

# Potential of Acetylcholine-induced Smooth Muscle Contraction in Rat Ileum by Lead

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## Abstract

*The aim of this study was to find out lead interaction on acetylcholine-induced ileal (0.8" to 1" longitudinal total strips) contractions in varying calcium ( $Ca^{++}$ ) or magnesium ( $Mg^{++}$ ) media and the cholinergic mechanism. Potentiation of ileal strips by lead ( $Pb^{++}$ ) remained dose-dependent as elicited by acetylcholine-induced contractions ( $1.81 \times 10^{-6} M$  to  $1.28 \times 10^{-4} M$ ). These were completely antagonized by atropine ( $1.01 \times 10^{-8} M$ ). Lead ( $1.2 \times 10^{-5} M$ ) potentiated contractions caused by acetylcholine in normal or in excess  $Ca^{++}$  media, but in low  $Ca^{++}$  or with various  $Mg^{++}$  media, lead failed to potentiate such contractions. Thus  $Pb^{++}$  has indirect cholinomimetic effect which involved extracellular  $Ca^{++}$ .*

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**Key words:** Acetylcholine, Calcium, Ileal muscle, Lead, Magnesium, Rat

## Introduction

It is known that exposure of animals and humans to heavy metals such as lead can result in neurological dysfunction.<sup>1</sup> Pharmacodynamic effects of lead ( $Pb^{++}$ ) on tension of smooth muscles,<sup>2</sup> interaction with catecholaminergic function<sup>3</sup> and neuromuscular transmission have been described.<sup>4,6</sup> Intracellular calcium ( $Ca^{++}$ ) regulation is an important process since  $Ca^{++}$  mediates numerous cell functions.<sup>7</sup> Interactions of heavy metals with cellular  $Ca^{++}$  signalling mechanisms are investigated as possible molecular mechanisms of heavy metal toxicity.<sup>8</sup>

The regulation of the cytoplasmic  $Ca^{++}$  concentrations is important particularly in the nervous system as the level of free  $Ca^{++}$  affects both axonal conduction and synaptic transmission.<sup>9</sup> It is well known that the release of acetylcholine from any site depends on the presynaptic transmembrane potential at that site and the concentration of  $Ca^{++}$  in the extracellular media.<sup>10</sup> The exact molecular mechanisms by which  $Pb^{++}$  alters cholinergic synaptic signalling are unknown. Lead toxicity may be in part due to its ability to react with sulfhydryl (-SH) containing enzymes,<sup>11</sup> involved in cellular energy production<sup>12</sup> and other vital processes.<sup>13</sup>

It has been suggested that lead can be substituted for  $Ca^{++}$ <sup>14-16</sup> and possibly zinc in ion-dependent events at the synapse, and further responsible for the observed im-

pairment by  $Pb^{++}$  on other neurotransmitter systems such as cholinergic, gabergic and dopaminergic systems.<sup>17,18</sup> Lead activates protein kinase C, a second messenger in the regulation of cellular metabolism.<sup>19</sup> Lead may also replace  $Ca^{++}$  in calmodulin-dependent reactions. It also inhibits membrane bound  $Na^+K^+$ -ATPase and thereby interfering with the mitochondrial release of  $Ca^{++}$  with impairment of energy metabolism.<sup>20,21</sup> These effects are potentially reversible if lead can be removed from active sites. Thus  $Pb^{++}$  is an effective substitute for various  $Ca^{++}$ -dependent processes.<sup>21-24,25</sup>

Magnesium at the physiological level may be a  $Ca^{++}$  antagonist as it prevents contractions of vascular and non-vascular smooth muscle.<sup>22-29</sup>  $Mg^{++}$  may either regulate membrane permeability of  $Ca^{++}$  or occupy membrane sites which are exchangeable with membrane bound  $Ca^{++}$ .<sup>28-30</sup> Further studies of  $Pb^{++}$  interaction with acetylcholine in rat isolated ileum especially in  $Ca^{++}$  utilisation are a void area. Hence, we undertook to study lead effects and the role of extra-cellular  $Ca^{++}$  on muscuranic-acetylcholine (ACh) receptor mediated smooth muscle contraction in rat ileum.

## Materials and Methods

Wister strain albino rats of either sex weighing 250 g to 300 g were employed. The live animals were sacrificed by a blow on the head, and the ileum (0.9" to 1" total strip)

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was immediately dissected out and placed in a petri dish containing Tyrode solution of the following composition (in mM): NaCl 137, KCl 2.7,  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$  1.4,  $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$  2.0,  $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$  0.32,  $\text{NaHCO}_3$  12 and glucose 5.55 in de-ionised distilled water. Ileal smooth muscle strips were placed into a 30 ml of organ bath containing Tyrode solution (pH 7.4) which was continuously bubbled with 95% oxygen and 5% carbon dioxide and maintained at  $37^\circ\text{C} \pm 0.05^\circ\text{C}$ . The preparation was allowed to stabilise for 30 min. Contractions of ileal muscle strips were recorded on a smoked drum with an isotonic frontal writing lever which was under 500 mg tension and given a ten-fold magnification. The acetylcholine response (muscurinic effect) on ileal strips was confirmed by an antagonist atropine ( $1.01 \times 10^{-8}\text{M}$ ).

The cumulative dose-response curve for acetylcholine was determined. The preparation was then exposed to  $\text{Pb}(\text{NO}_3)_2$  ( $1.2 \times 10^{-5}\text{M}$ ) for 30 min and the dose-response curve of acetylcholine was re-determined in the presence of  $\text{Pb}(\text{NO}_3)_2$  ( $1.2 \times 10^{-5}\text{M}$ ).

In a second set of experiments, after establishing an ACh cumulative concentration-response curve of acetylcholine in control Tyrode medium, the preparation was exposed to either  $\text{Ca}^{++}$  free or high  $\text{Ca}^{++}$  (2.8 mM) Tyrode solution for 30 min. The concentration response curves were then re-determined in the presence of  $\text{Pb}^{++}$  ( $1.2 \times 10^{-5}\text{M}$ ).

In a third set of experiments, after establishing an ACh concentration, response curves in control Tyrode solution were established. The preparation was exposed to either  $\text{Mg}^{++}$  free or high  $\text{Mg}^{++}$  (4.0 mM) solution for 30 min. The dose response curve was then re-determined in the presence of  $\text{Pb}^{++}$  ( $1.2 \times 10^{-5}\text{M}$ ). In another set of experiments the same protocol was repeated in the presence of EDTA ( $3.0 \times 10^{-5}\text{M}$ ).

## Results

The acetylcholine-induced contraction of the ileum was found to be concentration-dependent ( $1.8 \times 10^{-6}\text{M}$  to  $1.28 \times 10^{-4}\text{M}$ ). ACh effect on ileum was completely antagonised by atropine ( $1.01 \times 10^{-8}\text{M}$ ).

Rat ileum preparation incubated in  $\text{Ca}^{++}$  free Tyrode solution with EDTA for 30 min showed a decrease in both sensitivity and in maximal responses to acetylcholine. When such preparation was incubated in the presence of  $\text{Pb}^{++}$  ( $1.2 \times 10^{-5}\text{M}$ ) in normal  $\text{Ca}^{++}$  (1.4 mM) for 30 min, it exhibited an increase in sensitivity and enhanced maximal response to acetylcholine. Preparations incubated in excess  $\text{Ca}^{++}$  (2.8 mM) Tyrode solution for 30 min also showed a decrease in sensitivity and attenuated the maximal response to acetylcholine. When these preparations were incubated in excess  $\text{Ca}^{++}$  (2.8 mM) medium for 30 min in the presence of  $\text{Pb}^{++}$  ( $1.2 \times 10^{-5}\text{M}$ ), the responses were potentiated to acetylcholine more than potentiated preparations (Fig. 1).

Ileum strips incubated in  $\text{Mg}^{++}$  free Tyrode solution showed a decrease in sensitivity and reduced maximal responses to acetylcholine ( $60.15 \pm 0.3\%$  of the control,  $P < 0.05$ , Fig. 2, Table I). In contrast, incubation in medium containing excess  $\text{Mg}^{++}$  (4.0 mM) reduced the maximal responses to acetylcholine ( $40.69 \pm 1.2\%$  of the control,  $P < 0.05$ , Fig. 1, Table I). All the preparations when incubated in normal  $\text{Mg}^{++}$  (2.0 mM),  $\text{Mg}^{++}$  free or excess  $\text{Mg}^{++}$  (4.0 mM) media in the presence of  $\text{Pb}^{++}$  ( $1.2 \times 10^{-5}\text{M}$ ) failed to potentiate the maximal response to acetylcholine (Fig. 1, Table I).

Rat ileum preparation incubated in  $\text{Mg}^{++}$  free Tyrode solution with EDTA showed a less increase in sensitivity and an increase in maximal response to acetylcholine ( $60.35 \pm 2.2\%$  of the control; Fig. 3; Table I) which was less than that obtained in the absence of EDTA (Fig. 1).

However, incubation in medium containing excess  $\text{Mg}^{++}$  (4.0 mM) also showed a reduction in both sensitivity and maximal response ( $45.40 \pm 2.5\%$  of the control; Fig. 3, Table I). Here, in spite of a rise in maximal responses, it was less when compared to that obtained in the absence of EDTA (Fig. 2). When these preparations were incubated in the presence of  $\text{Pb}^{++}$  ( $1.2 \times 10^{-5}\text{M}$ ) in any  $\text{Mg}^{++}$  concentrations, there was decrease in sensitiv-

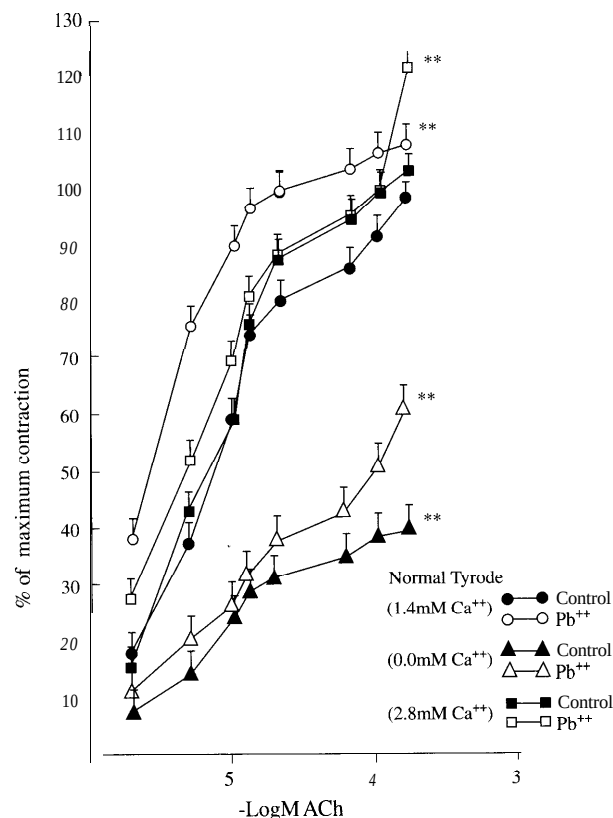


Fig. 1. Effects of  $\text{Pb}^{++}$  ( $1.2 \times 10^{-5}\text{M}$ ) on the concentration response curves of ACh obtained in rat ileum exposed to media containing  $\text{Ca}^{++}$  (1.4 mM), no  $\text{Ca}^{++}$  and excess  $\text{Ca}^{++}$  (2.8 mM) concentrations. Each point of the curves represents the mean of 6 experiments and vertical bars the SEM.  $^{**}P < 0.05$

TABLE I: EFFECT OF  $Pb^{++}$  ON MAXIMUM RESPONSES TO ACETYLCHOLINE IN RAT ILEUM EXPOSED TO DIFFERENT CONCENTRATION OF  $Mg^{++}$  TYRODE MEDIUM, WITHOUT AND WITH EDTA ( $3 \times 10^{-5}$  M).

| Treatment                                 | n | % maximum response $\pm$ SE |
|-------------------------------------------|---|-----------------------------|
| Without EDTA                              |   |                             |
| Acetylcholine                             |   |                             |
| $Mg^{++}$ (2.0 mM)                        | 6 | 100.00 $\pm$ 0.0            |
| $Mg^{++}$ (0.0 mM)                        | 6 | 60.15 $\pm$ 0.3*a           |
| $Mg^{++}$ (4.0 mM)                        | 6 | 40.62 $\pm$ 1.2*a           |
| ACh + $Pb^{++}$ ( $1.2 \times 10^{-5}$ M) |   |                             |
| $Mg^{++}$ (2.0 mM)                        | 6 | 102.16 $\pm$ 1.6            |
| $Mg^{++}$ (0.0 mM)                        | 6 | 80.35 $\pm$ 2.3*a           |
| $Mg^{++}$ (2.0 mM)                        | 6 | 50.66 $\pm$ 3.2*a           |
| With EDTA ( $3 \times 10^{-5}$ M)         |   |                             |
| Acetylcholine                             |   |                             |
| $Mg^{++}$ (2.0 mM)                        | 6 | 98.66 $\pm$ 1.5             |
| $Mg^{++}$ (0.0 mM)                        | 6 | 62.35 $\pm$ 2.2*a           |
| $Mg^{++}$ (4.0 mM)                        | 6 | 45.40 $\pm$ 2.5*a           |
| ACh + $Pb^{++}$ ( $1.2 \times 10^{-5}$ M) |   |                             |
| $Mg^{++}$ (2.0 mM)                        | 6 | 100.25 $\pm$ 2.6            |
| $Mg^{++}$ (0.0 mM)                        | 6 | 82.88 $\pm$ 1.5*a           |
| $Mg^{++}$ (4.0 mM)                        | 6 | 55.35 $\pm$ 3.5*a           |

n: number of experiments

a: significant difference from corresponding value in 2.0 mM  $Mg^{++}$

\*  $P < 0.05$

TABLE II: EFFECT OF  $Pb^{++}$  ON THE NEG LOG MOLAR ED., VALUES OF THE CONCENTRATION DOSE RESPONSE CURVES OF ACETYLCHOLINE IN ILEUM OBTAINED FROM RATS AND EXPOSED TO  $Mg^{++}$  IN TYRODE MEDIUM, WITHOUT AND WITH EDTA ( $3 \times 10^{-5}$  M)

| Treatment                          | n | Mean neg log molar<br>ED,, value ± SE | P value |
|------------------------------------|---|---------------------------------------|---------|
| Acetylcholine                      |   |                                       |         |
| Without EDTA                       |   |                                       |         |
| 2.0 mM Mg <sup>++</sup>            |   |                                       |         |
| Control                            | 6 | 5.12 ± 0.02                           | < 0.05  |
| Lead                               | 6 | 5.30 ± 0.06                           |         |
| 0.0 mM Mg <sup>++</sup>            |   |                                       |         |
| Control                            | 6 | 4.00 ± 0.16a                          | < 0.05  |
| Lead                               | 6 | 7.40 ± 0.1 la                         |         |
| 4.0 mM Mg <sup>++</sup>            |   |                                       |         |
| Control                            | 6 | 0.0 ± 0.0a                            | < 0.01  |
| Lead                               | 6 | 3.80 ± 0.06a                          |         |
| With EDTA (3 x 10 <sup>-5</sup> M) |   |                                       |         |
| 2.0 mM Mg <sup>++</sup>            |   |                                       |         |
| Control                            | 6 | 4.98 ± 0.06                           | < 0.05  |
| Lead                               | 6 | 4.90 ± 0.08                           |         |
| 0.0 mM Mg <sup>++</sup>            |   |                                       |         |
| Control                            | 6 | 4.18 ± 0.10a                          | < 0.01  |
| Lead                               | 6 | 7.85 ± 0.12a                          |         |
| 4.0 mM Mg <sup>++</sup>            |   |                                       |         |
| Control                            | 6 | 0.00 ± 0.0a                           | < 0.05  |
| Lead                               | 6 | 3.98 ± 0.12a                          |         |

n: number of experiments

a: significant difference from corresponding value in 2.0 mM  $Mg^{++}$

$P < 0.05$

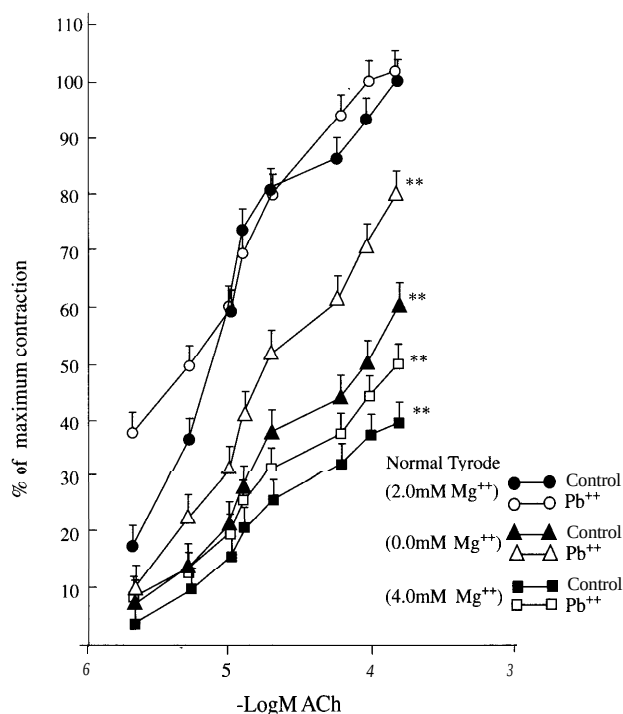


Fig. 2. Effects of  $Pb^{++}$  ( $1.2 \times 10^{-5}$  M) on the concentration response curves of ACh obtained in rat ileum exposed to normal  $Mg^{++}$  (2.0 mM),  $Mg^{++}$  free and excess  $Mg^{++}$  (4.0 mM) without EDTA. Each point of the curves represents the mean of 6 experiments and vertical bars the SEM. \*\* $P < 0.05$

ity and in maximal response to acetylcholine (Fig. 3). Thus, in the presence of  $Pb^{++}$ , any magnesium concentrations in the media without EDTA or with EDTA failed to potentiate such contractions in rat ileal preparations.

## Discussion

Smooth muscle contraction is initiated when an agonist binds to a receptor and activates a series of events which increase intracellular  $Ca^{++}$  concentration. Contractile response to 5-hydroxytryptamine in isolated rat ileum is inhibited by  $Pb^{++}$ ,<sup>28</sup> whereas in this study, acetylcholine resulted in an increase in tension of ileum, suggesting the existence of binding site for this agonist in the ileum. Contractions of rat ileum in response to acetylcholine were blocked by atropine indicating muscarinic receptor-mediated response. Present observations have shown that acetylcholine is able to elicit a small residual contractile response even in the absence of extracellular  $Ca^{++}$ . This would suggest that this agonist utilises another source of intracellular  $Ca^{++}$  storage.<sup>31,32</sup> Such responses are all similar to those of bovine coronary artery,<sup>33</sup> swine trachealis,<sup>34</sup> the circular layer of the guinea pig and human stomach,<sup>35,36</sup> rat duodenum<sup>37</sup>

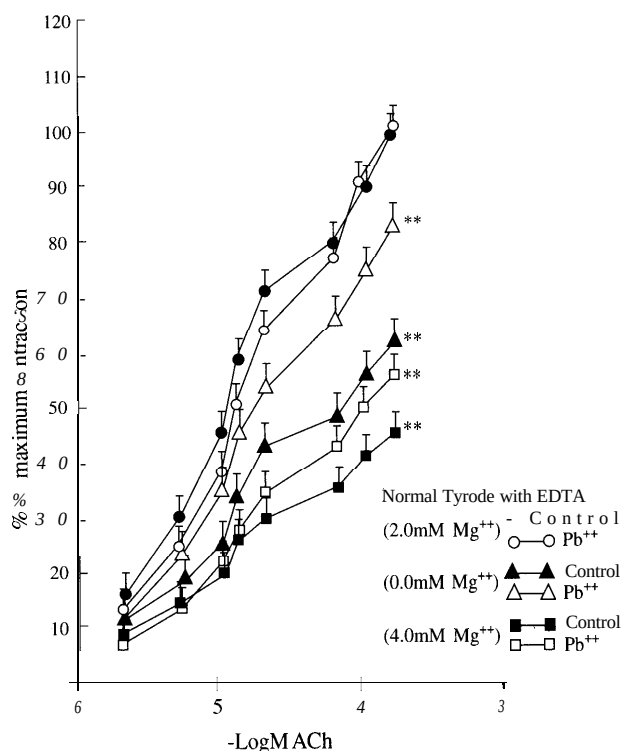


Fig. 3. Effects of  $Pb^{++}$  ( $1.2 \times 10^{-5} M$ ) on the concentration response curves of ACh obtained in rat ileum exposed to normal  $Mg^{++}$  (2.0 mM),  $Mg^{++}$  free (0.0 mM) and excess  $Mg^{++}$  (4.0 mM) with EDTA ( $3 \times 10^{-5} M$ ). Each point of the curves represents the mean of 6 experiments and vertical bars the SEM. \*\* $P < 0.05$

to acetylcholine. Our study provides an evidence for intracellular  $Ca^{++}$  release by acetylcholine and further shows that  $Pb^{++}$  has a role facilitating the entry of  $Ca^{++}$  inside the cell and serves as a  $Ca^{++}$  agonist enabling release of acetylcholine.

Lead is unique because it appears to resemble  $Ca^{++}$  sufficiently to produce normal or nearer to the biologic response when it interacts or substitutes for a  $Ca^{++}$  in  $Ca^{++}$ -dependent processes such as: (i)  $Ca^{++}$  channels in the membrane,<sup>14</sup> (ii)  $Ca^{++}$  pump and  $Ca^{++}$  ATPase,<sup>15</sup> (iii)  $Ca^{++}$  uptake into mitochondria,<sup>14</sup> (iv)  $Ca^{++}$  and calmodulin-dependent processes,<sup>11,36,37</sup> (v) binding to potential  $Ca^{++}$  binding protein (probably by  $Ca^{++}$  absorption)<sup>38</sup> and (vi)  $Ca^{++}$ -dependent  $K^{+}$  permeability.<sup>16</sup> This could lead to an increase in the ionised  $Ca^{++}$  level,<sup>13</sup> resulting in the disruption of a number of  $Ca^{++}$  regulated processes including acetylcholine release.

In this study, the peak-tension of acetylcholine stimulated the ileum by gradually adding an equimolar acetylcholine doses, which gradually decreased the maximal responses of ileum in the  $Ca^{++}$  free media, indicating the exhaustion of intracellular  $Ca^{++}$  stores. The present investigation does not provide direct evidence for acetylcholine-induced intracellular  $Ca^{++}$  release. However, several experimental observations support this hypothesis: (i) contractions of rat ileum to acetylcholine were

markedly attenuated by atropine indicating muscarinic receptor mediated response, (ii) muscarinic receptors in various tissues are coupled to phosphoinositide hydrolysis,<sup>39</sup> (iii) inositol-1,4,5-trisphosphate, a product of phosphoinositide hydrolysis can induce intracellular  $Ca^{++}$  release in a pancreatic cell<sup>40</sup> suggesting that acetylcholine utilises intracellular sources of  $Ca^{++}$  as well as extracellular ones to produce contraction in rat ileum, an effect that may be linked to its ability to increase phosphoinositide hydrolysis<sup>39,40</sup> which can induce intracellular release of  $Ca^{++}$  and that facilitates the release of neurotransmitter i.e. acetylcholine.

The dose-related augmenting effect of  $Pb^{++}$  on acetylcholine-induced contraction of the ileum was observed with different  $Ca^{++}$  concentrations in the media. These observations are consistent with a leftward shift in the concentration response curve (Fig. 1). This potentiating effect of acetylcholine could theoretically be the result of several mechanisms including increased levels of acetylcholine by increasing the synthesis of acetylcholine or a decreased turnover of acetylcholine or inhibition of acetylcholine esterase<sup>17</sup> and an increased affinity of agonist for the post synaptic muscarinic receptor. The contractions induced by acetylcholine were dependent on the presence of extracellular  $Ca^{++}$  in the media. The increased in sensitivity and enhanced maximal responses and foot of the concentration response in preparations incubated in normal or  $Ca^{++}$  excess media suggest that  $Pb^{++}$  acts on processes that rely upon extracellular  $Ca^{++}$ . The statement is supported by the decrease in concentration response curve responsiveness to acetylcholine by  $Pb^{++}$  in a preparation incubated in  $Ca^{++}$  free media. However, the slight rise in concentration response curve attained by  $Pb^{++}$  (compare with  $Ca^{++}$  free control, Fig. 1) could in part be related to intracellular  $Ca^{++}$  sources that were not totally depleted by incubation in  $Ca^{++}$  free media. Residual  $Ca^{++}$  within the tissue was a contributing factor for developing tension as no EDTA was added to the media. It is unlikely that  $Pb^{++}$  induced contraction by competing with  $Ca^{++}$  for activation. If so, it would be expected that the  $Pb^{++}$  induced contraction by competing with  $Ca^{++}$  in the  $Ca^{++}$  free media. However, the possibility remains that  $Pb^{++}$  may compete with  $Ca^{++}$  once they have entered the myoplasm. Their entrance into the cell could be mediated through  $Ca^{++}$  transport processes. All heavy metals may provoke changes to a specific ionflux i.e.,  $Na^{+}$ ,  $Ca^{++}$  or  $K^{+}$ , across the membrane due to -SH interaction. Modification of -SH with an alkylating agent results in an increased  $Ca^{++}$  efflux<sup>39</sup> rate indicating an increased myoplasmic  $Ca^{++}$  level<sup>41</sup> and increased leakage of  $Ca^{++}$  through the sarcoplasmic reticulum vesicles isolated from skeletal muscle.<sup>42</sup>

Changes in the membrane potential by firing of action potentials and also in a depolarised muscle by increas-

ing intracellular  $\text{Ca}^{++}$  affects the contractile response of the muscle cell by acetylcholine. The reduction in membrane potential increases permeability of  $\text{Ca}^{++}$  leads to influx of  $\text{Ca}^{++}$  from an extracellular source.<sup>43</sup> Lead produced potentiation of acetylcholine-induced responses of preparations which were bathed with the medium containing normal or excess  $\text{Ca}^{++}$ , whereas  $\text{Pb}^{++}$  failed to potentiate the acetylcholine-induced responses in preparations which were exposed to  $\text{Ca}^{++}$  free or any  $\text{Mg}^{++}$  concentration in media (Fig. 2). Interpretation of these results is complicated by the observation of an increased sensitivity of acetylcholine in rat ileum, which probably interferes with  $\text{Na}^{++}$ ,  $\text{Ca}^{++}$  exchange process mediated by membrane bound  $\text{Na}^{+}\text{-K}^{+}\text{-ATPase}$  or a reduction in enzyme-dependent  $\text{Na}^{+}\text{-Ca}^{++}$  exchange process or block of the entry of  $\text{Ca}^{++}$  via voltage gated  $\text{Ca}^{++}$  channels through which  $\text{Pb}^{++}$  at higher concentrations also penetrates and then acts as  $\text{Ca}^{++}$  to promote transmitter release.

The augmenting effect of  $\text{Pb}^{++}$  on acetylcholine-induced contraction was much less (but significant) in the presence of EDTA compared to that of its absence. The EDTA ( $3 \times 10^{-5} \text{ M}$ ) was present in Tyrode solution to protect exogenously administered acetylcholine in the bath from the oxidation catalysed by the presence of trace metals in the solution. The lack of observable  $\text{Mg}^{++}$  induced supersensitivity might have been due to the presence of EDTA which could interfere with increased membrane permeability to  $\text{Ca}^{++}$ . In excess  $\text{Mg}^{++}$  medium without EDTA, there was marked inhibition by  $\text{Pb}^{++}$  responses to acetylcholine (Fig. 2). In the presence of EDTA, the inhibition observed in excess  $\text{Mg}^{++}$  media was superimposable with the control curve (Fig. 3). This would suggest the possibility of antagonism between  $\text{Pb}^{++}$  and  $\text{Ca}^{++}$  at the membrane-binding sites, thus in a manner of  $\text{Ca}^{++}$ ,  $\text{Mg}^{++}$  antagonism. It has been suggested that there is a competition between  $\text{Pb}^{++}$  and  $\text{Ca}^{++}$  for the same binding sites at the membrane and or at intracellular sequestering sites like the sarcoplasmic reticulum and mitochondria.<sup>44</sup>

In conclusion, the present experiments and the data of the literature sighted suggest that lead potentiated contractions caused by acetylcholine in normal or excess  $\text{Ca}^{++}$  media, but this low  $\text{Ca}^{++}$  or any  $\text{Mg}^{++}$  concentrations in the media  $\text{Pb}^{++}$  failed to potentiate such contractions at cellular level by inducing a  $\text{Ca}^{++}$  accumulation or an increase of the intracellular exchangeable  $\text{Ca}^{++}$ . This was available from extracellular media facilitating contraction or activation of the intracellular  $\text{Ca}^{++}$  stores or  $\text{Ca}^{++}$  binding or the processes of  $\text{Ca}^{++}$  extrusion are inhibited. No single mechanism appears to account for the diverse effects of  $\text{Pb}^{++}$  and most likely it will eventually be found that  $\text{Pb}^{++}$  exerts its toxic effects by acting at several cellular and subcellular  $\text{Ca}^{++}$  regulatory sites.

Lead in many occupations results in morbidity, particularly tremors, memory deficits, anaemia, muscular

degeneration, cramps. The mechanism of lead induced impairment in smooth and striated muscles is not yet clearly known. The key factor is its intervention of the cholinergic dynamics. Invariably, elements play a vital role in acetylcholine as well as other effects of neurochemicals. In our study, the role of both intracellular and extra-cellular  $\text{Ca}^{++}$  in an in vitro smooth muscle set up explains the effects of lead and this enables a clinical importance of channel blockers in  $\text{Pb}^{++}$  toxicity.

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