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# ALTERATION OF THE LOCOMOTIVE ACTIVITY OF MICE POISONED WITH SUBLETHAL DOSES OF LEAD ACETATE

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It was shown that locomotometry allows for continuous recording of some injurious effects of lead poisoning upon mice. For detection of poisoning with lead acetate in mice, locomotometry was more sensitive than the recording of mortality rates, demonstration of stippled cells in the peripheral blood, or detection of manifest symptoms of illness by plain inspection. Single or multiple intraperitoneal injections of saline or distilled water, after the third post-injection day, do not induce detectable alterations of the locomotive activity (LA) of mice. Dose-dependent alterations of LA have been observed after intraperitoneal administration of lead acetate. Acute, single-dose poisoning induces dose-dependent depressions of the LA, while chronic poisoning with multiple injections tends to induce elevated LA, mostly combined with and exceptionally without depression of the LA. Time trends show intermittent alterations of the LA induced by lead poisoning. In some animals significant alterations of LA lasted as long as observed (from 4 to 6 months), while in others the normal LA was restored within 20 or 60 days after the last injection of lead acetate. Animals poisoned with lethal doses of lead acetate developed stippled cells during the 2nd or 3rd week, while significant alterations of the LA started to be manifest from the first day after the last injection. Mice poisoned with subletal doses (0.4, 0.8 and 1.0 mg) showed no stippled cells, but displayed alterations of LA. Possible applications of locomotometry for continuous measurements of degrees of illness or of clinical improvements, in cases of poisoning or of various treatments, were discussed briefly.

A photoelectric apparatus has been described (1) which permits continuous measurement of the locomotive activity (LA) of animals. It consists of a two – compartment cage interconnected with a tunnel. A photoelectric eye connected with a relay counter records the passings of the animal through the tunnel. Each interruption of the light beam, directed across the tunnel, causes the relay counter to advance one digit.

In the preceding report were described: the construction of the locomotometer, its operation and control, the procedures both of processing and evaluating the numerical data, as well as some possibilities of interpreting the results, obtained by locomotometry of mice.

This report presents experimental data, showing the applicability of locomotometry for studies of lead poisoning in mice.

## MATERIAL AND METHOD

1. *Animals.* White female mice, varying in weight from 21 to 33 gr and in age from 4 to 6 months, were used throughout these experiments. They were held on sawdust as bedding, and fed on oat-barley and water ad libitum, with addition of crushed pellets of a comercial mouse-diet, in amounts of about 0.8 gr per mouse daily. Only animals displaying prior to poisoning »normal LA« (as determined in the previous report) were used in these experiments.

2. *Treatment with lead acetate or water:* 2.0, 0.2 and 0.02 per cent solutions of lead acetate (m. w. 379.31) in distilled water have been injected intraperitoneally, in amounts of 0.5 ml. per mouse, in single doses or in 2, 4 or 8 doses, given at 48 hour intervals, to make the following 6 groups of poisoned animals: mice receiving 4 × 0.1 mg (0.4 mg) of lead acetate; 8 × 0.1 mg (0.8 mg); 1 × 1.0 mg (1.0 mg); 2 × 1.0 mg (2.0 mg); 1 × 10.0 mg (10.0 mg); and 2 × 10.0 mg (20.0 mg) of lead acetate.

From some of the treated animals were taken blood specimens, others were examined for LA, and in a few of them were tested both blood smears and the LA.

Also 10 animals injected intraperitoneally once, 4 times or 8 times, each with 0.5 ml of saline or distilled water, were tested for LA, to serve as controls.

3. *Smears* were prepared from blood samples obtained by cutting the tip of the tail. The smears were airdried, fixed with methanol, and stained for 40 minutes with a stain composed of Toluidin blue 5 gr and sodium tetraborate (m. w. 381.42) 0.5 gr, dissolved in 1000 ml of distilled water.

The stained smears were examined for the presence of stippled red blood cells (RBC). In each microscopic field were counted both the total number of RBC, and the number of stippled cells.

From 20 animals a total of 56 smears was taken, from each animal being examined 1 smear before the treatment with lead acetate, and 1 to 5 smears, at different days, after the last injection.

4. *The locomotive activity* of normal and treated animals was measured in 8 locomotometers. As shown earlier, the instrumental count recorded on the locomotometer is a measure of the animal's LA.

The »time per count« value (TPC) referring to single mouse – days (observed on individual mice for about 24 hour periods) was defined as the average number of minutes for which a mouse causes the counter to advance one digit. The TPC value is inversely proportional to the LA of the animal.

Before any »treatment« daily TPC values of each animal were recorded, on the average for about 11 days per animal. Means of the daily TPC values of normal mice were computed (Mn) for each animal separately. The Mn values of individual animals varied between 0.79 and 2.68. Also the interval limits 0.5 Mn and 1.5 Mn were computed for each normal animal separately. During the pretreatment period observed, none of the animals showed a TPC value $< 0.5$ Mn or $> 1.5$ Mn.

After the injecting, TPC values of each mouse were recorded daily. The statistical significance of the alterations of TPC values observed have been evaluated by the procedures described in a previous report (1).

Of the 10 normal animals, all were tested both before and, from the 7th day on, after injection with saline or distilled water. Only four of them were tested also during the first 6 days after the last injection.

Of the 22 animals poisoned with lead acetate, one was tested after the poisoning only, and the rest of 21 mice were tested for LA both before and after poisoning; individual animals were tested for different periods of time.

## RESULTS

### A. *Mortality rates and manifest signs of illness*

A shown in Table 1. of the 22 animals poisoned with 0.4, 0.8 or 1.0 mg of lead acetate, none succumbed to the treatment. Except 3 animals, all appeared normal during the 2–6 months period of visual inspection. The 3 exceptions were 2 mice receiving 4 injections, and one which received 8 injections of the poison. These 3 animals displayed quite unusual jumpiness, consumed increased amounts of food and revealed obvious hypermotility.

Of the 4 animals poisoned with 2 mg of lead acetate, only two revealed ruffled fur and decreased motility, detectable by plain inspection, for certain periods of observation time. One of these succumbed to the treatment.

Eleven mice were treated with 10 or 20 mg of lead aceate. All manifested obvious symptoms of illness, with depressed motility which was detectable also by plain inspection. Within 2 to 37 days after the last injection, all of these mice died. Only one of the mice treated with 10 mg of the poison was tested for LA, the one surviving the longest time after the poisoning.

Table 1.
*Lethality of poisoned animals*

| Doses of lead acetate in mg-s | Number of mice | Percent mortality | Survival in days[a] | Observed for days |
|---|---|---|---|---|
| 4 × 0.1 | 10 | 0 | — | 56 – 127 |
| 8 × 0.1 | 6 | 0 | — | 62 – 178 |
| 1 × 1.0 | 6[b] | 0 | — | 62 – 134 |
| 2 × 1.0 | 4 | 25 | 50 | 50 – 62 |
| 1 × 10.0 | 4 | 100 | 2, 2, 6, 37 | 2 – 37 |
| 2 × 10.0 | 7 | 100 | 3, 5, 8, 9, 13 23,30 | 3 – 30 |
| All | 37 | — | 2 – 50 | 2 – 178 |

(a) Counted from the last injection until death (survivors until sacrificed);
(b) Two more mice, poisoned with this dose and observed just for 8 days, are omitted from this table.

## B. *Finding of stippled red blood cells (RBC)*

More than 2000 RBC were examined in each of the smears, taken both from untreated and poisoned animals.

None of the pretreatment specimens, taken from animals to be treated with lead acetate, revealed stippled cells. Data in Table 2 show that in a

Table 2.
*Findings of stippled cells in blood smears of animals poisoned with lead acetate*

| Lead acetate given to single mice | Number of tested | | Smears taken at days after the last injection | Finding of stippled RBC |
|---|---|---|---|---|
| | mice | blood specimens | | |
| 0.4 (4 × 0.1) mg | 3 | 5 | 17, 27, 63 | 0 in 12439 RBC |
| 0.8 (8 × 0.1) mg | 5 | 7 | 2, 13, 23, 59 | 0 in 15612 RBC |
| 1.0 (1 × 1.0) mg | 3 | 3 | 61, | 0 in 7343 RBC |
| 2.0 (2 × 1.0) mg | 2 | 6 | 18, 28, 58 | 0 in 14340 RBC |
| 10.0 (1 × 10.0) mg | 2 | 6 | 2, 6, 12, 20, 35 | 35 in 19600 RBC |
| 20.0 (2 × 10.0) mg | 5 | 9 | 6, 10, 11, 19, 20 | 68 in 20813 RBC |
| 0.4 — 20.0 mg | 20 | 36 | from 2 to 63 | 103 in 90147 RBC |

total of about 50 thousand RBC examined, not a single stippled cell was found in the blood specimens of mice poisoned with sublethal doses of lead acetate.

Of the four animals poisoned with 2.0 mg, two mice, including the only one succumbing to poisoning were tested for LA, while the other two survivors were examined for stippled RBC.

All animals poisoned with 100 per cent lethal doses of lead acetate, after different periods of latency, showed RBC stippled with fine or coarse granules of characteristic appearance.

As shown in Table 1 three of the 4 animals treated with 10 mg, and four of the 7 animals treated with 20 mg of lead acetate, died within 10 days after the last injection. Consequently only 1 of the animals receiving 10 mg, and 3 mice treated with 20 mg, were available for observation after the 10th post-treatment day. Since from one of these animals were taken as many as 5 consecutive specimens, and on the average from each animal were taken 2 post-treatment specimens of blood, the data shown in Table 3 seem to allow for the following conclusions:

Table 3.

*Findings of stippled cells in blood smears of 7 animals poisoned with lead acetate (l. a.)*

| | 2 mice* treated with 10 mg (1 × 10 mg) of l. a. | | | | 5 mice treated with 20 mg (2 × 10 mg) of l. a. | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Smears taken at days | 2 and 6 | 12 | 20 | 35 | 2—35 | 6 | 10 and 11 | 19 and 20 | 6—20 |
| no. of mice bled | 3 | 1 | 1 | 1 | | 4 | 3 | 2 | |
| no. of stippled cells per no. of examined RBC | 0/7230 | 0/5000 | 15/5000 | 20/2370 | 35/19600 | 0/8285 | 31/7070 | 37/5458 | 68/20813 |
| no. of stippled cells per 1000 RBC | 0 | 0 | 3.00 | 8.44 | — | 0 | 4.39 | 6.78 | |

* From one of these 2 animals blood specimens were taken both the 2nd and 6th day as well as on the 12th, 20th and 35th day after the poisoning.

(a) After a period of latency, in the peripheral blood of mice treated with 100 per cent lethal doses of lead acetate, stippled RBC appear in proportions well above 1 : 1000, and persist until the death of the animal.

(b) In mice poisoned with 10 mg of lead acetate the stippled cells appear after a latency of 12–19 days. In the animal from which 5 specimens were examined, the number of stippled cells increased significantly until the death of the animal. The significance of the difference in the proportions observed on the 20th and 35th day, gives a chi square value of about 10 ($p = 0.001$).

(c) In mice poisoned with 20 mg of lead acetate, the stippled cells appeared earlier (between the 7th and 10th day after treatment) than in mice poisoned with 10 mg. The difference between proportions observed on the 10th and 20th day, showed to be highly significant.

## C. *Alterations of the locomotive activity*

### 1. *Sets of TPC values displayed by different categories of mice.*

Animals no. 1, 2, 15, 16, 17, 18, 19 and 20 received 0.4 mg. (4 times 0.1 mg); nos. 3, 4 and 5 received 0.8 mg (8 times 0.1 mg); nos. 6, 7, 8, 9, 11, 12, 13 and 14 received 1.0 mg (1 × 1 mg), nos. 21 and 22 received 2.0 mg (2 × 1 mg), and no. 10 received 10.0 mg (1 × 10 mg) of lead acetate. Due to the lack of a sufficient number of locomotometers, the measurements could not be conducted uninterruptedly throughout the whole observation period. Some animals showing pathological TPC values, for periods longer than about 50 days after the treatment, had been retested after an interval of about two months. Occasionally, a few days were skipped even from the observed periods.

The majority of the poisoned animals displayed intermittent alterations of the TPC values. In order to facillitate statistical evaluation of the periodic alterations observed, each time series of the daily TPC values, displayed by individual mice, was subdivided into two or more of the following four types of spells or periods (P.) of the LA:

(a) P. of normal LA, with TPC values between 0.5 Mn and 1.5 Mn.

(b) P. of elevated LA, characterised by TPC values $<$ 0.5 Mn. This period is limited by the first and last TPC value $<$ 0.5 Mn in the series.

(c) P. of depressed LA, characterised by TPC values $>$ 1.5 Mn. This period is limited by the first and last TPC value $>$ 1.5 Mn in the series.

(d) P. of combined alterations of the LA (TPC $<$ 0.5 Mn or $>$ 1.5 Mn). This period is limited by the first and last TPC value $<$ 0.5 Mn or $>$ 1.5 Mn in the series.

### 2. *Discrimination between »mechanical injuries« and peracute toxic effects upon the LA*

In intraperitoneal administration of the poison discrimination should be made between alterations of the LA due to toxic effects of the poison, and possible alterations due to mechanical injuries, caused by the act of intraperitoneal injection. With this in mind were introduced the water – controls, already described.

Alterations of the LA, observed in mice injected with saline or distilled water, all took place during the first 3 days after the last injection. Data in Table 4 show alterations of the LA during the first 6 post-injection days observed in 22 mice treated with saline, distilled water or lead acetate.

No correlation could be found between the number of injections given and the proportion of altered TPC values revealed. However, a positive correlation does exist between the dose of lead acetate administered and the proportion of altered TPC values displayed during the first 6 post-injection days. These observations supported with those presented in

Table 4.

*Alterations of the LA observed during the first 6 post-injection days*

| Mice | Treatment (per mouse) | | Number of mouse-days | | | Proportion of mouse-days with altered TPC values | | |
|---|---|---|---|---|---|---|---|---|
| | mg of lead acetate | no of injections | tested | with TPC values >1.5Mn | with TPC values <0.5Mn | > 1.5 | < 0.5 | All |
| 23 | 0 (sal.) | 1 | 6 | 0 | 0 | | | |
| 24 | 0 (sal.) | 1 | 6 | 2 | 0 | (3/24) 13% | (0/24) 0% | (3/24) 13% |
| 25 | 0 (d. w.) | 4 | 6 | 0 | 0 | | | |
| 26 | 0 (d. w.) | 4 | 6 | 1 | 0 | | | |
| 15 | 0.4 | 4 | 6 | 0 | 5 | | | |
| 16 | 0.4 | 4 | 5 | 0 | 1 | | | |
| 17 | 0.4 | 4 | 6 | 0 | 0 | (5/33) 15% | (6/33) 18% | (11/33) 33% |
| 18 | 0.4 | 4 | 5 | 1 | 0 | | | |
| 19 | 0.4 | 4 | 5 | 2 | 0 | | | |
| 20 | 0.4 | 4 | 6 | 2 | 0 | | | |
| 3 | 0.8 | 8 | 1 | 0 | 1 | (0/2) 0% | (1/2) 50% | (1/2) 50% |
| 4 | 0.8 | 8 | 1 | 0 | 0 | | | |
| 6 | 1.0 | 1 | 3 | 0 | 0 | | | |
| 7 | 1.0 | 1 | 3 | 0 | 0 | | | |
| 8 | 1.0 | 1 | 3 | 2 | 0 | | | |
| 9 | 1.0 | 1 | 6 | 2 | 0 | (21/39) 54% | (0/39) 0% | (21/39) 54% |
| 11 | 1.0 | 1 | 6 | 4 | 0 | | | |
| 12 | 1.0 | 1 | 6 | 4 | 0 | | | |
| 13 | 1.0 | 1 | 6 | 4 | 0 | | | |
| 14 | 1.0 | 1 | 6 | 5 | 0 | | | |
| 21 | 2.0 | 2 | 5 | 5 | 0 | (11/11) 100% | (0/11) 0% | (11/11) 100% |
| 22 | 2.0 | 2 | 6 | 6 | 0 | | | |

Data referring to the first 6 post-injection days of animals no. 1, 2, 5 and 10 are lacking.

sal. = saline

d. w. = distilled water.

Table 5 and Figures 1 and 2 allow for the following conclusions: (a) alterations of LA due to mechanical injuries caused by intraperitoneal injections are not detectable after the 3rd post-injection day, and (b) significant, dose-dependent, alterations of LA due to acute lead poisoning, do show up already during the first 6 post-injection days.

Table 5.

*Compared frequencies of TPC values > 1.5 Mn and < 0.5 Mn*

| Mouse. Dose of lead acetate | Limit interval 0.5 Mn – 1.5 Mn | Periods of »normal« LA activity | | | Spells of »altered« LA activity | | Significance of difference between compared frequencies |
|---|---|---|---|---|---|---|---|
| | | pre injection | post injection | all | post injection | all | |
| 1. 0.4 mg | 1.18 – 3.54 | 0/9(a) (5–18)(b) | 0/8 (51 – 59) | 0/17 | 5/15 (12 – 38) | 5/15 | $p = 0.1$ |
| 2. 0.4 mg | 1.25 – 3.74 | 0/9 (5 – 14) | 1/12 (22 – 38) 0/10 (57–126) | 1/31 | 6/6 (10 – 15) 4/6 (51 – 56) | 10/12 | $p < 0.002$ |
| 15. 0.4 mg | 0.68 – 2.05 | 0/16 (1 – 23) | | 0/16 | 44/49 (8– 56) | 44/49 | $p < 0.002$ |
| 16. 0.4 mg | 0.74 – 2.23 | 0/20 (1 – 22) | 0/28 (29 – 56) | 0/48 | 4/21 (7 – 28) | 4/21 | $p = 0.1$ |
| 17. 0.4 mg | 0.40 – 1.19 | 0/20 (1 – 20) | 0/18 (7 – 24) | 0/38 | 26/29 25 – 56) | 26/29 | $p < 0.002$ |
| 18. 0.4 mg | 1.34 – 4.02 | 0/22 (1 – 23) | 0/45 (12 – 56) | 0/67 | 1/4 (8 – 11) | 1/4 | $p > 0.2$ |
| 19. 0.4 mg | 0.62 – 1.86 | 0/13 (1 – 13) | 0/18 (38 – 56) | 0/31 | 10/27 (9 – 37) | 10/27 | $p = 0.002$ |
| 20. 0.4 mg | 0.71 – 2.13 | 0/20 (1 – 20) | 0/37 (7 – 43) 0/9 (48 – 56) | 0/66 | 1/4 (44 – 47) | 1/4 | $p > 0.2$ |
| 6. 1.0 mg | 0.53 – 1.58 | 0/5 (2 – 20) | 0/15 (c) (4 – 29) 0/14 (42–134) | 0/34 | 2/4 (37 – 41) | 2/4 | $p = 0.05$ |
| 7. 1.0 mg | 0.59 – 1.76 | 0/6 (2 – 16) | 0/5 (c) (6 – 14) 0/12 (19 – 42) | 0/23 | 2/4 (15 – 18) | 2/4 | $p = 0.1$ |
| 8. 1.0 mg | 0.70 – 2.09 | 0/4 (2 – 6) | – | 0/4 | 12/28 (7 – 124) | 12/28 | $p > 0.2$ |
| 9. 1.0 mg | 0.71 – 2.12 | 0/5 (1 – 16) | – | 0/5 | 23/31 (7 – 124) | 23/31 | $p = 0.02$ |
| 11. 1.0 mg | 0.58 – 1.73 | 0/13 (1 – 13) | 0/13 (8 – 20) 0/10 (24 – 33) | 0/36 | 1/3 (21 – 23) | 1/3 | $p > 0.2$ |
| 12. 1.0 mg | 0.78 – 2.33 | 0/14 (1 – 14) | – | 0/14 | 16/32 (8 – 39) | 16/32 | $p = 0.005$ |
| 3. 0.8 mg | 0.93 – 2.78 | 0/8 (9 – 16) | – | 0/8 | 44/46 (7 – 132) | 44/46 | $p < 0.002$ |

continued Table 5.

| Mouse. Dose of lead acetate | Limit interval 0.5 Mn – 1.5 Mn | Periods of »normal« LA activity | | | Spells of »altered« L activity | | Significance of difference between compared frequencies |
|---|---|---|---|---|---|---|---|
| | | pre injection | post injection | all | post injection | all | |
| 4.<br>0.8 mg | 0.70 – 2.10 | 0/6<br>(9 – 15) | 0/11<br>(7 – 28)<br>0/16<br>(47–122) | 0/33 | 3/5<br>(29 – 34) | 3/5 | $p = 0.01$ |
| 5.<br>0.8 mg | 0.78 – 2.34 | 0/5<br>(11 – 15) | 1/10<br>(17 – 26)<br>1/11<br>(103–178) | 2/26 | 3/5<br>(12 – 16)<br>9/13<br>(27–102) | 12/18 | $p < 0.002$ |
| 21.<br>2.0 mg | 0.73 – 2.19 | 0/9<br>(1 – 13) | – | 0/9 | 32/33<br>(8 – 40) | 32/33 | $p < 0.002$ |
| 22.<br>2.0 mg | 0.81 – 2.43 | 0/11<br>(1 – 12) | 0/8<br>(15 – 22)<br>0/4<br>(37 – 40) | 0/23 | 3/7<br>(8 – 14)<br>6/14<br>(23 – 36) | 9/21 | $p = 0.01$ |
| 10.<br>10 mg | – | – | – | – | 24/25 (d)<br>(7 – 34) | 24/25 | $p < 0.002$ |

(a) Number of »altered« (with TPC $>$ 1.5 Mn and $<$ 0.5 Mn) per all mouse-days observed.

(b) Period designating the 1st and last post-injection days observed.

(c) To increase the statistical mass, here are counted also a few days before the 7th post-injection day, but only those with normal TPC values. In all other cases were counted only the days after the 6th post-injection day.

(d) LA not tested before poisoning. Of the 25 post-injection days tested, 24 showed TPC values $>$ 10 (highly significant alteration of the LA).

In spite of this, for further analysis will be considered only data obtained after the 6th post-injection day. This precaution was taken to avoid misinterpretations due to possible interference of the effect of lead poisoning upon the LA of the poisoned animals, with that of traumatisation due to intraperitoneal injections.

### 3. *Significance of the alterations observed*

None of the 10 animals injected intraperitoneally once, 4 times or 8 times, with saline or with distilled water, showed alterations of locomotive activity. Of the animals treated with lead acetate, animal no. 10 displayed 24 days and animal no. 21 displayed 4 days TPC values $>$ 10, while mice nos. 3, 15 and 17 displayed 32, 29 and 20 days respectively TPC values $<$ 0.3 (see Table 6).

According to the procedure of »absolute threshold levels« described earlier (i), mice nos. 3, 10, 15, 17 and 21 showed highly significant alterations of LA, after the 6th post-injection day.

An evaluation of the significance of the alterations of LA, according the procedure of »relative threshold levels« can be performed on the basis of the data presented in Table 5.

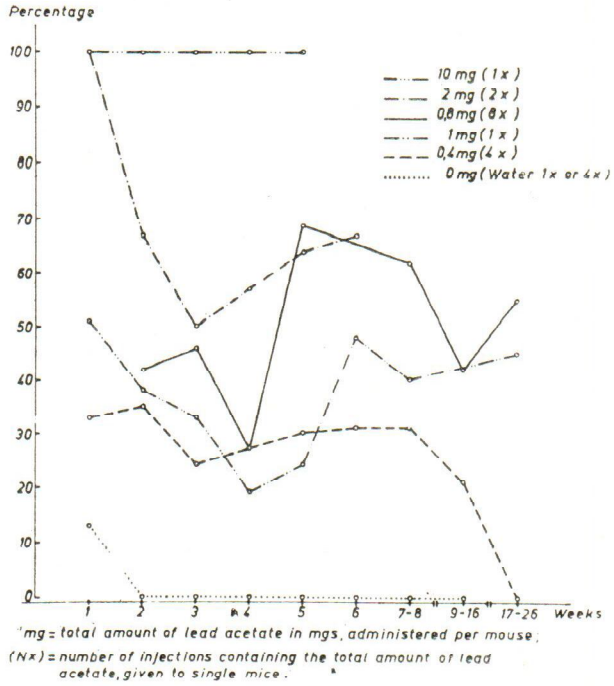


Fig. 1. *Percentage of altered TPC values (< 0.5 or > 1.5 Mn), at week after the last injection*

Frequencies of altered TPC values, occurring in periods of normal activities, have been compared with those occurring at spells of altered TPC values, as shown for each animal separately. The last column of table 5 shows that the proportion of mice, with statistically significant alterations of LA, in groups of animals poisoned with 0.4, 1.0, 0.8, 2.0 and 10.0 mg lead acetate, amounted to 4/8, 3/6, 3/3, 2/2 and 1/1 respectively. Comparing grouped data, as shown in Table 6, the procedure of »relative threshold levels« revealed rather quantitative information about the significance of the difference between alterations of the LA, displayed by different categories of mice. It is thus possible to compare, by chi-square test, the combined proportion of altered TPC values displayed by mice treated with single doses of 1.0 mg (59/169) with that displayed by mice poisoned with 0.8 mg of lead acetate in 8 successive doses (61/117). A chi-square value of 7.3 indicates ($p < 0.01$) that chronic poisoning with a dose of 0.8 mg induced actually more significant alterations of the LA than an acute poisoning with a dose of 1.0 mg of lead acetate.

4. *Dose-dependence and chronicity of the alterations*

Table 6 shows that the average percent – incidence of the altered TPC values observed, after the 6th post-injection day, in the 5 categories of animals, receiving 0.4 mg, 1.0 mg, 0.8 mg, 2.0 mg and 10.0 mg of lead acetate per mouse, amounted to 29, 35, 52, 61 and 100 per cent respectively. The correlation coefficient computed from these sets of data was $r = 0.9282$ ($p = 0.025$), indicating a significant positive association

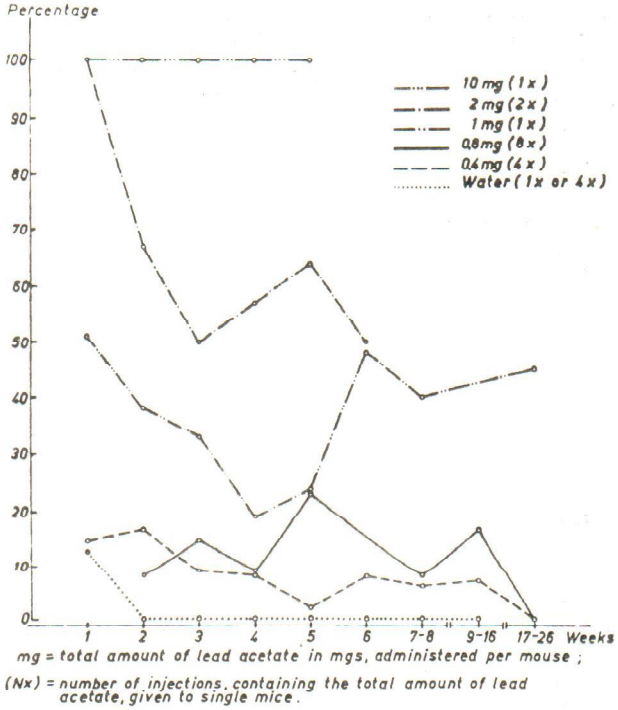


Fig. 2. *Percentage of TPC values > 1.5 Mn, at weeks after the last injection*

between the two variables observed. The actual correlation was somewhat impaired by the fact, that doses of 1.0 and 0.8 mg induced altered TPC values in 35 and 52 per cent proportions respectively. This was due to neglection of the »chronicity« of the poisoning, a parameter contributing significantly to the alterations of LA.

Comparing by chi-square test the combined proportions of altered TPC-values revealed by the various categories of mice, shown in the last column of Table 6, one may find reliable evidences also for an actual dose-dependence of the alterations of LA observed in poisoned animals.

Proportion 102/351 compared with 61/117, referring to groups of mice poisoned chronically with 0.4 and 0.8 mg respectively as well as propor-

Table 6.

*Frequency distribution (percentage) of mouse-days with altered TPC values, in single mice and groups of animals*

| Mouse. Dose of lead acetate | | Number of mouse days according TPC values found in single animals | | | | | Same data referring to groups of poisoned animals | | | | | Same data combined | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | $>10^a$ | $<1.5$ Mn | $<0.5$ Mn | $<0.3^b$ | All (100) | $>10^a$ | $>1.5$Mn | $<0.5$Mn | $<0.3^b$ | All (100) | $>1.5$Mn and $<0.5$Mn | $>10^a$ and $<0.3^b$ | All (100) |
| 1 | 0.4 mg | 0 | 2 (8) | 3 (12) | 0 | 25 | 0 | 27 (8) | 75 (21) | 49 (14) | 351 | 102 (29) | 49 (14) | 351 |
| 2 | | 0 | 11 (32) | 0 | 0 | 34 | | | | | | | | |
| 15 | | 0 | 1 (2) | 43 (88) | 29 (59) | 49 | | | | | | | | |
| 16 | | 0 | 1 (2) | 3 (6) | 0 | 49 | | | | | | | | |
| 17 | | 0 | 0 | 26 (55) | 20 (43) | 47 | | | | | | | | |
| 18 | | 0 | 1 (2) | 0 | 0 | 50 | | | | | | | | |
| 19 | | 0 | 10 (21) | 0 | 0 | 47 | | | | | | | | |
| 20 | | 0 | 1 (2) | 0 | 0 | 50 | | | | | | | | |
| 6 | 1.0 mg | 0 | 2 (7) | 0 | 0 | 30 | | | | | | | | |
| 7 | | 0 | 2 (11) | 0 | 0 | 18 | | | | | | | | |

Continued Table 6.

| Mouse. Dose of lead acetate | | Number of mouse days according TPC values found in single animals | | | | | Same data referring to groups of poisoned animals | | | | | Same data combined | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | $>10^a$ | $>1.5$ Mn | $<0.5$ Mn | $<0.3^b$ | All (100) | $>10^a$ | $>1.5$Mn | $<0.5$Mn | $<0.3^b$ | All (100) | $>1.5$Mn and $<0.5$Mn | $>10^a$ and $<0.3^b$ | All (100) |
| 8 | 1.0 mg | 0 | 12 (43) | 0 | 0 | 28 | 0 | 59 (35) | 0 | 0 | 169 | 59 (35) | 0 | 169 |
| 9 | | 0 | 23 (74) | 0 | 0 | 31 | | | | | | | | |
| 11 | | 0 | 1 (4) | 0 | 0 | 26 | | | | | | | | |
| 12 | | 0 | 16 (50) | 0 | 0 | 32 | | | | | | | | |
| 13 | | 0 | 1 (50) | 0 | 0 | 2 | | | | | | | | |
| 14 | | 0 | 2 (100) | 0 | 0 | 2 | | | | | | | | |
| 3 | 0.8 mg | 0 | 0 | 44 (96) | 32 (70) | 46 | 0 | 11 (9) | 50 (43) | 32 (27) | 117 | 61 (52) | 32 (27) | 117 |
| 4 | | 0 | 2 (6) | 1 (3) | 0 | 32 | | | | | | | | |
| 5 | | 0 | 9 (23) | 5 (13) | 0 | 39 | | | | | | | | |
| 21 | 2.0 mg | 4 (12) | 32 (97) | 0 | 0 | 33 | 4 (6) | 38 (58) | 2 (3) | 0 | 66 | 40 (61) | 4 (6) | 66 |
| 22 | | 0 | 6 (18) | 2 (6) | 0 | 33 | | | | | | | | |
| 10 | 10.0 mg | 24 (96) | 25 (100) | 0 | 0 | 25 | 24 (96) | 25 (100) | 0 | 0 | 25 | 25 (100) | 24 (96) | 25 |

This table presents data observed from the 7th post-injection day on.
a: Cases with TPC $>10$ are included in the number of cases with TPC $>1.5$ Mn.
b: Cases with TPC $<0.3$ are included in the number of cases with TPC $<0.5$ Mn.

tion 59/169 compared with 40/66, referring to groups of mice poisoned with 1.0 and 2.0 mg of lead acetate respectively, both yielded chi-square values indicating ($p = 0.005$) that the proportions of altered TPC-values, in the groups of mice compared, were significantly different.

From the data presented in Table 6 it appears, that the variation of percent-incidence of altered mouse days within groups of mice was much greater, than the variation of the same variable between the 5 groups of animals. However, in view of the following facts, this actually may not be true:

(a) After the administration of the poison, the LA of different mice has not been recorded continually, neither was it recorded in all animals for the same length of time, nor at the same time intervals.

(b) Consecutive spells of altered LA, observed in the different animals of the same group, varied both in duration and in the length of time intervals separating them.

These circumstances might be contributing significantly to the apparently enormous variation of the percent incidence of altered mouse – days within groups of animals treated with the same dose of the poison.

Table 7 shows the number of mouse days with TPC $>$ 1.5 Mn and TPC $<$ 0.5 Mn, over the number of mouse – days observed during the corresponding weeks. Expressed as percentages, these proportions are plotted against the time scale in Figures 1, 2 and 3.

As shown in Figure 1, the frequency of altered TPC – values observed in poisoned animals proved to be dose – dependant, excepting the

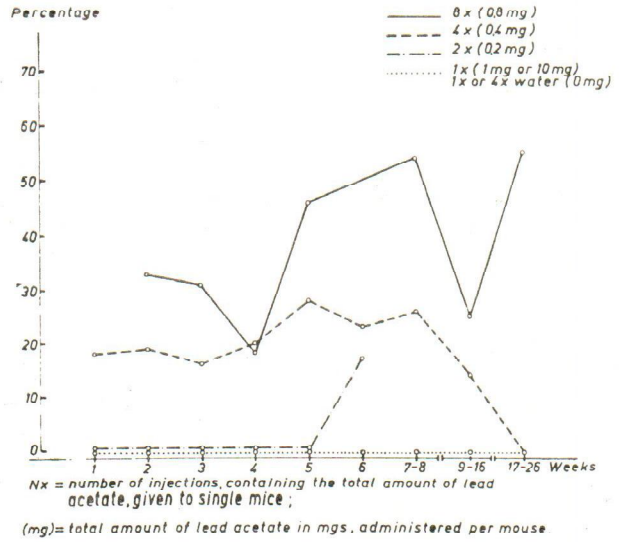


Fig. 3. *Percentage of TPC values $<$ 0.5 Mn, at weeks after the last injection*

Table 7.

*Frequencies of altered LA observed at weekly periods*

| Treatment | No. of mice | Weeks after the last injection | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1. | 2. | 3. | 4. | 5. | 6. | 7–8 | 9–16 | 17–26 | 1–26 |
| 1 × saline or 4 × distilled water | 4 | 3+0 * / 24 | 0+0 / 18 | 0+0 / 14 | 0+0 / 14 | 0+0 / 14 | 0+0 / 14 | 0+0 / 27 | 0+0 / 2 | – | 3+0 / 127 |
| 4 × 0.1 mg l. a. | 8 | 5+6 / 33 | 8+9 / 48 | 4+7 / 45 | 4+10 / 51 | 1+13 / 47 | 4+11 / 49 | 5+23 / 90 | 1+2 / 14 | 0+0 / 7 | 32+81 / 384 |
| 1 × 1.0 mg l. a. | 8 | 20+0 / 39 | 8+0 / 21 | 12+0 / 36 | 5+0 / 26 | 5+0 / 21 | 11+0 / 23 | 8+0 / 20 | – | 10+0 / 22 | 79+0 / 208 |
| 8 × 0.1 mg l. a. | 3 | 0+1 / 2 | 1+4 / 12 | 2+4 / 13 | 1+2 / 11 | 3+6 / 13 | 0+1 / 1 | 2+14 / 26 | 2+3 / 12 | 0−16 / 29 | 11+51 / 119 |
| 2 × 1.0 mg l. a. | 2 | 11+0 / 11 | 8+0 / 12 | 7+0 / 14 | 8+0 / 14 | 9+0 / 14 | 6+2 / 12 | – | – | – | 77 |
| 1 × 10.0 mg l. a. | 1 | 2+0 / 2 | 7+0 / 7 | 5+0 / 5 | 6+0 / 6 | 7+0 / 7 | | | | | 27+0 / 27 |
| All | 26 | | | | | | | | | | 201+134 / 942 |

* Nominators show the number of mouse – days with TPC > 1.5 Mn and TPC < 0.5 Mn, over the number of mouse – days observed during the corresponding weeks.

l. a. = lead acetate.

fact that mice treated with 8 doses of 0.1 mg showed a greater proportion of altered TPC-values, than animals poisoned with a single dose of 1.0 mg of lead acetate. However, if the frequencies of TPC-values $> 1.5$ Mn were plotted apart from the frequencies of TPC, values $< 0.5$ Mn, it became evident, that:

(a) depressions of LA (as measured by frequencies of TPC-values $\geq$ 1.5 Mn) show a fair dose-dependence, regardless of the poison being administered in single or multiple injections (see Figure 2);

(b) elevations of LA (as measured by frequencies of TPC-values $<$ 0.5 Mn) show a fair dependence on the number of injections given (of the chronicity of the poisoning), regardless of the dose of the poison administered (see Figure 3).

It was striking to notice (see Table 7), that in a mass of 942 mouse days, of a total of 335 altered TPC-values observed, the 134 mouse – days with TPC-values $< 0.5$ Mn occurred just in animals poisoned »chronically« with multiple doses of lead acetate. None of these 134 mouse days, with TPC $< 0.5$ Mn, occurred in animals treated with single or multiple doses of water or with single doses of the poison. This observation proved to be significant by several statistical calculations.

The observations described show clearly, that acute lead poisoning, induced by single doses, tends to depress the LA of mice, while chronic poisoning, induced by repeated administration of lead for an extended period of time, tends to induce, beside a depression, spells of hyperactivity, (in some animals hyperactivity only).

A notion that the observed hyperactivity could be caused by mechanical injuries, induced by multiple injections, must be dispelled by the following evidences. It was shown already, that single or multiple intraperitoneal injections of water, if inducing alterations at all, induced depressions (not elevations) of the LA, which took place within the first 3 days after the last injection. In contrast to that, elevations of the LA took place only in poisoned animals, as a rule increased in frequency after the 7th post-injection day, and in some instances could be observed for months after the last injection.

Data in Table 6 show also, that TPC values exceeding the critical treshold levels (TPC $< 0.3$ or $> 10.0$), were displayed by mice poisoned with doses of 0.4, 0.8, 2.0 and 10.0 mg of lead acetate. This observation too, supports the notion, that chronic poisoning with total doses as small as 0.4 or 0.8 mg of lead acetate was more injurious to mice, than an acute poisoning induced by a single injection of 1.0 mg.

Some of the poisoned animals displayed alternations of remissions and relapses.

## DISCUSSION

In Table 8 are compared the mortality rates, the findings of stippled cells, and the results of locomotometry, displayed by mice poisoned with various doses of lead acetate. These data allow for the following conclusions:

Table 8.

*Comparison of some measurable manifestations of lead poisoning in mice*

| Dose of lead acetate per mouse | Mortality (a) | Stippled RBC (a) | Altered LA (a) | Frequency of mouse days with altered TPC (%) |
|---|---|---|---|---|
| 0.4 (4 × 0.1) mg | 0/10 | 0/3 | 4/8 | 102/351 (29) |
| 0.8 (8 × 0.1) mg | 0/6 | 0/5 | 3/3 | 61/117 (52) |
| 1.0 (1 × 1.0) mg | 0/6 | 0/3 | 3/6 | 59/169 (35) |
| 2.0 (2 × 1.0) mg | 1/4 | 0/2 | 2/2 | 40/66 (61) |
| 10.0 (1 × 10.0) mg | 4/4 | 1/1(b) | 1/1 | 25/25 (100) |
| 20.0 (2 × 10.0) mg | 7/7 | 3/3(c) | — | — |

(a) Number of positive mice per number of examined animals.

(b) Before the 20th post-injection day smears from two animals were examined, both giving negative result. One of these succumbed to the treatment at the 6th day. The other survived for 37 days. This is the animal, which was examined both for stippled cells and locomotive activity. Stippled RBC revealed after the 20th day.

(c) Before the 10th day were tested 4 animals, all giving negative result. Smears were examined altogether from 5 mice, of which 2 died before the 10th day. The 3 positive animals displayed stippled cells on or after the 10th day.

(a) Chronic or acute poisoning with sublethal doses of lead acetate (0.4, 0.8 and 1.0 mg) stimulated no appearance of stippled cells, whereas displayed significant alterations of the LA of the poisoned animals. It is obvious, that for studies of poisoning, locomotometry proved to be more sensitive than the examination of blood for stippled cells.

(b) Acute poisoning with lethal doses of lead acetate stimulated the appearance of stippled cells in the course of the 2nd or 3rd week after the administration of the poison (see Table 3). In contrast to that, animals poisoned with doses of lead acetate inducing 25 to 100 per cent fatality, started to manifest significantly altered LA immediately after the last injection given.

(c) Locomotometry appears to allow for a continuous, daily follow-up of the injurious effect of the poison for any length of time, and its conduction yields no additional injury whatsoever to the animal tested.

The duration of the alterations of the LA observed in mice, appears to be consistent with some findings disclosed by other methods on rats. *Castellino* and *Aloj* (2) reported on slow removal of lead from the organism of poisoned rats. *Schepers* (3) described significant lesions, in various organs of rats sacrificed, as late as 21 weeks after poisoning with sublethal doses.

The toxicity of lead acetate injected intraperitoneally into rats, seems to be comparable (4) with that we found for mice.

Both depressions and elevations of the LA, observed in poisoned mice, appear to have their clinical equivalents in some of the well-known manifestations of lead poisoning in men, characterised by paralyses and other symptoms of depression of the neuromuscular activity, irritability of the nervous system and other symptoms of psychomotoric excitations, with wide variations in the time of onset, severity and duration of symptoms (5, 6, 7 and other textbooks). It is well known also that the clinical manifestation of neuromuscular disturbances observed in men may be temporary, intermittent or permanent. As shown in this report, the alterations of the LA of poisoned mice too displayed a similar diversity both in manifestation and in outcome.

It is known further that the intensity of the hæmatological and histological findings, the results of various biochemical tests, and the amounts of excreted or deposited lead, do not correlate with the severity or duration of the clinical symptoms in men poisoned with lead. It also appears that in animals, poisoned with sublethal doses, no reliable tests are available to measure the »clinical severity« of plumbism. Consequently, a large array of various treatments with claimed or possible preventive or therapeutic value, lacks the opportunity of being evaluated properly.

For the study of such problems, locomotometry seems to be a suitable model, which allows for measurement of degrees of illness or discomfort in mice poisoned, or treated in other ways.

*Sadržaj*

## ALTERACIJA LOKOMOTORNE AKTIVNOSTI (LA) MIŠEVA OTROVANIH SUBLETALNIM DOZAMA OLOVNOG ACETATA

Metod lokomotometrije omogućuje kontinuirano registrovanje patološkog dejstva otrovanja olovom na miševe. Za detekciju otrovanja olovom metoda lokomotometrije pokazala se je znatno osetljivijom od praćenja mortaliteta, od nalaza bazofilnih granulacija u eritrocitima, i od registrovanja manifestnih simptoma oboljenja. Od trećeg dana posle aplikacije jedne ili više intraperitonealnih injekcija vode, LA miševa ne ispoljava merljive alteracije. Intraperitonealne injekcije rastvora olovnog acetata izazivaju alteraciju LA, koja je proporcionalna dozi unetog otrova. Akutna otrovanja, izazvana pojedinačnim dozama izazivaju usporavanje pokretljivosti, dok je u slučajevima hroničnih otrovanja, izazvanih multiplim dozama olova, zapaženo i povišenje LA miševa. U nekih životinja zapažane su mesecima značajne, većinom intermitirajuće, alteracije LA, dok je u drugih LA normalizovana u roku od 20 do 60 dana posle poslednje injekcije olovnog acetata. Bazofilne granulacije eritrocita pojavljuju se tek oko desetog dana posle otrovanja letalnim dozama olova, dok je značajna

alteracija LA zapažena već prvih dana posle davanja otrova. U eritrocitima miševa otrovanih subletalnim dozama otrova bazofilne granulacije nisu nađene. Diskutovane su mogućnosti primene lokomotometrije u cilju kontinuiranog merenja jačine bolesti usled otrovanja ili u cilju ocene kliničkog poboljšanja pod dejstvom raznih tretmana.

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*References*

1. *Terzin, A. L.:* Brit. J. Exp. Path., *49* (1968) 107.
2. *Castellino, N., Aloj, S.:* Brit. J. industr. Med., *21* (1964) 308.
3. *Schepers, G. W. H.:* Arch. Environ. Health, *8* (1964) 277.
4. *Spector, W. S.:* Handbook of Toxicology, Vol. I, W. B. Saunders Co., Philadelphia–London, 1956.
5. *Cecil, R. L., Loeb, R. F.:* A Textbook of Medicine, W. B. Saunders Co., Philadelphia–London, 1960.
6. *Sollmann, T.:* A Manual of Pharmacology, W. B. Saunders Co., Philadelphia–London, 1957.
7. *Stewart, C. P., Stolman, A.:* Toxicology, Vol. I, Academic Press, New York–London, 1960.

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