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# METHYL MERCURY AND THE METABOLIC RESPONSES OF BRAIN TISSUE


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# METHYL MERCURY AND THE METABOLIC RESPONSES OF BRAIN TISSUE

by

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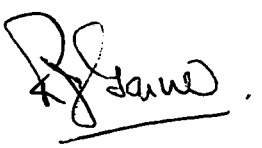
## FOREWORD

Man and his environment must be protected from the adverse effects of pesticides, radiation, noise and other forms of pollution, and the unwise management of solid waste. Efforts to protect the environment require a focus that recognizes the interplay between the components of our physical environment--air, water, and land. In Cincinnati, the Environmental Research Center possesses this multidisciplinary focus through programs engaged in

- studies on the effects of environmental contaminants on man and the biosphere, and
- a search for ways to prevent contamination and to recycle valuable resources.

The Health Effects Research Laboratory conducts studies to identify environmental contaminants singly or in combination, discern their relationships, and to detect, define, and quantify their health and economic effects utilizing appropriate clinical, epidemiological, toxicological, and socio-economic assessment methodologies.

Some years ago, methyl mercury was found to be responsible for the Minimata Bay incident and, more recently, a similar tragedy in Iraq. Damage to the central nervous system typifies the effects of methyl mercury, somewhat in contrast to the effects produced by inorganic salts of mercury. This study was initiated to determine the minimal ingestion of methyl mercury required to produce subtle alterations in brain metabolism.



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ABSTRACT

Weanling, Sprague-Dawley rats have been exposed to methyl mercuric chloride (concentrations from 0.01 to 10.0 mg/liter) in their drinking water. At 10 mg/liter the animals exhibited neurological symptoms typical of methyl mercury. Also, in this group a considerable decrease in growth occurred which was associated with a decreased consumption of food.

Responses of the respiratory intermediates to stimulation were found to be altered in cerebral cortex slices taken from exposed animals. Effects on tissue pyridine nucleotides predominated. An enhancement of the rate of pyridine nucleotide reduction by electrical stimulation was observed at 0.1 mg/liter. This rate progressively decreased at higher dose levels. Reoxidation of reduced pyridine nucleotide was also inhibited at 0.1 mg/liter at both 90 and 180 days of exposure.

Potassium stimulated aerobic glycolysis was found to be enhanced in its initial stages at 0.10 mg/liter of methyl mercuric chloride in the drinking water but progressively declined at 1.0 and 10 mg/liter. A close parallel was observed between the time constant of pyridine nucleotide oxidation following electrical stimulation and the "responsiveness" of the aerobic glycolytic rate to stimulation by potassium. These results suggest an initial defect in the oxidation of cytoplasmic NADH which progresses to loss of metabolic control of cytoplasmic oxidations.

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# INTRODUCTION

The occurrence of trace elements in drinking water supplies has been well documented (McCABE, et al., 1970). Many of the metals are present in raw water and others are, to a greater or lesser extent, from the distribution systems. Since several toxic metals have been associated with the development of disease states which take several months to many years to become clinically evident, it is imperative that their concentrations be limited to safe levels in drinking water. Although drinking water standards have been established for many of these chemicals, there are many questions as to the adequacy of the data available for such purposes, particularly in relationship to the long-term development of certain types of disease (INDRAPRASIT, et al., 1974).

Some metals are known to produce alterations in nervous system function when chronically ingested. The clinical syndromes which arise are poorly defined and often confused with infectious disease (JAIN, et.al., 1970). Metals which are known to produce central nervous system toxicity include lead, manganese, thallium, copper, lithium, organic mercury and tin. Other metals known to affect the central nervous system when locally applied, but for which little data exists in terms of the oral route of exposure are cobalt, cadmium, aluminum and inorganic mercury. Some of these elements have been implicated or suggested as possible etiological factors in the development of, or their chronic toxicity resembles, certain chronic diseases of the nervous system, most notably Parkinson's disease (COTZIAS, et al., 1971), multiple sclerosis (CAMPBELL, et al., 1950), amyotrophic lateral sclerosis (PETKAU, et al., 1974), and hyperkinesis (DAVID, et al., 1972).

Detection of marginal damage to the central nervous system is extremely difficult because of the organ's diverse function. There are four basic ways of approaching the problem experimentally: by using the basic tenets and techniques of neurophysiology, neurochemistry, behavioral research or neuropathology. The approach of each of these disciplines has basic advantages and disadvantages. Neuropathology is perhaps the most widely applicable, but suffers from the fact that it is primarily a study of structure and lacks sensitivity in terms of function. Behavioral research addresses itself in a very general way to function, but is most often criticized on the basis of species specificity of much animal behavior. Neurophysiology and neurochemistry both address themselves specifically at function (at different levels of biological organization), but often suffer from being too specific in their approach for wide application. Our own efforts have combined certain elements of both neurochemistry and neurophysiology. We have attempted to avoid falling into

the trap of randomly examining very specific functions of the nervous system and have developed an approach we feel has quite general application to the study of chemically-induced alterations in the central nervous system. All questions concerning the effects of heavy metals on brain function will not be answered with our techniques; it would be presumptious for us to pretend that they will. However, their application will yield sensitive measurement of the alterations produced and form a framework for more specific pursuit of a particular chemicals effect within the central nervous system.

Because of the central role energy plays in the operation of any biological system, we have chosen to use parameters of energy and intermediary metabolism to assess damage to brain. The functional activities of tissues are universally dependent upon a constant availability of cellular energy. Conversely, each functional activity of a tissue will have a discrete kinetic input to the overall energy metabolism of that tissue. The functionalities of a given tissue can be activated by various means. If the stimulus were selective enough, the intactness of the function could be indirectly judged by its kinetic impact upon energy metabolism. Brain tissue is excitable and functions by utilizing ionic movements to conduct electrical signals. For this reason we employ electrical pulses and ionic stimulation to evoke changes in "functional" state within the tissue. The change in state is accompanied by increases in the rate of energy metabolism (McILWAIN, 1966). Of course, in making measurements of energy metabolism, direct effects of a chemical will also become evident.

The present report represents the results of our first application of these techniques. We have examined the effects of methyl mercury, administered in vivo, on the metabolic responses of brain tissue to in vitro stimulation. Several presentations and publications have been made concerning the background data validating the experimental techniques used (CUMMINS, 1971; CUMMINS and BULL, 1971; BULL and LUTKENHOFF, 1973; BULL and CUMMINS, 1973; BULL and O'NEILL, 1975; BULL, 1975). The basic form of the responses measured has also been confirmed independently (LIPTON, 1973).

Short-term experiments have shown that metabolic responses of cerebral cortex slices to stimulation were altered when animals were exposed to low intraperitoneal doses of methyl mercuric chloride (BULL and LUTKENHOFF, 1975). The observed changes centered around the oxidation and reduction of cellular electron transport intermediates in response to electrical pulses or to elevated potassium concentrations. Cerebral function is known to be highly dependent upon the provision of adequate energy in the form of ATP (RIDGE, 1971). High doses of methyl mercury have been previously shown to decrease the ATP/ADP ratio, in vivo (PATERSON and USHER, 1971). Consequently, we decided to determine if similar changes in the relationship of energy metabolism to function could be observed with long-term oral exposures to methyl mercury.

## CONCLUSIONS

Alkyl mercurials differ in their effects on animals from other forms of mercury in that neurological changes predominate the clinical picture. Mercury contamination of drinking water supplies appears to be quite uncommon. However, in considering the possibility of a mercury-contaminated water supply, one would have to assume that some considerable portion of the mercury in solution would be in the methylated form unless shown otherwise. Inorganic mercury tends to be tightly complexed in bottom sediments of reservoirs (GAVIS and FERGUSON, 1972). Under appropriate conditions, bacterial conversion of inorganic mercury to methyl mercury has been shown to occur and serves as a means of mobilizing mercury (JENSEN and JERNELOV, 1969). By virtue of their almost complete absorption in the gastrointestinal tract, the alkyl mercurials also present the greatest danger in terms of their toxicity. It is appropriate, therefore, that the drinking water standard for mercury be based upon the toxic effects of methyl mercury.

Short-term experiments (BULL and LUTKENHOFF, 1975) revealed complex changes in the metabolic responses of cerebral cortex slices taken from animals exposed to methyl mercury. Effects of methyl mercury tended to primarily involve tissue pyridine nucleotide (NAD(P)) responses to stimulation. Changes were also observed in the cytochrome chain, but these appeared secondary to the NAD(P)H responses. Very low doses of methyl mercury (as the chloride, 0.01 to 0.1 mg/Kg per day for 14 days) substantially increased the activation of substrate oxidation as measured by an enhancement of NAD(P) reduction with electrical and/or potassium ion stimulation. In this dose range, small but measurable increases in the mercury content of the cerebral cortex could be measured ($0.026 \pm 0.004$ ug/g at 0.02 mg/Kg/day vs. $0.005 \pm 0.002$ ug/g in control animals). Progressive increases in methyl mercury dosage lead to increased mercury content of the cerebral cortex ($0.78 \pm .06$ and $9.9 \pm 0.9$ ug/g with 0.2 and 2.0 mg/Kg/day, respectively), but began to progressively inhibit the activation of substrate oxidation produced by electrical stimulation. At the highest dose (2.0 mg/Kg/day), this could be correlated with an inhibition of potassium-stimulated aerobic glycolysis. In addition, at doses exceeding 0.05 mg/Kg/day, measurable increases in the duration of the NAD(P) response indicated some inhibition of NAD(P)H reoxidation. Only at the highest dose were there any substantial losses in body weight or signs of definite neurological impairment.

Long-term oral exposures to methyl mercury confirmed the results seen with short-term exposures. Weanling rats were exposed to concentrations of methyl mercuric chloride of 0.01, 0.1, 1.0 and 10 mg/liter in their

drinking water for six months. Animals in the highest dose range showed substantial inhibition of growth (measured as body weight gained over the experimental period) which was associated with a decrease in the consumption of food. Again this was the only dose at which substantial neurological impairment was observed. Enhancement of the stimulated reduction of tissue (NAD(P)) was observed at low doses (0.1 and 1.0 mg/liter) followed by inhibition at higher doses (10 mg/liter) with 90 days of exposure. At 180 days, the enhancement of the responses was no longer observed and significant inhibition of NAD(P) reduction was observed at both 1.0 and 10 mg/liter methyl mercuric chloride. These results also coincided with an inhibition of potassium-stimulated aerobic glycolysis which had been reported by earlier investigators (YOSHINO, et al., 1966). Inhibition of NAD(P)H reoxidation following electrical stimulation was apparent at 0.1 mg/liter at both 90 and 180 days, but appeared to become less prominent at higher doses, perhaps as a result of the inhibition of NAD(P) reduction. These results can all be reconciled with previous observations (PATERSON and USHER, 1971; SALVATERRA, et al., 1973) dealing with in vivo changes in the concentrations of the adenine nucleotides and glycolytic intermediates produced by low acute intraperitoneal doses of methyl mercury.

From this work it can be concluded that methyl mercury induces substantial changes in the organization and control of energy metabolism of brain as it relates to changes in functional activity. This occurs at exposure levels far below those required to produce overt toxicity. If sufficient time and personnel had been available, it may have been possible to apply more sophisticated behavioral parameters and functional deficits in the whole animal may have been apparent at lower dosage levels. However, the deficits in metabolism are more than sufficient to explain the neurological changes seen with the highest doses.

## RECOMMENDATIONS

In terms of the drinking water standard for mercury, we can make the following generalizations:

1. Intake of mercury was not constant with time over the duration of the long-term experiments, because animals drank less than half the amount of water/unit of body weight after they matured. Taking, however, the minimum intake/unit of body weight, the doses of methyl mercuric chloride to the animal consuming 0.01, 0.1, 1.0 and 10 mg/liter correspond closely to 0.001, 0.01, 0.1 and 1 mg/Kg/day.

2. Inhibition of NAD(P)H reoxidation was observed at a dose of 0.01 mg/Kg/day. Reduction of NAD(P) by electrical stimulation was enhanced at this dose in 90 days and slightly, but not significantly, inhibited at 180 days of exposure.

3. No significant differences were observed with doses in the drinking water corresponding to 0.001 mg/Kg/day. To this, however, must be added approximately another 0.001 mg/Kg/day as being derived from the standard lab chow.

4. Extrapolating these data to man would yield a minimum effect level of:

   $$70 \text{ Kg} \times 0.01 \text{ mg/Kg/day} = 0.7 \text{ mg/day}$$

   and a "no-effect" level of:

   $$70 \text{ Kg} \times 0.002 \text{ mg/Kg/day} = 0.14 \text{ mg/day}.$$

FRIBERG (1971) estimated a "no-effect" dose rate in man of 0.3 mg/day. This figure is somewhat less than a previous estimate of BERGLUND and BERLIN (1969) of 0.6 to 1.0 mg/day. To the extent that our data may be extrapolated to man, it is clear that our estimate is more compatible with that of FRIBERG. It was on this estimate that the proposed drinking water standard was established. The 0.002 mg/liter figure proposed would lead to a maximum intake of 4 ug/day, assuming a 2-liter daily consumption of water. If water were the sole source of mercury, this allows a more than adequate safety factor of 75. This safety factor is lowered if dietary sources are considered. If the maximum "safe" dose recommended by FRIBERG, of 30 ug is not exceeded from dietary sources, it is doubtful that an additional contribution of 4 ug from drinking water would significantly add to the overall risk.

## METHODS

Sixty, weanling, Sprague-Dawley rats were divided into 5 groups of 12 animals each. Methyl mercuric chloride was added to the drinking water at levels of 0.01, 0.10, 1.0 and 10 mg/liter in the first four groups, respectively, and the fifth group served as control. Double-distilled (glass) water was utilized for all groups. The rats were housed three to a cage and water consumption was taken daily on a cage basis. Corrections for spillage were made for the first 80 days of the study by catching spilled water in a screened jar containing a film of paraffin oil over the collected water to prevent evaporation. These jars were periodically weighed and the accumulated water subtracted from the water consumption of the particular cage of rats. This exercise corrected an error of only 2-3% and was discontinued for the remainder of the study. Food consumption was measured twice weekly and the animals were weighed on a weekly basis for the first 60 days of the study and at longer intervals (usually 2 weeks) for the remainder of the experiment. On the basis of the periodic measurements of body weight and the daily water consumption an average dose per unit of body weight was derived for each group exposed to methyl mercury. These data are found in Fig. 1. As can be seen, the dose per unit weight decreased with time. This was the result of a decrease in water consumption per unit of body weight as the animals increased in size. Because the nature of this curve precludes the use of any meaningful average dose for the entire experimental period we will refer to doses in terms of the concentration added to the drinking water. Food was supplied ad lib. and was the standard laboratory chow used in our laboratory (Rockland). The chow was assayed for mercury by atomic absorption spectrophotometry and was found to contain $<0.006 \pm 0.002$ ug/g (SEM). Using this figure, it was determined that this source of mercury contributed significantly to the total intake of mercury only at the 0.01 mg/liter level. This amounted to a dose 30% greater than indicated for this group in Figure 1. This group may then be calculated to be receiving a total mercury dose averaging approximately four times the dose of mercury that the control group was receiving from their diet.

Six animals from each group were sacrificed after 90 days of exposure and the remainder were sacrificed at 180 days. As only 3 animals could be examined on a given day the sacrifice period was in fact 10 successive days. To avoid prejudicing the results, the animals were sacrificed in rotation rather than by group. Some instrument problems required a prolongation of this period at 180 days and it was extended to 20 days.

Spectrophotometric changes and lactic acid production of brain slices taken from exposed animals were measured basically as described earlier

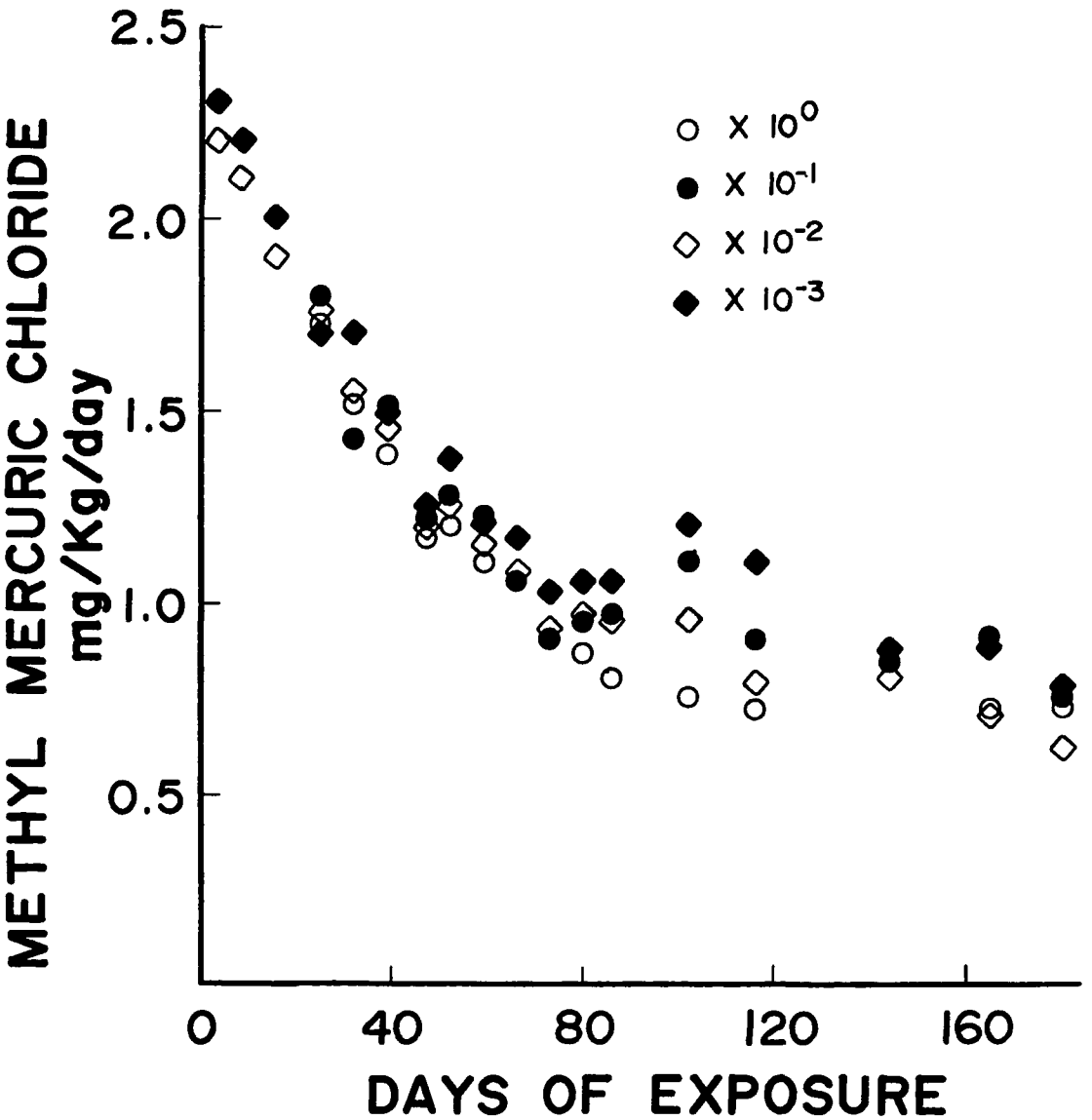


Figure 1. Dosage levels of methyl mercuric chloride in differing experimental groups with age. ◆ 0.01 mg/liter; ◇ 0.10 mg/liter; ● 1.0 mg/liter; ○ 10 mg/liter.

(BULL and LUTKENHOFF, 1974). Two specific changes must be noted. Since the aerobic glycolytic response to addition of potassium proved more sensitive than the respiratory response the perfusion method was modified slightly in hopes of increasing the time resolution of the measurements of changing lactic acid production. This involved primarily an increase in the perfusion rate to 1.3 ml/min, roughly twice that utilized in the short term studies. At this flow rate measurements of changes in oxygen consumption were not reliable and consequently will not be presented.

Statistical analysis of the results was done by analysis of variance and t-test. Unless otherwise noted a P of $<0.05$ is reported as significant.

## RESULTS

Only in the 10 mg/liter group was evidence of gross toxicity apparent which could be attributed to methyl mercury. One measure of this toxicity was a decrease in growth rate over the experimental period. The body weight of animals in this group failed to keep pace with that of the control group (Fig. 2). Lower levels of methyl mercury in the drinking water had no measurable effect on body weight (data not shown). The lessened weight gain of the 10 mg/liter group was associated with significantly decreased consumption of lab chow (Fig. 3), suggesting that anorexia may have been responsible. At this dose level two deaths resulted in only four animals being available for sacrifice following 180 days of exposure. Additional signs of toxicity were noted in this group which were similar to observations of other investigators (e.g., KLEIN, et al., 1972), and thus will not be dealt with here.

The effects of methyl mercury on electrically stimulated responses of cerebral cortex slices were similar, but not identical, to those observed in short-term experiments (BULL and LUTKENHOFF, 1975). As was seen in the short-term experiments methyl mercury did produce an inhibition of pyridine nucleotide reoxidation following electrical stimulation at an exposure level of 0.1 mg/liter of drinking water (Fig. 4). Some hint of inhibition was observed at 0.01 mg/liter at 90 days ($P < 0.05$) of exposure, but this was not confirmed at 180 days of exposure. Possibly the falling dose with increasing body weight could account for this difference. At 0.1 mg/liter the time constants for reoxidation were greater on the average than the maximum observed in the short term studies (130 sec vs. 100 sec, respectively). This appeared as a maxima, however, and at 1.0 and 10 mg/liter methyl mercuric chloride this effect of methyl mercury appeared to decrease. Little difference was observed in this result in animals sacrificed at 90 or 180 days of exposure.

Methyl mercury also reproduced the biphasic effect on the initial rate of pyridine nucleotide reduction with electrical stimulation seen in the short-term studies at 90 days of exposure, but not at 180 days. A stimulatory effect of methyl mercury was clearly evident at 0.1 mg/liter for 90 days but this effect appeared to progressively decline until at 10 mg/liter, it again approached control levels (Fig. 5). At 180 days of exposure only a progressive decline in the phase II response was noted at 1.0 and 10 mg/liter. At this time, the values obtained at both dose levels were significantly decreased relative to the control responses. Essentially the same result was observed with the cytochrome intermediates (data not shown).

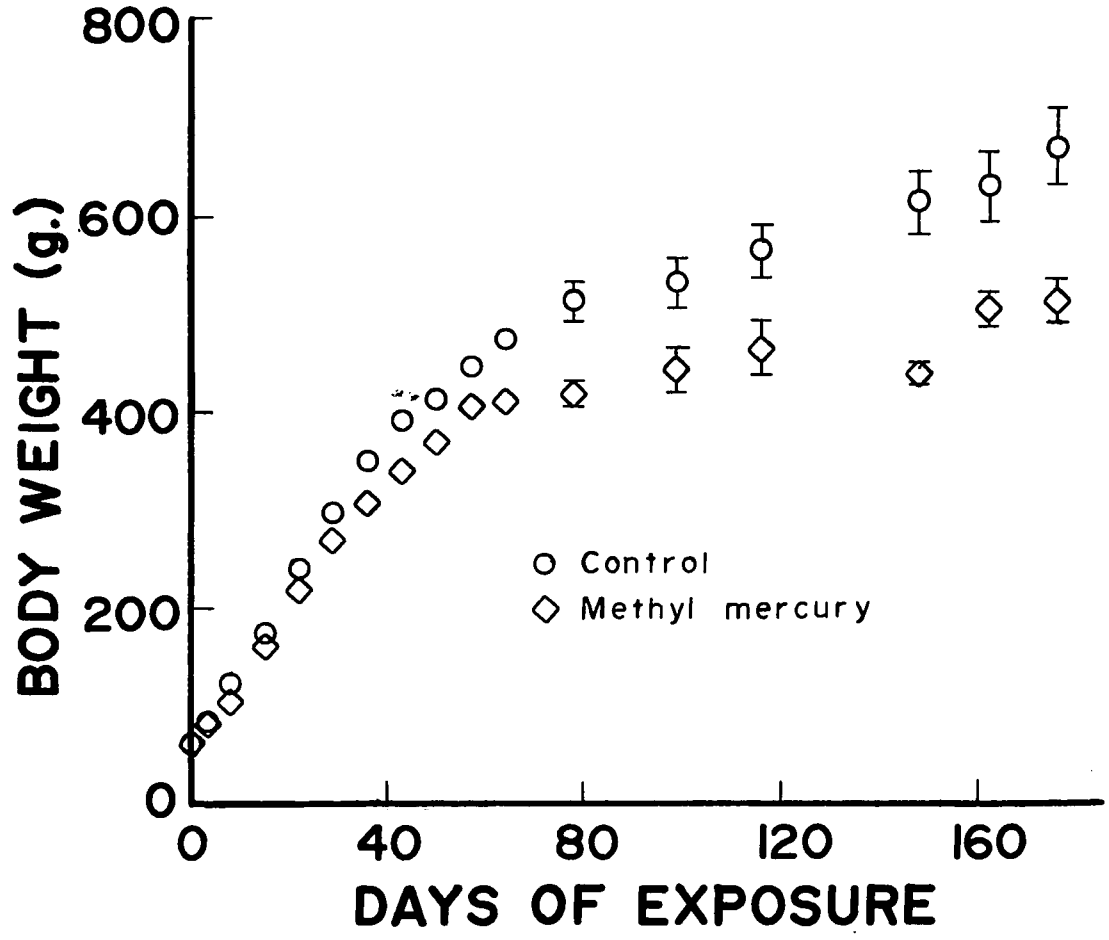


Figure 2. Effect of methyl mercuric chloride (10 mg/liter) on body weight gain. Vertical bars represent ± SEM.

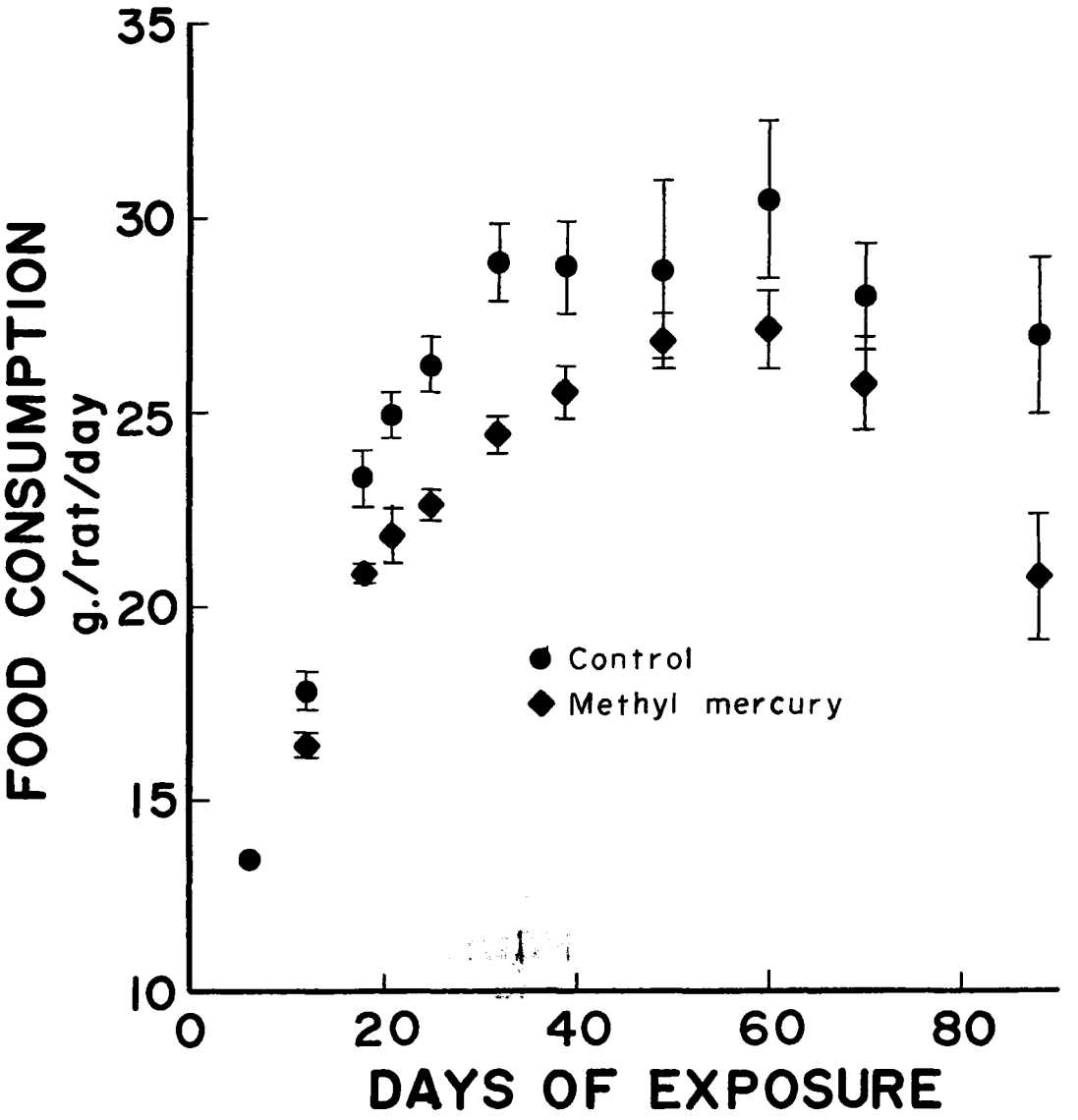


Figure 3. Methyl mercuric chloride (10 mg/liter) effect upon consumption of laboratory chow. Vertical bars represent ± SEM.

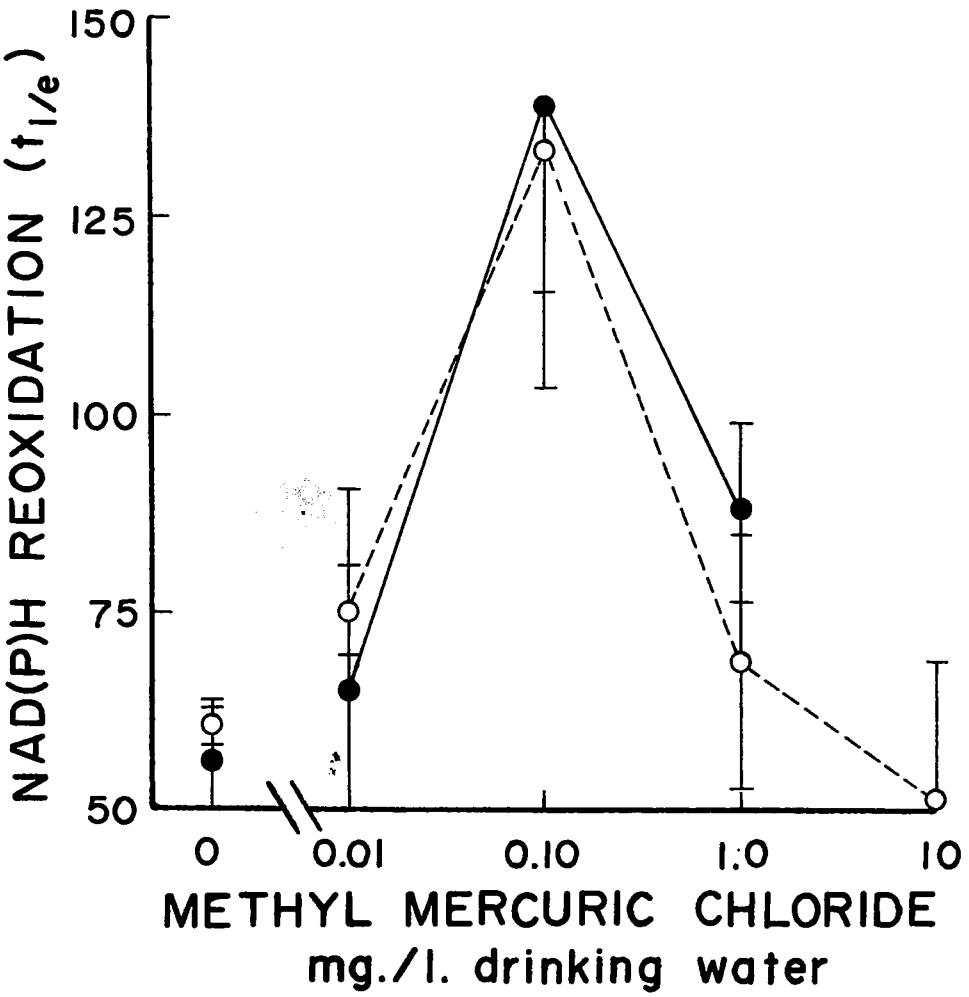


Figure 4. Pyridine nucleotide reoxidation in electrically stimulated brain slices derived from rats exposed to methyl mercury, ○ 90 days; ● 180 days. Vertical bars represent ± SEM.

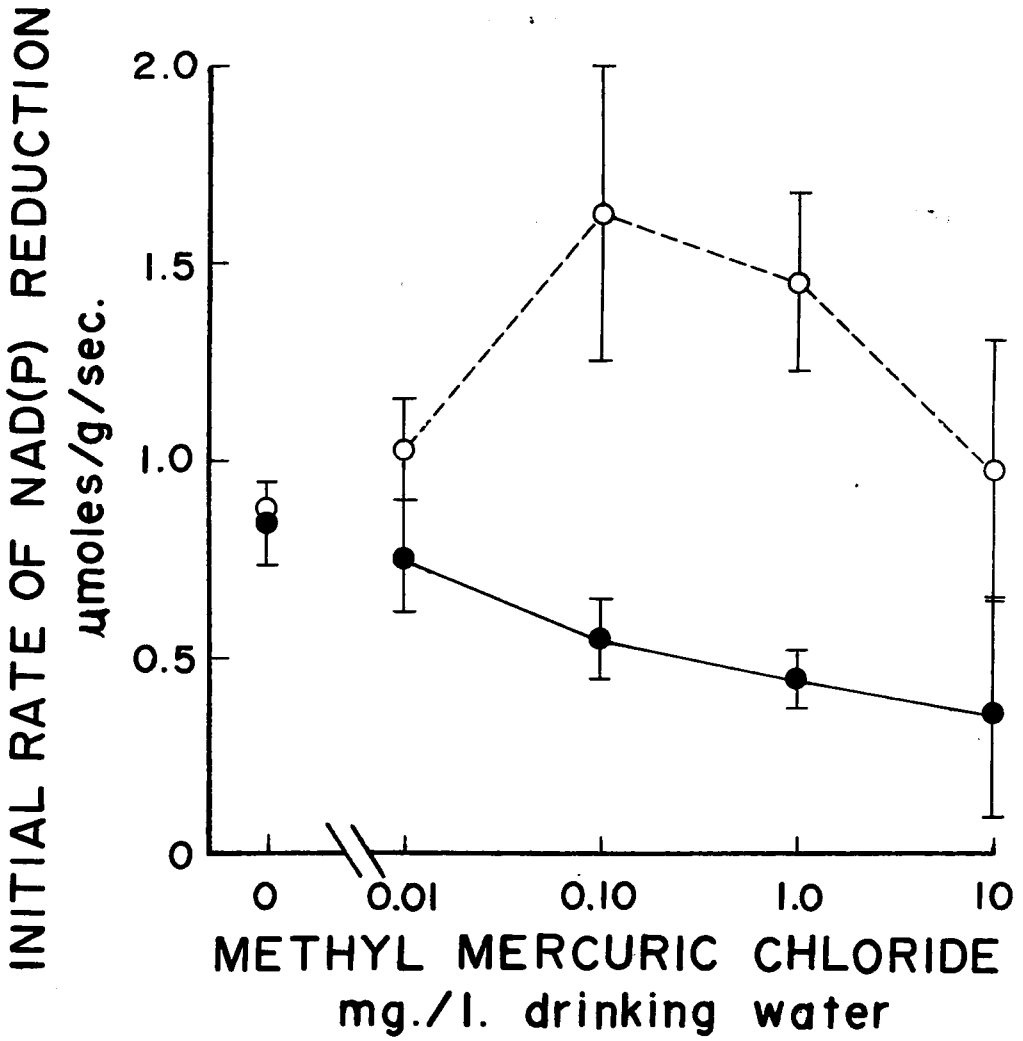


Figure 5. Initial rate of pyridine nucleotide reduction following electrical stimulation of brain slices taken from animals exposed to methyl mercury, ○ 90 days; ● 180 days. Vertical bars represent ± SEM.

The data in Figure 6 illustrate more completely the changes in the pyridine nucleotide responses to electrical stimulation. These responses were not necessarily representative of the particular dose, but are used to illustrate what appears to be the progression of alterations produced by methyl mercury. The typical control response, measured at 340-374 nm, distinctly shows the characteristic changes in absorbance observed following 10 sec of electrical pulses. Little change in these responses was observed at 0.01 mg/liter. At 0.1 mg/liter, the example illustrates the considerable prolongation of the response typical of this dose, although the one pictured is among the longest recorded. At 1.0 mg/liter, the response to electrical stimulation tends to assume a somewhat different configuration involving both a decrease in size and apparent duration of the response. In both slices from which the two examples of this dose were drawn, responses recorded in the cytochrome chain appeared very little changed, in either magnitude or duration. In the example shown taken from the 10 mg/liter group, the pyridine nucleotide response to electrical stimulation was purely oxidative in direction. This type of response has never been observed at this wavelength pair under the same conditions of incubation in a control animal (total of 29 in this and the short-term study and 12 in preliminary experiments). Although only 1 of 6 displayed this response at 90 days and 1 of 3 at 180 days, results from the short-term study confirm such an alteration in the response to electrical stimulation at high doses of methyl mercury.

Methyl mercury produced an alteration in the aerobic glycolytic response to an increase of media potassium concentration from 3 to 30 mM not observed in shorter term experimentation. At 180 days of exposure, the increase in lactic acid produced by tissues was markedly augmented, particularly during the initial stages of the response, at 0.1 mg/liter in the drinking water (Fig. 7). At higher doses a definite decrease in the rate at which the response developed was observed, consistent with the inhibition observed at 2.0 mg/Kg/day for 14 days in the short-term study. The lactic acid response was not measured at 90 days of exposure because of a shortage of technical personnel.

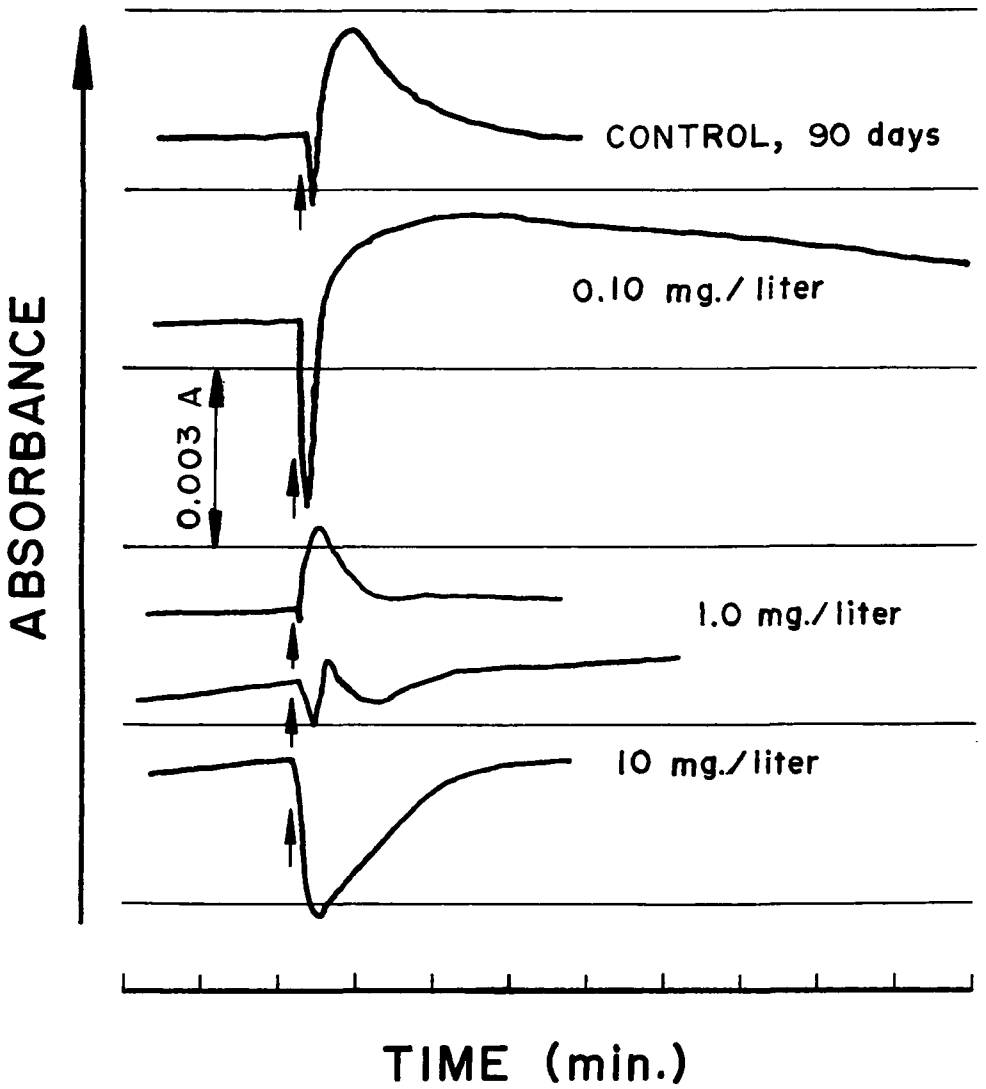


Figure 6. Effect of increasing doses of methyl mercuric chloride on the characteristics of the electrically-induced metabolic responses of tissue pyridine nucleotides. Taken at 180 days of exposure.

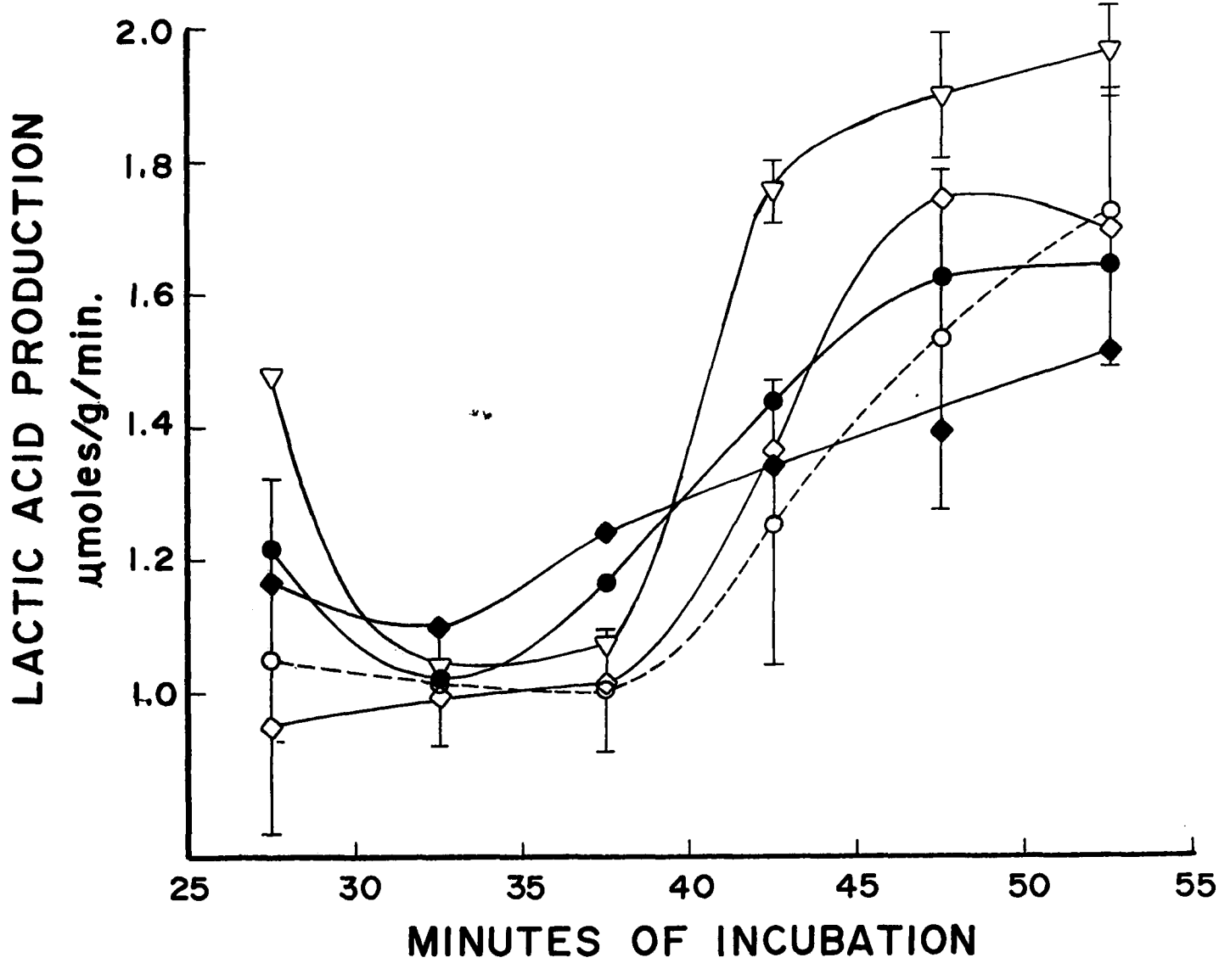


Figure 7. Effect of increasing doses of methyl mercuric chloride on potassium-stimulated glycolytic rate of brain tissue. ○ Control; ◇ 0.01 mg/liter; ▽ 0.1 mg/liter; ● 1.0 mg/liter; ◆ 10 mg/liter. Taken at 180 days of exposure. Vertical bars represent ± SEM.

## DISCUSSION

In appraising the results of the present paper it is well to keep in mind that the results obtained in our experimental system cannot necessarily be attributed exclusively to direct effects on the parameters measured. The measurements were not made with the limited view of measuring direct effects on these individual components of the tissue. If that had been the case it would have been much simpler to use isolated mitochrondrial preparations as the experimental system. However, our efforts have been directed towards measurement of methyl mercury induced alterations in the relationship of energy metabolism to function. This can only be accomplished when some functional capability is retained by the test preparation. Tissue slices retain such properties (e.g., electrical excitability and neurosecretory properties) but isolated mitochondrial preparations do not. There are certain advantages to be gained from such an approach as opposed to measurements directed towards specific enzymes or enzyme systems. Most obvious of these was the measurement of the respiratory intermediates under conditions which more closely approximate their in vivo environment. More important, however, is that such measurements allow an integrated approach to the effects of toxic chemicals on brain function. Inhibition of a specific enzyme by a toxic chemical necessitates adaptations in cellular metabolism. The adaptations are not merely functions of affinity constants and maximum velocities of various enzymes and metabolic pathways, but involve a complex array of feedback mechanisms of which only the barest outlines are clearly understood at present (ATKINSON, 1971). The possibility of this type of interference with tissue function has been demonstrated by methyl mercury's ability to reverse the effects of allosteric effectors on glutamate dehydrogenase (BITENSKY, et al., 1965) and the dissociation of subunits of glyceraldehyde-3-phosphate dehydrogenase by mercurials (SMITH and SCHACHMAN, 1971), in vitro. For these reasons we have made our first effort in the study of the central nervous system effects of methyl mercury at the tissue level of organization where many of the control mechanisms alluded to were still present. In addition, we have perturbed the tissue in such a way as to increase the functional activity of the tissue and thus activate these control mechanisms. Analysis of the kinetics of the responses allow an evaluation based upon alterations in the integrative aspects of function and metabolism. The result has not been a complete explanation of the central nervous system effects of methyl mercury. However, changes in the biochemistry and physiology of brain tissue taken from animals exposed to quite low doses of methyl mercury have been documented.

It is perhaps not surprising that long term exposures to methyl mercury produced a different gradation of responses that observed in shorter term

experiments. The basic difference in the long-term data is that the inhibition of pyridine nucleotide reoxidation, indicated by the increased time constant following electrical stimulation, appeared to be reversed at higher dosage levels. The qualitative nature of these changes were described in Figure 6 and the probable meaning of these changes is discussed later in this paper. In terms of a difference between long- and short-term exposures, however, it would seem reasonable to assume that longer term accumulations of methyl mercury gave brain tissue a chance to adapt to a given situation that it is not capable of or is unnecessary in the short-term. It was interesting to observe that the average time constant of pyridine nucleotide reoxidation following electrical stimulation remained little changed at 180 days relative to that observed at 90 days of exposure. This was despite an apparent loss of the enhancement in the reductive response to electrical stimulation at 0.1 mg/liter observed at 90 days. The enhancement of responses to electrical stimulation was also observed in shorter term exposures (BULL and LUTKENHOFF, 1975). Consequently, its apparent absence at 180 days of exposure requires explanation. It was possible that this effect of methyl mercury was confined to a dose between 0.01 and 0.1 mg/liter with the longer exposure time. The alternative explanation would be that this apparent adaptation to the inhibition of pyridine nucleotide oxidation cannot be sustained indefinitely. In either case the rather large shift in the dose-response curve relative to the doubling of the exposure time appears to indicate a cumulative effect of methyl mercury in depressing the reductive phase of the response to electrical stimulation.

There appeared to be a parallel between the rapidity of the aerobic glycolytic response to addition of potassium and the time constant of pyridine nucleotide reoxidation following electrical stimulation. This result strongly suggests that inhibition of the oxidation of cytoplasmic NAD(P)H may be involved in the observed effects of methyl mercury on electrically stimulated responses. This was another point of departure from the results obtained from short-term experiments, where little tendency for such an increased responsiveness to potassium was observed in lactic acid production by the tissue. On the other hand, higher levels of reduced pyridine nucleotide were achieved with addition of potassium at low doses of methyl mercury relative to controls in these short-term exposures. Consequently, the observation of a more "responsive" glycolytic pathway could be associated with the higher average time constant of pyridine nucleotide oxidation observed in the long-term results. One cannot, however, neglect the possibility that the increased time resolution afforded by the higher perfusion rate in the present study may have influenced the results.

The change in character of the response to electrical stimulation at the higher doses of methyl mercury (i.e., 10 mg/liter in the long-term study, with some indication of such a change at 1.0 mg/liter, and at 2.0 mg/Kg/day in the 14-day experiments) tends to fit well with the idea that the reducing equivalents for mitochrondrial oxidations may be derived from different pools. Differing pools of reduced pyridine nucleotides would be expected to display differing kinetic relationships with the

process of oxidative phosphorylation depending primarily on how direct the access to the cytochrome chain was (VANDAM and MEYER, 1971). We have previously shown that the reductive response to addition of potassium is glucose dependent (BULL and LUTKENHOFF, 1973) and that the pyridine nucleotide response to electrical stimulation is considerably attenuated with pyruvate as the sole substrate (unpublished results) relative to responses in the cytochrome chain. This may be taken as evidence of compartmentation of pyridine nucleotides in the tissue.

Experiments performed subsequent to the present work has shown that the form of the NAD(P) response is dependent upon the cytochrome redox potential (BULL, unpublished results). If the reduction of cytochrome c is reduced to somewhat less than 35% in the steady-state, the NAD(P) response becomes strictly oxidative in direction, similar to the observation made here with high doses of methyl mercury. Maintenance of a high steady-state level of reduction in isolated brain slices appears to be a peculiar property of glucose--presumably accounted for by its donation of a cytoplasmic reducing equivalent (BULL, 1975). The present data suggest, therefore, that delivery of cytoplasmic reducing equivalents to mitochrondrial oxidations in brain is severely impaired in rats exposed to high levels of methyl mercury.

The alterations in the metabolic response of rat cerebral cortex slices is consistent with observations made by PATERSON and USHER (1971) on in vivo levels of the adenine nucleotides of rat brain. These authors found an elevation of the ATP/ADP + AMP ratio in rats administered low doses of methyl mercury, but a decrease in this ratio at higher doses. High ATP levels would tend to inhibit oxidative phosphorylation and thus reduce the oxidation of reduced pyridine nucleotides. The increased levels of glycolytic intermediates noted by these authors may also account for the enhancement of reductive responses to stimulation noted throughout the respiratory chain (BULL and LUTKENHOFF, 1975) at the lower doses of methyl mercury. Lowered ATP levels accompanied by increases in ADP and P would act to stimulate oxidative phosphorylation and thus reduce the reductive response at higher doses of methyl mercury. SALVATERRA and co-workers (1973) documented the increase in ATP levels with low doses of methyl mercury in the mouse, but failed to show a decrease in these levels at higher doses. These authors observed certain behavioural changes which coincided with ATP elevations. The absence of a decrease in ATP with higher doses of methyl mercury may represent a species difference or may indicate a requirement for higher or repeated exposures in mice to produce the effect.

This preliminary investigation of the effects of methyl mercury on brain metabolism frankly arouses more questions than it answers. It is clearly evident that very low doses of methyl mercury, on the order of 0.01 mg/Kg/day, produce measurable changes in the pattern of cerebral metabolism following stimulation. It has been well established that the stimulated state, in vitro, more closely approximates in vivo rates of metabolic activity and that glucose serves as the primary substrate

of brain in vivo (MCILWAIN, 1966). With these facts in mind and viewing the preliminary nature of the findings, we find it advantageous to think of the results as indicating a progression from no effect, to compensation, to frank impairment of the coordination of the energy conserving mechanisms to the functional needs of the tissue. It appears that compensation is required for the decreased availability of one pyridine nucleotide pool for mitochrondrial oxidations. The cellular location of this pool cannot be stated for certain, but data from 180 days of exposure suggest that it may reside in the cytoplasm. When the tissue is required to rely exclusively on glucose as substrate, several problems of metabolic control can be envisioned. The aerobic glycolytic rate must be maintained in order to provide reducing equivalents for mitochondrial oxidations, either as reduced pyridine nucleotide and/or pyruvate. If oxidation of pyruvate or mitochrondrial oxidation of glycolytic NADH is impaired, a problem of coordination is introduced. In order for glycolysis to continue NAD must be regenerated either by conversion of pyruvate to lactate or by mitochrondrial oxidations through various shunts. If the tissue is required to use the lactic dehydrogenase step for regeneration of NAD, the relative amount of pyruvate available as a mitochondrial substrate is reduced, compounding the problem associated with a reduced mitochondrial oxidation of NADH. Consequently, one can see a need for an increase in the aerobic glycolytic rate upon increasing the energy demand of the tissue. This means of compensation, however, would eventually break down as inhibition increases because of the need to regenerate the catalytic quantities of NAD for glycolysis to remain in operation. This cannot be done without depleting pyruvate as a mitochondrial substrate. We feel that this progression is eventually responsible for the conversion of the pyridine nucleotide response to purely oxidative in direction. At this point, it is necessary for the tissue to draw on NADH generated during the resting state, a time at which energy demand is relatively low, in order to meet the energy demands of a brief burst of activity. Further work is planned to substantiate this hypothesis.

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16. ABSTRACT

Weanling, Sprague-Dawley rats have been exposed to methyl mercuric chloride (concentrations from 0.01 to 10.0 mg/liter) in their drinking water. At 10 mg/liter the animals exhibited neurological symptoms typical of methyl mercury. Also, in this group a considerable decrease in growth occurred which was associated with a decreased consumption of food.

Responses of the respiratory intermediates to stimulation were found to be altered in cerebral cortex slices taken from exposed animals. Effects on tissue pyridine nucleotides predominated. An enhancement of the rate of pyridine nucleotide reduction by electrical stimulation was observed at 0.1 mg/liter. This rate progressively decreased at higher dose levels. Reoxidation of reduced pyridine nucleotide was also inhibited at 0.1 mg/liter at both 90 and 180 days of exposure.

Potassium stimulated aerobic glycolysis was found to be enhanced in its initial stages at 0.10 mg/liter of methyl mercuric chloride in the drinking water but progressively declined at 1.0 and 10 mg/liter. A close parallel was observed between the time constant of pyridine nucleotide oxidation following electrical stimulation and the "responsiveness" of the aerobic glycolytic rate to stimulation by potassium. These results suggest an initial defect in the oxidation of cytoplasmic NADH which progresses to loss of metabolic control of cytoplasmic oxidations.


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