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Variations in rat biochemical parameters after buckshot implant

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Abstract

Twenty eight albino Wistar rats were implanted with two 100 mg lead spheres: 14 received the implant in the peritoneum (P) and 14 in the thigh (T). Variations in the activity of delta-aminolevulinic dehydratase (ALAD), of urinary delta-aminolevulinic acid (ALAU), of hematoporphyrins (HP) and of lead blood levels (BPb) were then determined at 30, 60 and 90 days with respect to basal values. Parallel determinations were performed by the same schedule in 7 rats implanted with two glass beads and in 8 sham animals receiving surgical incision alone. Techniques employed for ALAD were Berlin and Schaller; for ALAU, Tomokuni and Ogata; for HP, Piomelli; and for BPb, atomic absorption spectrophotometry. As indicators of lead presence, HP and ALAU proved better, both in P and in T rats. The replacement of lead buckshot for small game hunting by other less toxic elements is recommended.

Keywords: Biochemical parameters; Rat; Lead; Buckshot

1. Introduction

The hazard inherent in the traumatic introduction of lead particles into a living organism would seem to involve two aspects as far as the biological subject is concerned, although the mechanism by which the metal exerts its toxic action is presumably one and the same. Biological subjects may be broadly split into two classes:

1. man himself;
2. the remainder of warm-blooded mammals making up the ecosystem.

As regards such subjects, a number of queries may be posed.

(1) Faced with the dilemma whether or not to remove metallic particles lodged in man:

- Is the organism capable of compensating anatomically and functionally the biochemical alterations liable to be induced by the foreign body?
- Is it advisable to expose the subject to surgical trauma?
- What cost/benefit ratio may be established?
- How may toxic lead effects be evaluated?
- Which biochemical parameters are more suitable to evaluate toxicity?

(2) Regarding the ecosystem organisms mentioned above, when they survive the action of small or big game hunting (can it be considered really sporting?), the same queries may be posed together with others of a moral nature, such as to what extent it is lawful to subject animals that survive hunting to a noxious agent.

In the case of man himself, the aggressive use of lead bullets partially covered with other substances such as tinplate or bronze makes it difficult to evaluate the usefulness of intervention solely on the basis of determining the quantity and volume of missiles, since it is difficult to establish how the uncovered portion of the bullet is exposed.

Diverse studies carried out on human beings (which make up a second stage in this line of research), though incomplete from the biochemical point of view, have employed rather non-specific parameters such as reticulocytosis and basophil stippling.

The first paper on exposure to lead from firearm bullets is a review of 40 cases reported in the literature from the middle

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of last century up to 1936 [1]. Obviously, the diagnosis of lead impregnation was made by clinical observation and by poorly sensitive, non-specific laboratory assays, including blood lead by the old-fashioned methodology and erythrocyte determination by basophil stippling.

In 25 cases collected in a critical review by Magos [2], the presence of reticulocytes, erythrocytes with basophil stippling, hemoglobin, HP, BPb, UPb and UCP are taken into account, but only 4 cases include HP, BPb and another 4 UCP with BPb.

Furthermore, out of the 7 only cases studied within one year of the accident, HP and BPb were determined in 3, BPb and UPb in 2 and BPb alone in the remainder [3–8]. There is no information on temporal changes in these parameters.

Such paucity of data prompted us to study the variations in certain biochemical parameters in animals (albino Wistar rats) implanted with buckshot in the thigh and peritoneum.

The quantity of urine required for UCP determination becomes troublesome due to the number of samples needed, so that the ALAU test was adopted as it requires less volume. Besides, HP tests cover an aspect of heme metabolism.

However, since no reports have been found describing studies performed on rats, here albino Wistar rats have been employed in order to determine BPb, ALAU activity, HP and ALA pre- and post-implant of buckshot (as used for hunting).

2. Experimental

2.1. Equipment

A Varian atomic absorption spectrophotometer, model AA475 and carbon rod atomizer CRA 90.
An Aminco Bowman spectrophotometer.
A Shimadzu UV-VIS spectrophotometer, model 2100.

2.2. Reagents for analysis

Reagents indicated for original techniques for BPb, HP, ALAU and ALAD determination.

2.3. Materials for animal experiments

Buckshot 2 mm in diameter and 100 mg in weight.
Glass spheres 2 mm in diameter.
Suture materials mentioned in the text.

Male albino Wistar rats, weighing from 200 to 250 g, and kept in a room at 22.5°C with a 12 h day–night cycle. Animals were acclimatized for 15 days pretreatment and fed a conventional diet with water ad libitum.

2.4. Experimental design

A total of 43 animals was randomly distributed into 4 groups, as follows:

control group (C) (sham), $n=8$;
glass group (G), $n=7$;
thigh group (T), $n=14$;
peritoneum group (P), $n=14$.

Each Group T and P animal was implanted with 2 buckshot spheres, weighing 100 mg each, as follows:

thigh group (T): the implant was made by means of an incision in the posterior aspect of the thigh, with dissection of muscular layers of the crural triceps; sutures by planes were made with linen thread;

peritoneum group (P): the implant was made by means of a sagittal incision in the abdomen and later suture with linen thread on the anterior peritoneum, with closure by planes.

The glass group (G) received an implant in the crural triceps of two glass spheres washed in nitric acid, rinsed in plentiful distilled water and stove-dried, employing the same technique as for Group T.

In the control group (C) an incision was made similar to that performed in Group G, but without making any implant.

BPb, HP, ALAD and ALAU determinations were carried out 7 days before and again 30, 60 and 90 days after incision.

3. Methodology

3.1. Biochemical analysis

Blood lead (BPb) was determined by atomic absorption spectrophotometry. The technique consisted in extraction with methylisobutylketone of a lead complex with amino pyrrolydin diethyl dithiocarbamate. Ten μl were seeded in the atomization device in a graphite furnace [9].

Urinary delta-aminolevulinic acid (ALAU) was determined by the technique described by Tomokuni and Ogata, based on the appearance of a colored complex with *p*-dimethylamino benzaldehyde [10].

For hematoporphyrin (HP), the fluorometric technique described by Piomelli [11] was employed.

Delta-aminolevulinic dehydratase (ALAD) activity was determined by the technique described by Berlin and Schaller, adopted by the EEC [12].

3.2. Histopathology

Tissues were fixed in formol buffer and included in paraplast to perform the following light microscopy assays: hematoxylin–eosin, periodic acid–Schiff and Masson's trichrome.

4. Results

4.1. Biochemical parameters

Results are listed in Tables 1–4.

4.2. Histopathology

Macroscopic observation of lead implants in thigh showed granulomatous development. At light microscopy staining,

Table 1
Concentration of lead in blood (μg%)

	Control (C)	Glass (G)	Thigh (T)	Peritoneum (P)
Basal	6.3 ± 3.1	11.2 ± 6.1	14.6 ± 6.2	15.9 ± 9.3
1st month	6.3 ± 2.7	12.2 ± 6.1	31.9 ± 18.4	26.1 ± 16.0
2nd month	5.9 ± 2.9	10.7 ± 6.5	32.7 ± 12.5	26.5 ± 12.7
3rd month	6.0 ± 3.1	11.0 ± 5.9	30.5 ± 18.2	20.1 ± 12.7

Values are expressed as means ± SD.
P, G and C inter se: $p > 0.05$.
T: basal vs. 1st or 2nd month: $p < 0.05$; all other correlations: $p > 0.05$.
P: all correlations: $p > 0.05$.

Table 2
ALAD activity in U/l of erythrocytes

	Control (C)	Glass (G)	Thigh (T)	Peritoneum (P)
Basal	6.8 ± 2.5	5.3 ± 1.0	5.3 ± 1.1	5.9 ± 2.7
1st month	5.5 ± 2.6	4.7 ± 1.0	4.6 ± 1.3	3.1 ± 2.2
2nd month	6.0 ± 3.5	4.2 ± 0.7	2.8 ± 1.9	4.5 ± 1.2
3rd month	5.5 ± 1.9	4.7 ± 1.0	3.8 ± 1.2	4.8 ± 1.2

Values are expressed as means ± SD.
C and G inter se: $p > 0.05$.
T: basal vs. 2nd month: $p < 0.05$; 2nd vs. 1st month: $p < 0.001$; all other correlations: $p > 0.05$.
P: basal vs. 1st month: $p < 0.01$; all other correlations: $p > 0.05$.

Table 3
Concentration of hematoporphyrin (μg/ml)

	Control (C)	Glass (G)	Thigh (T)	Peritoneum (P)
Basal	23.8 ± 4.2	26.6 ± 4.1	21.9 ± 7.0	26.0 ± 8.4
1st month	22.0 ± 7.5	17.0 ± 1.7	36.1 ± 10.2	39.0 ± 15.3
2nd month	22.0 ± 2.6	26.8 ± 5.8	36.3 ± 13.8	38.0 ± 7.8
3rd month	23.1 ± 3.6	27.3 ± 5.2	35.4 ± 8.1	26.0 ± 9.5

Values are expressed as means ± SD.
C: all correlations inter se: $p > 0.05$.
G: basal vs. 1st month: $p < 0.05$; 1st month vs. 2nd or 3rd month: $p < 0.01$; all other correlations: $p > 0.05$.
T: basal vs. 2nd month: $p < 0.001$; basal vs. 1st or 3rd month: $p < 0.01$; all other correlations: $p > 0.05$.
P: 1st month vs. basal or 3rd month: $p < 0.01$; 2nd month vs. basal or 3rd month: $p < 0.05$; all other correlations: $p > 0.05$.

Table 4
Urinary aminolevulinic acid (μg/ml)

	Control (C)	Glass (G)	Thigh (T)	Peritoneum (P)
Basal	0.67 ± 0.47	0.54 ± 0.32	1.2 ± 0.5	0.8 ± 0.6
1st month	0.38 ± 0.40	0.38 ± 0.29	1.2 ± 0.5	1.8 ± 0.4
2nd month	0.36 ± 0.17	0.23 ± 0.25	1.9 ± 0.6	1.5 ± 0.3
3rd month	0.43 ± 0.16	0.26 ± 0.24	1.1 ± 0.4	1.1 ± 0.3

Values are expressed as means ± SD.
C and G: all correlations inter se: $p > 0.05$.
T: basal vs. 2nd month, 1st vs. 2nd month and 2nd vs. 3rd month: $p < 0.05$; all other correlations: $p > 0.05$.
P: basal vs. 1st or 2nd month, 1st vs. 3rd month: $p < 0.001$; 2nd vs. 3rd month: $p < 0.05$; all other correlations: $p > 0.05$.

inflammatory granulomas with giant cells and atrophic muscle tissue, were visualized in the presence of fibrous tissue (Fig. 1).

Granulomas were likewise observed in peritoneal implants, featuring newly-formed tissue with plentiful lymphocytes, vascularization and connective tissue (Fig. 2).

Fig. 1. Lead-implanted rat thigh showing inflammatory granulomas.

Fig. 2. Lead-implanted rat peritoneum showing granulomatous features.

Implants with glass beads disclosed minimal non-specific lesions.

5. Discussion

Recorded BPb values showed a marked increase at the first month in both lead-implanted groups. Although in peritoneum-implanted animals this proved to be the peak value, in thigh-implanted rats the maximum value was recorded at two months. Statistical significance was lower in the peritoneum group and the finding that levels remained high is explained by the plentiful vascularization of the resultant granuloma, which fosters greater interchange between the live organism and the foreign body. Sham and glass groups failed to display appreciable differences.

ALAD activity reached its minimum value at one month post-implant in the peritoneum group and at two months in the thigh group, concomitantly with the peaks found for lead in blood. In both cases, there were statistically significant differences for basal versus 2nd month and for 1st versus 2nd month values for the thigh-implanted group, but only for basal versus 1st month levels for the peritoneum-implanted group.

A similar decrease, though lesser in degree and lacking statistical significance, was observed in the sham and glass groups.

Hematoporphyrins (HP) were raised in both lead-implanted groups at the second month, to level off in the thigh group and to decline in the peritoneum group at the third month, with statistically significant differences in every case.

In the above determinations it should be pointed out that glass-implanted animals likewise displayed strikingly significant negative differences for the first month, a finding to be discussed later on.

Recorded ALAU values reproduced the pattern observed for ALAD activity, with raised levels at the first month in Group P and at the second month in Group T, in both cases showing highly significant differences, together with low significance for Groups C and G.

HP and ALAU values in Groups P and T proved particularly noteworthy as they increased significantly during the first two months, whereas levels in Groups C and G remained stable or dropped with variable statistical significance.

It may be assumed that the earliest values recorded for biochemical parameters in peritoneum-implanted rats are attributable to fast absorption by this route, a hypothesis confirmed by histopathological findings indicating greater vascularization of the granuloma originating in the peritoneal implant.

The reversal in the trends shown by BPb and ALAD values, both in peritoneal and thigh implants, prompted us to deimplant buckshot at 105 days and subject developed granulomas to light microscopy obervation, whose results are given above under the respective heading.

Out of all biochemical parameters studied, it may be inferred that the best evaluators of peritoneal implant effect are ALAU and HP, while the most satisfactory indicators of thigh implant effect are HP at three months post-implant and ALAU only at the first and second months.

Blood lead values increased rapidly up to the second month to level off thereafter at a raised level, though with limited statistical significance.

ALAD activity, whose drop is widely regarded as an excellent indicator of lead impregnation effect, seems of limited value as there is also a decrease in glass-implanted and even in sham animals, though without reaching statistical significance.

The finding that lead implanted in this way is expressed by relatively long-lasting effect indicators suggests that this metal may be advantageously replaced by another less toxic element for hunting sports.

6. Abbreviations

C	control group
G	glass-implanted group

T	thigh-implanted group
P	peritoneum-implanted group
BPb	blood lead
HP	hematoporphyrin
UPb	urine lead
ALAD	delta-amino-levulinic acid dehydratasey
ALAU	delta-amino-levulinic acid in urine
UCP	urinary coproporphyrins

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