogen peroxide pre-treatment regime
has a pronounced protective effect against MMC intoxication. That the protective mechanism can be overloaded is indicated by the reaction of large adult males to the 10 mg/kg body weight dosage. This phenomenon is further explored below.

B. Response of 90-Day-Old Female Rats to Graded Doses of MMC

As shown in Table 4 and Fig. 14 there is a rather direct mortality response on the part of 90-day-old female rats to increments in per capita dose of MMC. All three differences in accumulative mortality between peroxide pretreated and saline pretreated groups are shown significant by chi square, suggesting that the hydrogen peroxide induced protective mechanism (PIPM) functions at all dose levels tested. This experiment suggests that a dose rate between 12.5 and 15 mg/kg of body weight is sufficient to cause 100% lethality in 90-day old female rats. The experiment did not directly establish the point at which the protective mechanism is completely overloaded, but a crude least squares line suggests that a dose of 19.4 mg of MMC/kg of body weight will completely overload the PIPM in 90-day-old female rats (Fig. 15). However, the sampling was not large enough to yield good confidence limits in estimating values by using the regression line obtained. The experiment does, however, suggest that there exists a dose level which will overload the PIPM.
TABLE 4. Percentage survival of hydrogen peroxide pretreated and non-hydrogen peroxide pretreated 90-day-old female rats after graded doses of MMC. Each group consisted of 15 animals.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>MMC Dosage (mg/kg body wt.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10</td>
</tr>
<tr>
<td>Control (Saline Pretreated)</td>
<td>47</td>
</tr>
<tr>
<td>H₂O₂ Pretreated</td>
<td>93</td>
</tr>
</tbody>
</table>

C. The Effect of Hydrogen Peroxide Post-Treatment on MMC Induced Mortality

Rats were injected with a post-treatment regime of hydrogen peroxide beginning 17 hours after dosing with MMC. On the third day following MMC injection all animals were either dead or moribund and the experiment was terminated. These observations do not indicate any therapeutic value in administering hydrogen peroxide after MMC intoxication.

Part VI. Histological Analyses of Gomori Positive Cells in the Periventricular Glia of the Nucleus Arcuatus

Counts of Gomori positive cells in the nucleus arcuatus of the rat brain indicated a numerical increase in the number of P-L organelles following either the pretreatment regime with hydrogen peroxide, treatment with 800 R whole body gamma radiation, or treatment with 10 KR X-irradiation to the head (Table 5). These differences were not significant, however, according to Student's 't' test.
Fig. 14. Response of hydrogen peroxide pretreated and non-pretreated 90-day-old female rats to graded doses of methylmercuric chloride (MMC). MMC doses in mg/kg body weight.
Fig. 15. A regression line analysis of the response to graded doses of methylmercuric chloride exhibited by hydrogen peroxide pretreated and non-pretreated 90-day-old female rats.
TABLE 5. Mean number of gomori-positive glial cells in 0.01337 mm
3 of the nucleus arcuatus of the rat brain. The number of animals in each group is given in parenthesis.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>800 R Whole Body</th>
<th>10 KR X-Irradiation To Head</th>
<th>Hydrogen Peroxide Pretreatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>139.4</td>
<td>164.3</td>
<td>146.5</td>
<td>183.4</td>
</tr>
<tr>
<td>(5)</td>
<td>(4)</td>
<td>(4)</td>
<td>(5)</td>
<td></td>
</tr>
</tbody>
</table>
DISCUSSION

Rat Mortality Following Single and Co-Insults with MMC and X-Irradiation

The mortality response obtained with the MMC treated rats is largely as expected from a survey of the literature. However, the large kill (70% male and 90% female) is of interest in that it suggests a high degree of variability in rat mortality response to MMC when compared to the $LD_{50}$ data available. The dose rate (10 mg/kg body weight) given in these experiments is near the $LD_{50(30)}$ dose (10.1 mg/kg body weight) proposed by Hoskins (1972) for MMC, but considerably less than the 40 mg/kg suggested by Ulfvarson (1969) for methylmercury hydroxide. Although the anion is not generally considered important relative to methylmercury toxicity (Klein et al., 1972), a comparison of the results of this study, the $LD_{50(30)}$ dose rate reported for MMC, and the $LD_{50(30)}$ response to methylmercuric hydroxide indicated by Ulfvarson (1969) suggests that the role of the anion be reconsidered.

The mortality response of the rats to the co-insult treatment is intermediate to their responses exhibited after the single insult treatments, indicating a neutralization pattern in which the X-irradiation effect appears to be
overriding the mortality effect of MMC. If a more rapid influx of MMC into the brain means earlier neurological damage and death, an assumption involved in the construction of this experiment, then it may be concluded from the results that X-irradiation has not increased the permeability of the BBB to MMC. In fact, the implication is that the accessibility of the MMC to the neurons has been decreased by X-irradiation treatment, providing some protection against the mortality effects of MMC.

Comparison of the graphs of Figs. 4 and 5 indicates a greater protective effect for male rats. This difference, however, is probably not a result of sexual constitution. It is more likely a dose related phenomenon, since the 2.75 mg per male rat dose represented an average dose rate of 8.1 mg/kg body weight, whereas the females received 10 mg/kg body weight.

Effects of Single and Co-Insults of MMC and X-Irradiation Upon the Uptake of Sulfur-35 Sodium Sulfate by Various Brain Areas

Insults of both X-irradiation and MMC demonstrate the presence of a BBB acting against radioactive sulfate, irradiation disrupting and MMC enhancing the barrier effect. The 10 KR single insult, for the most part, corroborates the work of Nair and Roth (1964). They demonstrated a BBB against sulfate and showed that increased brain-uptake of
sulfate occurs as a result of lesions caused by X-irradiation. The specific site of the lesions was not shown.

The current study demonstrated that the BBB to sulfate may be enhanced by the intraperitoneal administration of MMC. This finding is consistent with the observations of Steinwall and Olsson (1969) that methylmercury dicyandiamide given at a dose rate of 200 mg/kg body weight resulted in a decreased uptake of $^{75}$Se-selenomethionine by the brain. That the BBB is complex is indicated by the fact that these investigators also found an increased permeability of the cerebral blood vessels to Evans blue dye. They obtained the same results with $^{75}$Se-selenomethionine and Evans blue after treatment with the inorganic mercuric chloride. This suggests that the greater toxicity of organic mercurials may be a result of phenomena other than BBB damage.

Although the mechanism for transporting sulfate across the BBB is not known, there are indications that in a number of both procaryotic and eucaryotic cells sulfate-uptake is accomplished by active transport (McCready, 1974). Since, in general, mercurials are powerful but unspecific enzyme inhibitors (Clarkson, 1972), it seems likely that reduced sulfate-uptake in the rat brain results from MMC poisoning of an active transport enzyme system.

The specific locality of BBB lesions caused by X-irradiation is not known. However, if the capillary
endothelium with its tight intercellular junctions is the major anatomical component of the BBB (Holman, 1972), then it is likely that the radiation-induced lesion occurs somewhere in that structure. Maximum disruption has been shown to occur at 48 hours after irradiation (Nair and Roth, 1964; Nair et al., 1964), the delayed action suggesting that products such as lipoperoxides resulting from the ionization activities of radiation are involved in causing the barrier lesions. Possibly damage is done to either the intercellular tight junction or to some other component of the cell membrane. Evidence for in vitro radiation damage to cancer cell membranes, through the vehicle of H$_2$O$_2$, has been reported (Rosenberg and Matthews, 1972).

Whatever the operative mechanisms involved in the BBB altering abilities of X-irradiation and MMC, they tend to neutralize or counteract each other when administered as a co-insult. This suggests that they are attacking different transport pathways. It seems possible that X-irradiation may be causing anatomical leaks, while MMC is inhibiting an enzyme system mediating the transport of sulfate. For example, consider the cancellation of effects seen in sulfate uptake by the hypothalamus. The significant increase in sulfate uptake may result from damage to the tight junctions, whereas the significant decrease in uptake after
MMC treatment may be due to poisoning of the active transport system.

Hupp et al. (1974), in assaying for brain serotonin and norepinephrine after single and co-insult treatments with X-irradiation and MMC, found indications that the effect of the single insults upon neurotransmitter levels tended to be neutralized in the co-insult treatment. Neutralization, then, appears to be a common pattern of interaction between MMC and X-irradiation, having been observed in connection with brain neurotransmitter levels, with the uptake of sulfate by various brain regions, and with the mortality study above.

The Effect of Hydrogen Peroxide Pretreatment on MMC-Induced Mortality

The apparent neutralization effect, inferred from the mortality response study, indicates that X-irradiation affords a measure of protection against MMC toxicity. This observation stimulated the idea that if the part of the radiation effect resulting in the protection could be mimicked by a less severe insult, the protective mechanism could be investigated further.

There is clear evidence that lipid peroxidation occurs in irradiated tissue homogenates (Dawes and Wills, 1972). Ahlers et al. (1972) have shown an accumulation of liperoxides in rat liver after 1.5 KR whole body X-irradiation.
There is some \textit{in vivo} evidence that radiation-induced peroxidation is responsible for cell damage (Ershoff and Steers, 1960). Rosenberg and Matthews (1972) have done work which shows that radiation-produced hydrogen peroxide can alter permeability of murine lymphoma cells \textit{in vitro}.

The neutralization effect observed in the mortality response of rats to the co-insult with MMC and X-irradiation suggests that X-irradiation affords a measure of protection against the toxicity of MMC. Based upon this suggestion and upon evidence given in the literature that radiation-induced peroxides are responsible for the secondary effects of ionizing radiation, hydrogen peroxide was chosen for a pretreatment regime designed to simulate radiation effects and to trigger the proposed protective mechanism.

That a peroxide-induced protective mechanism (PIPM) is operable in the rat is clearly demonstrated by results obtained when peroxide pretreated and non-peroxide pretreated rats are subjected to a lethal dose of MMC. The experimental results show significant differences which are repeatable in both sexes and in different age groups. A size and/or age factor appears to be involved, however, since mature male rats, weighing from 372 g to 427 g, were not protected against the 10 mg/kg dose rate from which 90-day-old males and females, weighing 235 g to 301 g, were well protected. The literature generally indicates a
greater susceptibility of embryonic and juvenile individuals to mercurialism (Pierce, 1972; Kojima and Fujita, 1973). Furthermore, since the central nervous system appears to be the primary target of MMC and since increase in the total mass of the central nervous system represents only a small fraction of rat weight increase after 90 days of age, it appears more likely that the factor mentioned above is one of weight and not age. The older and larger males did show the PIPM intact when treated with a smaller dosage of MMC.

Although the manner in which the PIPM operates is not understood, there are some facts which suggest an hypothesis for the functioning of this mechanism. First, it is unlikely that any type of direct chemical relationship exists between $\text{H}_2\text{O}_2$ and MMC. Since MMC is administered 48 hours after the last dose of peroxide, it appears likely that catalase activity will have considerably reduced the peroxide level by the time MMC is administered. If some type of MMC-hydrogen peroxide complex is responsible for the PIPM, it is reasonable to expect that some protection might be achieved with an early hydrogen peroxide post-treatment. Complexing agents such as D-penicillamine, British antilewisite and mercaptodextran are used as therapeutic agents in treating mercurial toxicity because they contain sulfhydryl groups which are able to complex with various forms of
mercury and facilitate their removal by excretion (Aaseth, 1973; Aposhian, 1958; Ishihara, 1974). When hydrogen peroxide is injected intraperitoneally 17 hours after an acute lethal dose of MMC, no reduction in mortality is achieved. The hypothesis that the protection afforded by the hydrogen peroxide pretreatment regime results from a MMC-hydrogen peroxide complex is therefore rejected. If this is the case, then one must look for a more indirect protective mechanism.

At this point the peroxisome-like system of organelles (P-L organelles) demonstrated in the periventricular glia by Srebro et al. (1970) becomes extremely attractive as a possible response mechanism for the protection against MMC toxicity. The presence of such a system has been described and shown to be rich in thiol and disulfide groups (Srebro and Cichoki, 1971; Srebro, 1970; Noda, 1959). Since the P-L organelles have been shown to be strongly positive for peroxidase and to proliferate after X-irradiation, it appears likely that they function primarily in the decomposition of organic peroxides. Because of their perivascular localization and high concentration of sulfydryl and disulfide groups, the P-L organelles of the glia appear to be suited for an important role of protection against heavy metals. There is good evidence for such a protective phenomenon in the BBB studies of Wislocki and Leduc (1952) in which they
fed silver nitrate to rats and found a high concentration of the metal in glia. Breeman and Clemente (1954) also observed that silver deposition in the BBB did not occur beyond the glia cell membranes adjacent to the perivascular spaces. This phenomenon was demonstrated in the medulla, cerebrum and cerebellum. It is also interesting to note that Scott (1967), in attempting to demonstrate BBB damage by low levels of radiation, found no increase in brain uptake of silver. Electronmicroscopic analysis showed the silver to be deposited within BBB structures. All of these reports and the PIPM demonstrated in this study tend to favor the hypothesis of Srebro et al. (1972).

Ganther et al. (1967) found that low concentrations of selenium in the diets of quail and rats reduced methylmercury toxicity in these organisms. Relationships between the protective effects of selenium and hydrogen peroxide are not clear at present, though the selenium has afforded protection when consumed in small quantities in the diet along with methylmercury (Potter and Matrone, 1974; Stoewsand, 1974; Ganther et al., 1967) and when given as a pretreatment (Stoewsand, 1974). Hydrogen peroxide protection has been demonstrated only with a pretreatment regime. More work is needed, however, to determine the minimum dose of peroxide which will give measurable protection. Also, more investigation is required to determine the temporal limits
between the administration of peroxide and MMC within which protection will be provided. It is interesting to note that the following observations made by investigators who have worked with selenium bear some resemblance to concepts involved in the proposed PIPM:

(1) El Bergearmi et al. (1973) observed that 6 ppm of selenium and less offered protection against 20 ppm methylmercury. Potter and Matrone (1974) felt that such low molar ratios of selenium make it unlikely that simple, equimolar mercury-selenium interactions are responsible for the protective mechanism.

(2) A number of investigators felt "that the selenium protection must involve a change in the distribution of mercury on a subcellular level" (Potter and Matrone, 1974; Stillings et al., 1972; Ganther et al., 1972).

The observations above indicate that the protective mechanism engaged by selenium is somewhat different from the MMC-SH complexing activities of the therapeutic drugs mentioned earlier. In fact, Stillings et al. (1972) have concluded that increased mercury excretion does not occur after treatment with selenium as is the case with the therapeutic agents. The interpretation that selenium protection must involve a change in mercury distribution at the subcellular level is in agreement with the hypothesis that methylmercury becomes localized in the P-L organelle system.
Response of Peroxide and Non-Peroxide Pretreated 90-Day-Old Female Rats to Graded Doses of MMC

If the P-L organelles described in the literature do, in fact, comprise the PIPM or a portion of it, it is reasonable to assume that the saturability of this system is dependent upon the quantity of sulfur groups available for bonding with MMC. It is also reasonable to conclude that when the bonding sites are filled, excess MMC will move through the glia and into the neurons, causing neurological damage. The results of the graded dose study (Fig. 15) is consistent with this hypothetical scheme. As the dosage of MMC in increased, fewer animals are protected from mortality.

Also, if the proliferated P-L organelle system can give dramatic protection against MMC intoxication, it may be inferred that the normal, unproliferated P-L organelle system offers a measure of protection from background quantities of MMC. Individual differences in this system may explain the variability in mercurial symptoms which have been observed at similar blood-mercury levels (Hammond, 1971). Rustam and Hamdi (1974) found that while members of the same family had equally high blood-mercury levels, some of them showed mild symptoms, while others were severely poisoned and even died. This disparity between blood-mercury levels and toxic symptoms appears to be particularly important in view of the observations that brain mercury
content has been rather closely correlated with the onset of symptoms. Hirota (1969) found that the methylmercury content in the brain was slightly higher in rats with nervous manifestations than in those without them. Suzuki and Miyama (1971) correlated a brain concentration of 10 μg Hg/g in rats with the specific symptom of the inability to maintain the head in a horizontal position when being suspended by the tail. These findings suggest rather strongly that factors other than blood-mercury concentration may be involved in the passage of MMC into the brain. The role of the P-L organelle system, as presented in this paper, could account for the lack of correlation between blood-mercury concentration and observable symptoms.

That the PIPM can be overloaded with MMC is indicated not only in the graded dose study but also in two earlier experiments. When large mature male rats were given MMC at a dose rate of 10 mg/kg body weight, all animals died within 24 hours. However, when the dose rate was reduced to 3 mg per animal the PIPM was detected. Still another observation which appears relevant to the concept of over-loading the PIPM results from a comparison of the response curves of the co-insulted rats shown in Figs. 4 and 5. The co-insulted animals represented in Fig. 4 appear to be better protected than those shown in Fig. 5. However,
the difference probably results from the fact that the animals represented in Fig. 5 received a somewhat heavier dose of MMC.

Histological Analysis of the P-L Organelle System of the Nucleus Arcuatus After Treatment with 800 R Whole-Body Gamma Radiation, 10 KR Head X-Radiation and Hydrogen Peroxide Pretreatment

The radiation treatment administered in these experiments were designed to replicate the work of Srebro et al. (1970 and 1972) in which they showed a significant proliferation of glia cells containing P-L organelles after acute radiation treatment. The experiment involving the hydrogen peroxide pretreatment regime was designed to determine if the treatment which protects rats against MMC mortality will also cause a proliferation of the P-L organelle system. Numerical increases of P-L organelle-containing glia cells were shown after each of the three insults (Table 5). Although these data tend to agree with the work of Srebro et al., the lack of statistically significant differences detracts from the very strong argument which might otherwise have been made. However, in the light of other evidence presented in this paper and in the literature, it seems reasonable to consider the numerical trend observed in these experiments as an argument for the P-L organelle proliferation hypothesis. An attempt was made to follow the methodology of Srebro et al. (1970), and the difference
in statistical significance is unexplained. It should be pointed out, however, that the counting technique, although performed in the blind, does involve some difficulties. Also, a simple count of the Gomori positive glia cells does not take into consideration the greater density of Gomori positive material which some cells may possess. This factor, which will undoubtedly influence the quantity of mercury to be bound, is not considered in the statistical analysis.

A considerable degree of individual variability may exist in the P-L organelle system. By using relatively small numbers of animals, as is dictated by the time-consuming nature of these histological studies, it seems highly probable that skewed samples of animals may be obtained, especially when litter mates are used. This may explain the apparent discrepancy between the current findings and those of Srebro (1970, 1972). As was pointed out earlier, individual differences in the P-L organelle system may account for varying responses to blood-mercury concentration. Such individual differences may also account for the lack of complete group protection after peroxide pretreatment. For example, the 4% mortality shown for peroxide pretreated animals in Fig. 13 may be a result of such differences.

A survey of the rat brain (Srebro, 1969) indicates that the P-L organelle-containing glial cells are rather
widespread in the rat brain. Therefore, what appears as a very slight increase in Gomori positive material in the small sample area investigated by light microscopy may represent a significant increase toxicologically. That is, what may appear as a very small proliferation of P-L organelles in a 0.01mm$^3$ section of rat brain may represent an increase in available sulfur groups able to bind a relatively large dose of MMC.

**Summary of Data Supporting the P-L Organelle System (Srebro, 1972) as the PIPM**

1. P-L organelle containing glia have been demonstrated in the literature (Noda, 1959; Srebro, 1970; Srebro and Cichoki, 1971) and observed during this study.

2. The P-L organelle containing glial cells have been shown to proliferate after acute X-irradiation (Srebro, 1970). Srebro et al. (1972) also showed these cells to be rich in thiol and disulfide groups and to be positive for peroxidase.

3. In the present study, histological analyses of rat brain tissue have shown average numerical increases in Gomori positive glia after 800 R whole-body irradiation, 10 KR head irradiation and hydrogen peroxide pretreatment.

4. Silver localization has been observed in the perivascular glia (Wislocki and Leduc, 1952; Breeman and Clemente, 1954).

5. Mercury exhibits a slower turn-over rate in the brain than in other organs. This suggests that it is
strongly bound. Ulfvarson (1969b) found that after i.v. injection of methylmercuric hydroxide, cerebellar mercury peaked slowly and then declined slowly.

6. It has been observed that methylmercury thioacetenide symptoms may be delayed even though analysis of brain tissue indicates a peak concentration of mercury (Yoshino, 1966). When a sample of brain tissue is taken and homogenized for analysis, the exact locality of the mercury in the intact brain cells cannot be determined. It is possible that MMC is bound in the P-L organelles and is, therefore, not causing neuronal damage.

7. Variability in symptomatic responses to blood-mercury concentration suggests that some MMC exclusionary mechanism is operating between the blood and the neurons (Hammond, 1971).

8. The high lipid solubility of MMC suggests that it freely diffuses through membranes of the BBB (Hughes, 1954; Holman, 1972).

9. In the present study a post-treatment of MMC treated rats with hydrogen peroxide was without therapeutic value. This suggests that there is no direct action between hydrogen peroxide and MMC, but that the peroxide pretreatment stimulates a responsive mechanism.

10. Observations in the present study indicate that the PIPM can be saturated with MMC.
11. The fact that the hydrogen peroxide pretreatment regime provides protection against a heavy metal is a strong argument for Srebro's hypothesis. Although the experiment was not originally designed to test his hypothesis, in retrospect it would have been a logical experiment to perform for that purpose.

12. The apparent protection against MMC stimulated by X-irradiation also strongly favors the hypothesis of Srebro.

Hypothetical Functioning of the PIPM

Based upon the supportive data above, which were obtained from the literature and from experimental results and observations in the present study, the following hypothetical sequence emerges.

1. The MMC cation \((\text{CH}_3\text{Hg}^+)\) diffuses from the blood into the endothelium where it may alter endothelial permeability to other blood-borne substances such as \(^{35}\text{S}\)-sodium sulfate and \(^{75}\text{Se}\)-selenomethionine. These alterations in permeability may result from the poisoning of enzymes involved in transport.

2. MMC, being fat soluble, passes rather easily through the endothelium, the basement membrane, and into the glia. Since MMC is a non-specific inhibitor, it will react with available sulfur groups as it moves through the BBB.
3. Upon reaching the cytoplasm of the perivascular glia, MMC encounters a system of organelles especially rich in peroxidase and thiol and disulfide groups. These substances are much more highly concentrated in the P-L organelles than in the surrounding cytoplasm. Since the reactive groups are localized in the P-L organelles, the MMC cations in reacting with them also become localized in these organelles. Mercapitides are formed, detaining MMC that would otherwise diffuse into the neurons.

4. The larger the number of P-L organelles and the greater their content, the larger is the quantity of MMC cations which will become bound. Individual differences resulting from different genotypes or from environmental stresses may affect the number of the P-L organelles and the density of their contents.

5. As blood-MMC concentration increases, the P-L organelle system becomes increasingly burdened with bound methylmercury. If the blood-MMC level continues to rise after the P-L organelle system has reached its methylmercury binding capacity, the excess MMC will diffuse into the neurons, resulting in neurological damage.

6. In case of extraordinary insults, such as large doses of X-irradiation or injected hydrogen peroxide, the P-L organelle system proliferates in response to the elevated peroxide level in the blood. This compensatory
proliferation of P-L organelles provides a fortuitous barricade against dose levels of MMC which would normally overload the P-L organelle system and result in a net movement of MMC into the neurons, causing severe symptoms and/or death.

7. Blood-MMC levels begin dropping soon after an acute dose of MMC is administered, the drop resulting from both movement of MMC into organs and from excretion. Brain tissue mercury remains high, at least partially due to the mercaptide formed in the P-L organelles. After a period of time the blood-MMC concentration drops below the brain-MMC concentration and the diffusion gradient favors a movement of mercury into the blood. The sulfur component of the mercaptide will eventually be reduced and MMC will diffuse into the blood and be excreted.
SUMMARY

1. An experiment, designed and performed to investigate the interaction of large acute doses of MMC and X-irradiation upon mortality in male and female rats, demonstrated an effect which was intermediate to the single insult effects. The early X-irradiation effects (days 1-7) tended to override the effect of MMC, offering a measure of protection. The same pattern was observed for both males and females. Assuming that more rapid entry of MMC into the brain would cause more rapid neurological damage and earlier death, the experiment was also designed to ask if 10 KR would increase the speed with which MMC traverses the BBB. The answer was negative.

2. The second major part of the study was designed to investigate the interaction of MMC and 10 KR X-irradiation relative to the uptake of the indicator $^{35}$S-sodium sulfate by various regions of the brain. Several general trends emerged. First, as previously reported in the literature, acute doses of X-irradiation tend to cause an increase in uptake of sulfate by the brain areas examined. Second, MMC was found to have an opposite effect upon the passage of sulfate across the BBB, decreasing its rate of passage. Third, the general interaction pattern shown by the co-insult was neutralization. In a few tissues a net cancellation
of effects was seen. However, more often an overriding of effects by one or the other insult was seen. Neutralization appears to be a typical pattern for the co-insult with MMC and X-irradiation occurring in the sulfate uptake study and in the mortality study.

3. The third major part of the study arose directly from the results obtained in the mortality experiments of the first part. If X-irradiation stimulated a protective mechanism against MMC, then a less severe insult which mimicked X-irradiation effects might also be able to stimulate the mechanism. A hydrogen peroxide pretreatment regime was designed which caused a highly significant reduction in mortality of MMC treated rats. The protective mechanism was stimulated in different age groups of both sexes.

4. A hydrogen peroxide post-treatment was found to be non-therapeutic.

5. A study of mortality response to graded doses of MMC indicated that female rats respond rather directly to dose rate increments. The experiment also suggested that the PIPM may be saturated with MMC.

6. Histological analyses of tissue from the nucleus arcuatus of the rat brain demonstrated Gomori positive glia which contain the peroxisome-like organelles (P-L organelles). Treatment of rats with 800 R whole body gamma irradiation,
10 KR head X-irradiation and the hydrogen peroxide pre-treatment regime resulted in numerical increases of the Gomori positive glia. Although the differences did not show statistical significance, the proliferation pattern is consistent with reports in the literature.

7. Based upon reports from the literature and the results of this study, a hypothetical scheme is described for the mechanism which protects against MMC.
LITERATURE CITED


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