Table 1 Summary of Effects of Test Drugs

Drug (μg ml^{-1})	Atria	Ventricles
Control	Exponential decay.	Exponential decay. Beating rate slower than atria and ceases sooner.
Tyramine 40	Overall depression of beating rate. Decrease in survival time.	Similar to controls.
Oxyfedrine 20	The curve swung to a more horizontal position. 0–8 d beating slowed. 9–19 d rate speeded up. Slight increase in survival time.	Curve swung to more horizontal position. Rate decreased up to day 4, rate increased from day 5–16. Survival time increased.
Oxyfedrine 40	Slight but uniform increase in rate from day 2 to day 15. No increase in survival time.	Very slight decrease in rate up to day 6, increase in rate from day 7 to 14. No increase in survival time.
Oxyfedrine + Tyramine 40	Similar to control; the two drugs cancelling each other out.	Similar to control. Drugs cancel.
Oxyfedrine 80	Similar to control; striking reduction in the variability of the rate. (Scatter.)	Uniform slight increase in rate. Reduction in scatter as for atria.
Isoprenaline 50	Moderate overall increase in rate but no increase in survival time.	Moderate overall increase in rate; no increase in survival. Greater scatter.
L-Noradrenaline 15	Similar to oxyfedrine 20. Curve swung to horizontal position. Decreased rate 0–8 d, increased rate days 9–23. Survival time increased more than by oxyfedrine 20.	No significant effect from day 0–5; increased rate from day 6–17. Increase in survival time.
Practolol 50	0–8 d similar control, 9–18 d rate increased. Slight increase in survival time.	Overall increase in rate starting slight and becoming large. Large increase in survival time.

The four hearts under each set of conditions represent a range of foetal ages, and therefore a range of developmental stages, giving a certain amount of variation in beating performance.

The performance of the youngest and oldest groups of hearts were examined separately, and predictably the youngest hearts lived longer and beat better than the oldest. The atrial and ventricular beating rates of the oldest control hearts were erratic in this preliminary series, and we suggest that for drug evaluation, hearts of less than 60% of term give more meaningful results.

Some interesting differential effects were demonstrated. Oxyfedrine, for example, at 20 μg ml^{-1} showed a positive effect on both atria and ventricles in the young hearts with no apparent effect on the beating of the older atria and ventricles; whereas isoprenaline affected both the young and old hearts equally.

As well as being a potentially useful assay for cardioactive drugs, it seems also that any substance which is to be given to pregnant women should be examined for its direct effect on the mouse foetal heart. Further study with human foetal heart should follow if any effect is demonstrated. It cannot be assumed, however, that the substance is harmless if there is no effect on mouse or human foetal heart; many drugs are not pharmacologically active in their pure form, whereas their breakdown products are. Complete drug evaluation will require cultures with each of the intermediate breakdown products at their likely concentrations. If after this screening a drug seems to have no effect, or even a beneficial one, it would

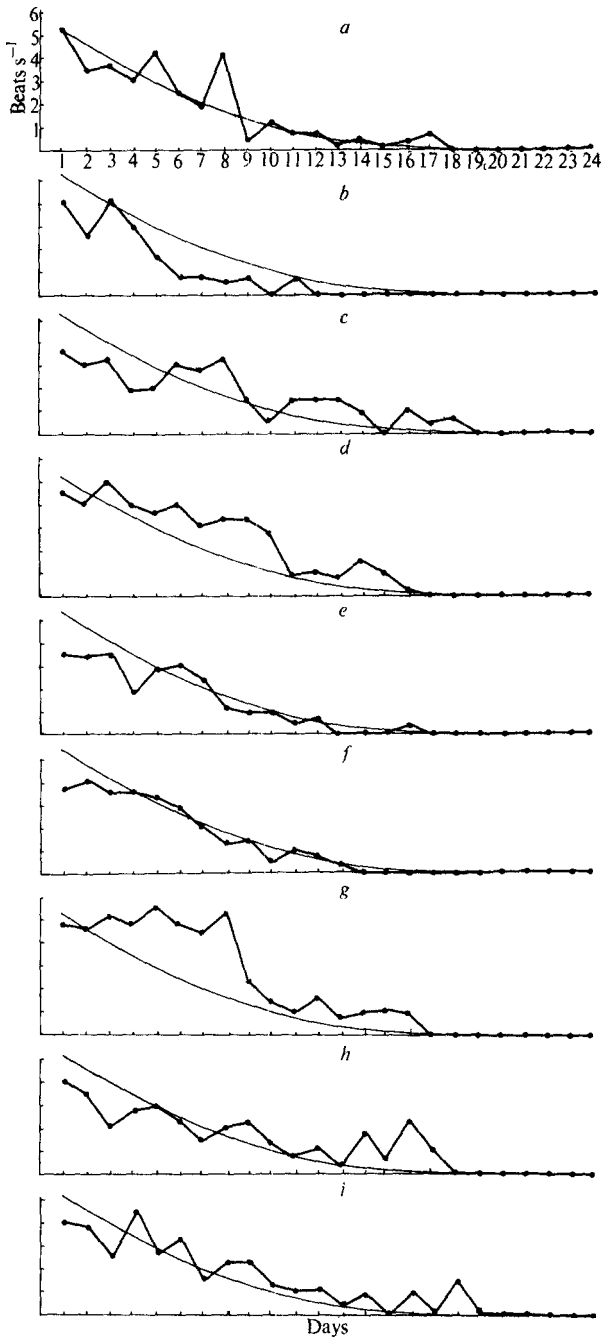

Fig. 1 Atrial rates of the control hearts compared with the atrial rates of the test hearts for the first 23 d of culture. Hearts from nine litter mates of comparable size were selected from pregnant mice for each set of experiments. (When one pregnant mouse contained only seven foetuses, two foetuses of the same crown rump size from another litter had to be used.) The experiment was repeated successfully four times, using a range of foetal ages 54% to 80% term (crown rump 9 to 16 mm). Hearts were cultured as previously described[5] on stainless steel mesh so that their lower surfaces were wetted by 65% medium 199 (Burroughs Wellcome), with antibiotics, 35% foetal calf serum, 50 μg ml^{-1} insulin, 0.1 μg ml^{-1} cortisol; in a water vapour saturated atmosphere of 95% oxygen and 5% CO_2 and at a temperature of 37° C ± 2° C. Six drugs were added to this standard medium to give the nine test media, as follows: (*a*) standard medium (control); (*b*) medium + tyramine hydrochloride 40 μg ml^{-1}; (*c*) medium + oxyfedrine 20 μg ml^{-1}; (*d*) medium + oxyfedrine 40 μg ml^{-1}; (*e*) medium + tyramine hydrochloride 40 μg ml^{-1} plus oxyfedrine 40 μg ml^{-1}; (*f*) medium + oxyfedrine 80 μg ml^{-1}; (*g*) medium + isoprenaline 50 μg ml^{-1}; (*h*) medium + L-noradrenaline 15 μg ml^{-1}; (*i*) medium + practolol 50 μg ml^{-1}. The medium was replaced every other day after observations had been made. The cultures were examined daily using a dissecting microscope, during which examination special efforts were made to maintain the same constant conditions of temperature, atmosphere and water vapour saturation.

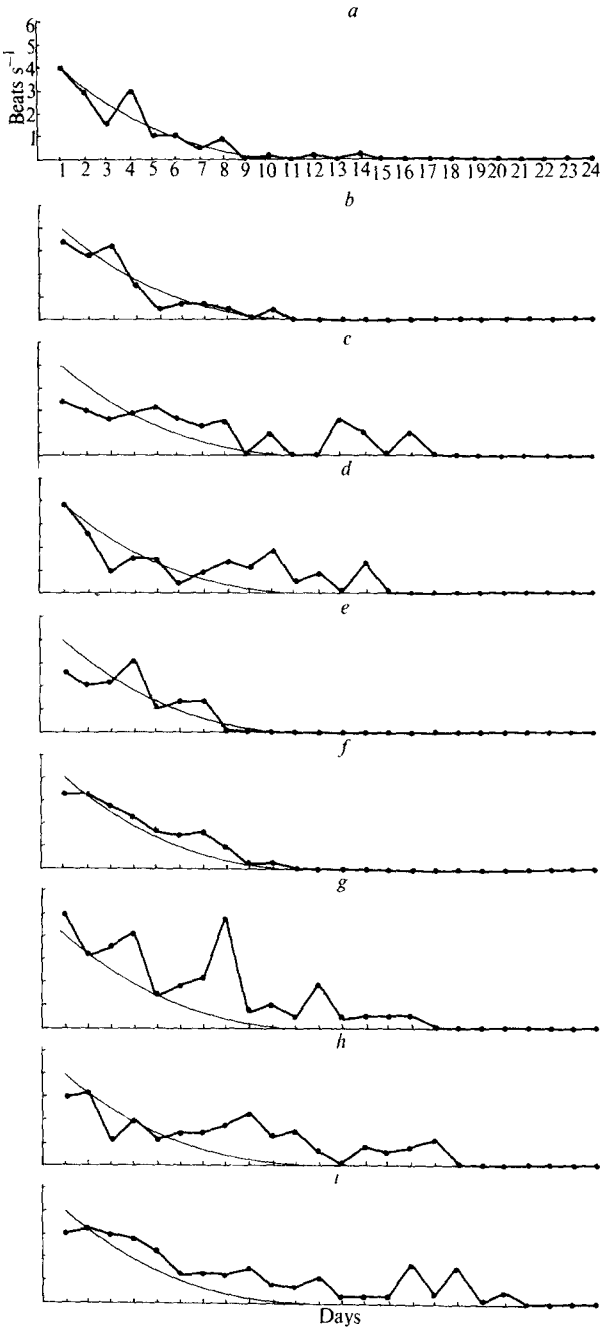

Fig. 2 Ventricular rates of the control hearts compared with the first 23 d for the test hearts.

seem reasonable to continue with further conventional clinical trials. If a new drug is depressant to the foetal myocardium above a certain concentration, it would be unwise to exceed this in a subsequent clinical trial. If the substance were to affect only young foetal hearts it might still be safe in late pregnancy.

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Penetration of Asbestos through the Digestive Tract of Rats

MESOTHELIOMAS of the pleura and peritoneum[1-3] have been linked with the inhalation of asbestos fibres. We have shown[4] that asbestos fibres are present in drinking water, beer, wine and other beverages. Asbestos fibres can penetrate the mucosa of the stomach and intestine[5] and Telischi and Rubenstone[6] have found asbestos material in a gastric carcinoma. Godwin and Jagatic[7], reporting on mesotheliomas of the pleura caused by asbestos, noted that asbestos particles were widely distributed in various tissues. They suggested that particles may migrate from the lung through the blood and lymphatic system to all parts of the body. In the light of these reports, we surmised that asbestos consumed orally can pass through the gut into the blood stream and accumulate in various tissues.

To determine if this is the case we injected a suspension of asbestos fibres into the stomachs of rats. The suspension was prepared by shaking 2.5 g of chrysotile asbestos (Johns Manville No. 7RFO2) in 500 ml of water in a graduated cylinder. The suspension was allowed to settle for 30 min and the top 250 ml, which contained about 9.4×10^9 (1 mg) fibres ml^{-1}, was drawn off. Most of these fibres are 0.2 μm–2.0 μm long (Fig. 1). To ensure that fibres passed through the digestive tract without rats inhaling any of them, the asbestos was introduced into the gastrointestinal tract by opening the abdomen under anaesthesia and injecting the fibres directly into the stomach. Two to 4 d later the rats were killed and blood and tissues analysed for asbestos. To ensure that any fibres on the rat hair could not contaminate the organs removed during surgery, the rats were first thoroughly vacuumed and washed with double distilled water and methanol. During the removal of tissues wet towels were placed over the animals and around the incisions. Uncontaminated blood was drawn from the orbital sinus using a capillary tube.

To avoid formation of excess ash, which interferes with examination of specimens under the electron microscope, the tissues were first solubilized with soluene (Packard Instrument Company); the fibres were then centrifuged down, washed with methanol and ashed as previously described[4]. The ash was taken up in 1 ml of distilled water which had been filtered through a 0.2 μm filter, and 5 μl of this mixture was dropped on hydrophilic carbon-coated electron microscope grids supported on a ring peg. The grids were dried in a closed glass container (to avoid air contamination) under a heat lamp and were examined at magnifications of 20,000 and 80,000 with an electron microscope. The presence of chrysotile asbestos fibres was verified by electron diffraction. They were counted and the numbers corrected for dilution to determine the number in the original 1 ml sample. Results of recovery of asbestos fibres from rats treated in this way are shown in Table 1.

Rats in group 1 were given 9.4×10^9 fibres (1 ml) and killed 2 d later; those in group 2 received 94×10^9 fibres and were killed 4 d later. No asbestos fibres were detected in the blood of non-injected rats (controls) but 4.65×10^6 g^{-1} (a statistically significant increase) were found in the first group.

This is the first time direct physical evidence for the presence of asbestos fibres in the blood has been obtained. Four days after treatment the amount of asbestos in the blood of rats in group 2 had dropped to 1.19×10^6 g^{-1} even though these rats received ten times as much asbestos. This indicates that blood

Table 1 Numbers of Asbestos Fibres Recovered from Treated and Non-Treated Rats

	Controls mean *		Group 1 † mean *		Group 2‡ mean *	
Blood	0.00	—	4.65¶	1.02	1.19	0.77
Spleen	2.33	0.67§	4.11	0.49	3.45	1.20
Omentum	2.46	0.46	2.74	0.87	18.25	7.79
Heart	2.09	0.41	—	—	2.28	0.40
Brain	0.05	0.02	0.31‖	0.19	0.29**	0.11
Lungs	1.06	0.26	1.80	0.54	1.74	0.26

* Average of five rats in all cases except where noted. † Animals injected with 9.4×10^9 fibres and tissues examined 2 d later. ‡ Animals injected with 94×10^9 fibres and tissues examined 4 d later. § Standard error of the mean. ‖ Four rats only. ¶ $P < 0.01$; ** $P < 0.05$.
(The figures in the table are fibres $g^{-1} \times 10^{-6}$.)

can clear itself of the fibres. Tissues such as the spleen, heart and lung also seem to have, to a lesser extent, the ability to clear themselves of the asbestos fibres, as the figures obtained from rats in group 2 indicate. They are lower than those for group 1 rats even though they received a larger dose, but the tissues had longer to clear themselves. Indeed, recent work in our laboratory with radioactive asbestos indicated that some tissues, such as the lungs, can reduce their fibre content by half in 2 d. This clearing action could possibly have quite an effect on the numbers of fibres found in the liver and kidney of the rats but unfortunately, because of the considerable amount of ash present in these organs even after soluene treatment, we could not examine these specimens under the electron microscope.

Another factor may contribute to the higher fibre count obtained for the organs of rats in group 1 compared with animals in group 2, namely, that there were four times as many fibres in the blood of rats in group 1. The residual blood in the tissues on removal from the animal would of course contribute to the total fibre count of a particular tissue.

This factor could have created the anomaly in the fibre counts in brains. In the brains of treated rats this was about six times higher than in the controls, and the increase in fibre count is statistically significant for rats in group 2 but not, oddly, for rats in group 1 even though there were more fibres in these animals. The greater number of fibres per gram in the blood of rats in group 1 could be responsible for the larger standard error in the counts of rats in group 1 and therefore did not allow the values obtained to be significant.

The brain seems to have little ability to clear itself of asbestos fibres, but the omentum has even less. This tissue, which

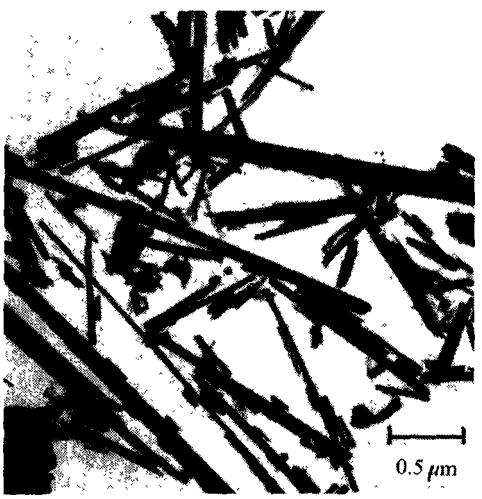

Fig. 1 Electron micrograph of the suspension of asbestos fibres injected into the rat stomach. Note the varying sizes of the bundles of fibres present.

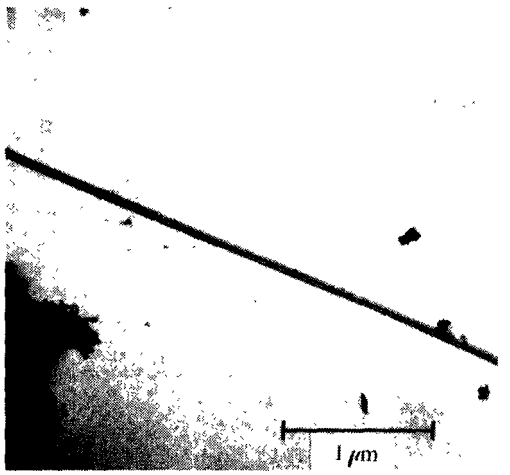

Fig. 2 Small bundle of asbestos fibres from the peritoneum of a rat. This bundle is at least 15 μm long.

surrounds the small intestine, appeared to accumulate most asbestos. Although the asbestos fibre content of the omentums in rats in group 2 may seem rather high (one extreme count contributed to the high standard error) the omentum does not seem to respond as do the other tissues. As well as having little ability to clear itself, fibre accumulation in the omentum may take place much more slowly than it does in other tissues. As the omentum consists partly of peritoneum and partly adipose tissue, asbestos fibres may enter the peritoneum by direct penetration of the intestinal wall. Concerning the prolonged retention of asbestos fibres in the omentum in women asbestos workers, Keal[8] found a higher incidence of peritoneal cancer than of lung cancer.

The amount of asbestos found in the tissues of the controls is higher than we had anticipated, considering the age of the rats and the number of fibres they might have consumed in their drinking water[4]. But we do not know how much was in their feed or air. We have analysed the tissues of some people who died of natural causes and found somewhat similar levels. In one person there was twice as much asbestos in the brain as in the brains of our control rats and about one quarter as much in the omentum, but the omentum content was still higher than the brain.

Most of the fibres found were about 0.2–2.0 μm long but some measured 15 μm (Fig. 2) and one fibre in the blood of a rat was 23.55 μm long. We do not know how the fibres pass through the intestinal wall. Some long ones may pierce the gut like a needle, whereas pinocytosis may account for the absorption of the small ones.

We demonstrated earlier the presence of asbestos fibres in air, snow, city drinking water and a number of beverages[4]. The experiment described here shows that fibres of a similar size administered into the stomach of rats appear in the blood and accumulate in various tissues in the body.

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Presynaptic and Postsynaptic Effects of Lead at the Frog Neuromuscular Junction

LEAD has long been known to be toxic to animals and humans, with some of its effects being attributable to actions on the neuromuscular system[1,2]. Since several other polyvalent cations have potent effects on neuromuscular transmission[3-19], the question arose as to whether one of the manifestations of lead toxicity might be an interference with neuromuscular transmission. Answers to this question might elucidate some mechanisms of lead toxicity and, at the same time, improve our understanding of junctional transmission. This report shows that lead influences both pre and postsynaptic events in neuromuscular transmission with the presynaptic ones being most sensitive.

There have been no previous electrophysiological studies of the effects of lead on neuromuscular transmission. However, Kostial and Vouk[20], in a study of the perfused superior cervical ganglion of the cat, found that lead nitrate, in concentrations as low as 12.1 μM, blocked ganglionic transmission. The authors concluded that this effect was due to a presynaptic action of lead because: (*a*) the acetylcholine (ACh) output of the eserinized ganglion, resulting from preganglionic nerve stimulation, was reduced by lead, and (*b*) the postganglionic response to perfused ACh was not affected by lead.

Experiments were performed *in vitro* on the isolated sciatic nerve-sartorius muscle preparation of the frog (*Rana pipiens*). Superficial neuromuscular junctions were located optically with a compound microscope (magnification ×400). Standard microelectrode and photographic techniques were used to monitor intracellular responses from individual end-plates. Responses were recorded first while the preparation was bathed in a control Ringer solution, then in the presence of lead, added as $PbCl_2$, and finally after the lead had been washed out. Solutions entered the experimental chamber by gravity and left by suction. The composition of the normal Ringer solution was: 111 mM NaCl, 2.5 mM KCl, 2.0 mM $CaCl_2$, 4 mM Tris-maleate. All Ringer solutions were maintained at *p*H 6.9 and at a temperature of 15° C. Preparations were exposed to lead by bathing them in a Ringer solution to which 0.01–0.10 mM $PbCl_2$ had been added. To record subthreshold end-plate potentials, nerve-muscle preparations were bathed in 0.8 mM Ca^{2+}/5.5 mM Mg^{2+}-Ringer solution[3,21]. Acetylcholine was applied directly to the end-plate receptors by iontophoresis from a micropipette[22,23] in order to test for postsynaptic effects of lead.

We have consistently observed that lead increases the frequency of miniature end-plate potentials (Figs 1 and 2). The

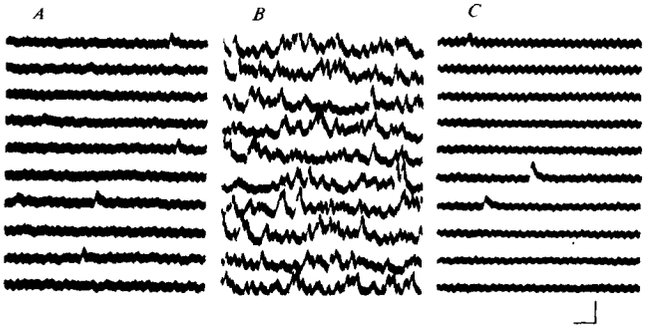

Fig. 1 Effect of lead on the frequency of miniature end-plate potentials. *A*, Control: the preparation was bathed in normal Ringer solution. *B*, Five minutes after exposing the preparation to 0.10 mM Pb^{2+}-Ringer solution: the frequency increased greatly while the amplitudes of the miniature end-plate potentials were essentially unchanged. *C*, Five minutes after washing the preparation with normal Ringer solution: the frequency was no longer elevated. Oscillograms *A–C* were taken from the same neuromuscular junction. Vertical calibration: 1 mV. Horizontal calibration: 50 ms.

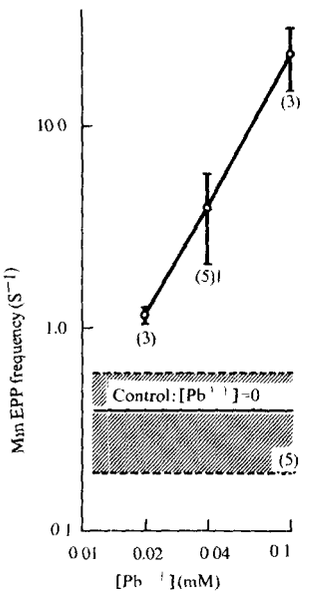

Fig. 2 A double logarithmic plot showing the dose-response relationship between the lead concentration and the frequency of miniature end-plate potentials. Each point is the mean ± s.e. for measurements made from three to five fibres. The control frequency was measured in the absence of lead and is depicted by horizontal lines (mean ± s.e.). All measurements were made after the preparations were exposed to the Pb^{2+}-Ringer solution for 5 min. Although not shown in the above plot, the mean frequency decreased to 0.42 ± 0.10 s^{-1} 5 min after washing the preparations with normal Ringer solution (see also Fig. 1*C*).

records shown in Fig. 1*A* were made while the preparation was bathed in normal Ringer solution; those in Fig. 1*B* were made after bathing the preparation in 0.10 mM Pb^{2+}-Ringer solution for 5 min; and those in Fig. 1*C* were recorded after washing with normal Ringer solution for 5 min. Data taken from several fibres are shown graphically in Fig. 2 which is a log-log plot of the relationship between the lead concentration and the frequency of miniature end-plate potentials. The frequency during control conditions was 0.39 ± 0.20 s^{-1} (mean ± s.e.), and it increased by almost two orders of magnitude in the presence of 0.10 mM Pb^{2+}, the actual value being 22.1 ± 7.6 s^{-1}. When the fibres were washed with normal Ringer solution, the frequency returned to 0.42 ± 0.10 s^{-1} within 5–10 min. Thus, this effect of lead is reversible (see also Fig. 1*C*).

In 1954, del Castillo and Katz postulated that two modes of transmitter release exist at the frog neuromuscular junction—one for the spontaneous release of individual quanta and another for the synchronous or phasic release of several hundred quanta of the transmitter, ACh[11]. This hypothesis is based on the observation that magnesium did not reduce the frequency of the spontaneous release of transmitter even though this ion had been shown previously to inhibit the phasic release following a nerve impulse[3]. Calcium antagonized this blocking effect of magnesium[3], but it had no clear effect on the frequency of miniature end-plate potentials[24]. Our experiments show that lead depresses the phasic release of transmitter (Fig. 3). Subthreshold end-plate potentials were recorded in 0.8 mM Ca^{2+}/5.5 mM Mg^{2+}-Ringer solution. The motor nerve was stimulated with pairs of pulses to facilitate transmitter release during the second transmission, thereby producing easily detectable (facilitated) end-plate potentials. The interval between pulses in a pair was 5–7 ms, and the pair of stimuli were applied at a rate of 3/s. Since the amplitude of end-plate potentials recorded from preparations bathed in high magnesium and low calcium Ringer solution varies according to the quantal hypothesis[25], many recordings were made in a particular condition to obtain the mean ± s.e. of the end-plate potential. The facilitated end-plate potential during the control period was 4.71 ± 0.15 mV ($n = 18$) (Fig. 3*A*). The preparation was then exposed to 0.01 mM Pb^{2+}, the Ca^{2+}/Mg^{2+} ratio