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THE EFFECT OF EXPERIMENTAL LEAD POISONING ON SOME ENZYMATIC ACTIVITIES OF THE KIDNEY

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SUMMARY

The alterations of lactate dehydrogenase and its isoenzyme fractions, of glucose-6-phosphate dehydrogenase, and of glutamate dehydrogenase were studied in the kidneys of 12 guinea pigs poisoned with a daily dose of 120 mg/kg body weight of lead nitrate given by means of an oesophageal catheter, and in the kidneys of 12 guinea pigs made ischemic by partial constriction of the renal artery. The investigation was made on homogenates of kidneys *in toto* and on homogenates of cortex and medulla separately.

In the kidneys of lead-poisoned guinea pigs and in the kidneys with ischemia as a result of stenosis of the renal artery, similar metabolic alterations were observed. In both experimental situations a marked increase in type M isoenzymes of lactate dehydrogenase (LDH_4 and LDH_5), an increase in glucose-6-phosphate dehydrogenase up to values twice those found in normal kidneys, and a marked reduction in glutamate dehydrogenase activity were observed. The increase of glucose-6-phosphate dehydrogenase activity was only moderately prevalent in the cortical zone, and the other enzymes examined did not undergo alteration of their percentage distribution between cortex and medulla.

These results are interpreted as indicating a metabolic adaptation of the kidney to a condition of tissue hypoxia, in both experimental situations.

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The following abbreviations are used:
G6PD: Glucose-6-phosphate dehydrogenase (D-Glucose-6-phosphate:NADP oxidoreductase E.C. 1.1.1.49).
6PGD: Phosphogluconate dehydrogenase (6-Phospho-D-gluconate:NADP oxidoreductase E.C. 1.1.1.44).
LDH: Lactate dehydrogenase (L-Lactate:NAD oxidoreductase E.C.1.1.1.27).
LDH_1, LDH_2, LDH_3, LDH_4, LDH_5: Lactate dehydrogenase isoenzymes.
GlDH: Glutamate dehydrogenase (L-Glutamate:NADP oxidoreductase E.C.1.4.1.4).

INTRODUCTION

Renal involvement in human and experimental lead poisoning is clearly established by pathological and clinical findings.

In the acute stage of lead poisoning, the onset of renal damage has been attributed to acute ischemia[1–8], with transitory and reversible reduction of the renal blood flow and of the glomerular filtration rate. Moreover, the presence of amino aciduria is an indication of early tubular damage, which has also been demonstrated by microscopic and ultramicroscopic studies[9–12]. The prevalence of renal insufficiency in subjects exposed for many years to lead is proof that lead-nephropathy can evolve towards an irreversible nephroangiosclerosis[2,3,5–7,13–15].

In the last few years, several studies carried out on kidneys made ischemic by partial constriction of the renal artery, have drawn attention to alterations of enzymatic activities related to the metabolism of pyruvate and glucose-6-phosphate. Hess and Pearse[16] showed, by histochemical methods, an increase in G6PD and 6PGD activities in the macula densa of ischemic kidneys. This finding has been confirmed by Gross and Hess[17], Fisher[18], Hess and Regoli[19], Peart[20] and Vander[21]*. A positive correlation between an increase in G6PD activity in the macula densa, hypertrophy of the juxta-glomerular apparatus, increase in juxta-glomerular granularity, and a rise in renin synthesis have been clearly demonstrated in the ischemic kidney[20,22–27]. More recently, in clamped kidneys a significant increase was demonstrated in the M-type isoenzymes of LDH (LDH_4 and LDH_5)[28,29].

Therefore, in the kidneys of guinea pigs poisoned with lead nitrate, we have examined the activities of those enzymes known to be modified by renal ischemia. We compared the LDH isoenzymatic pattern and G6PD activity in guinea pig kidneys made ischemic by constriction of the renal artery and in the kidneys of guinea pig poisoned with lead nitrate. At the same time we studied the activity of GlDH, an enzyme localized in the mitochondria, which are the site of the main cellular oxido-reductive processes. Since, at least as far as G6PD is concerned, the alteration in activity has been observed previously only in the macula densa, our investigation was performed not only on whole kidney, but also on cortical and medullar homogenates of the same organs.

MATERIALS AND METHODS

Male guinea pigs, weighing 400–500 g were divided in three groups of 12 each.

The first was used as control. The second was subjected to ligature of the right renal artery as described by Schroeder *et al.*[30]. The degree of stenosis was standardized, as advised by these authors, by inserting a 0.5-mm diameter metal filament between the renal artery and the silk thread, which was gently removed after the knot had been tied. The animals were sacrificed by decapitation after 20 days, and only those animals exhibiting a weight reduction of clamped kidney below 35–40% in respect to the contralateral kidney were utilized for our experiments. This result was obtained in 80% of the animals treated.

* Due to the low specificity of histochemical methods used the true identity of these enzymatic activities is uncertain: in fact, Capelli *et al.*[27] propose a more generic denomination, *i.e.* "NADPH tetrazolium reductase".

The third group was poisoned, in accordance with the method of Pernis *et al.*[31], with a daily dose of 120 mg/kg body weight of lead nitrate given by means of an oesophageal catheter. All animals were decapitated after 40–50 days of treatment.

The kidneys were dissected longitudinally into equal parts, one of which was used for the studies on the kidney *in toto*. In the other half, the cortex and medulla were separated. The samples of tissue were cut into thin slices which were then twice dipped in isotonic NH_4Cl at 4° in order to hemolyze the red cells and were afterwards washed in Tris-buffered saline. The organs were finally homogenized at 4° with 9 volumes of buffered saline (v/v) in a Potter–Elvejhem tissue grinder. The homogenates were twice frozen and dried. The protein content was determined by Lowry's method.

Enzyme determinations

Lactate dehydrogenase (E.C.1.1.1.27—LDH) was determined by Warburg's optical test according to the method of Bergmeyer *et al.*[32]. The values were expressed as micromoles NADH/min/mg protein. The identification of LDH isoenzymes was made by electrophoresis on Cellogel, using Veronal 0.06 *M*, pH 8.6 buffer. The isoenzyme fractions were identified with a method based on the reduction of tetrazolium salts according to Barnett[33]. The quantitative evaluation of the fractions was obtained by reading on Chromoscan photodensimeter (Joyce, Loeb).

Glucose-6-phosphate dehydrogenase (E.C.1.1.1.49—G6PD) was determined by the method of Löhr and Waller[34]. The values were expressed as μmoles NADPH/min/mg protein.

Glutamate dehydrogenase (E.C.1.4.1.4—GlDH) was determined according to Schmidt's method[35]. The values were expressed as micromoles NADH/min/mg protein.

EXPERIMENTAL RESULTS

Lactate dehydrogenase

The amount of LDH activity was found to be raised in the kidneys of both

TABLE I

QUANTITATIVE DETERMINATION OF LACTATE DEHYDROGENASE IN KIDNEYS OF NORMAL AND LEAD-POISONED GUINEA PIGS, AND IN KIDNEYS MADE ISCHEMIC BY PARTIAL CONSTRICTION OF THE RENAL ARTERY

The values of lactate dehydrogenase of kidney homogenate *in toto*, expressed as micromoles NADH/min/mg protein, and of the cortical and medullar fractions, expressed as a percentage of the total activity, are given.

Groups	*Kidney in toto*	*Cortex (%)*	*Medulla (%)*
Normal	6.8	41.5	58.5
SD ±	0.64	1.4	1.4
Lead-poisoned	9.5	39.7	60.3
SD ±	2.1	2.6	2.6
Ischemic	7.4	43.6	56.4
SD ±	3.2	1.5	1.5

Statistical evaluation:
Normal/Lead-poisoned $p < 0.001$.
Normal/Ischemic $p > 0.05$.

TABLE II

CHARACTERIZATION OF LDH ISOENZYMES IN KIDNEYS OF GUINEA PIGS

Normal and lead-poisoned guinea pig's kidneys and kidneys made ischemic by partial constriction of the renal artery were examined. Values are expressed as percentage of total activity.

Groups	LDH_1 (H H H H)	LDH_2 (H H H M)	LDH_3 (H H M M)	LDH_4 (H M M M)	LDH_5 (M M M M)
Normal					
Kidney *in toto*	37 (SD ± 4.2)	24 (SD ± 1.9)	20 (SD ± 2.0)	14 (SD ± 3.0)	5 (SD ± 2.3)
Cortex	37 (SD ± 6.7)	24 (SD ± 2.8)	19 (SD ± 1.5)	14 (SD ± 3.5)	6 (SD ± 3.0)
Medulla	37 (SD ± 10.5)	23 (SD ± 3.0)	20 (SD ± 4.4)	14 (SD ± 3.0)	6 (SD ± 2.6)
Lead-poisoned					
Kidney *in toto*	26 (SD ± 3.4)	22 (SD ± 2.6)	21 (SD ± 0.6)	19 (SD ± 2.5)	12 (SD ± 3.4)
Cortex	25 (SD ± 1.9)	22 (SD ± 2.6)	21 (SD ± 1.9)	19 (SD ± 1.2)	13 (SD ± 2.6)
Medulla	24 (SD ± 4.9)	25 (SD ± 3.8)	22 (SD ± 2.7)	18 (SD ± 2.5)	11 (SD ± 4.3)
Ischemic					
Kidney *in toto*	26 (SD ± 3.7)	22 (SD ± 3.7)	22 (SD ± 0.9)	19 (SD ± 3.3)	11 (SD ± 3.7)
Cortex	28 (SD ± 3.7)	23 (SD ± 2.0)	22 (SD ± 1.3)	17 (SD ± 1.7)	10 (SD ± 3.5)
Medulla	27 (SD ± 4.4)	22 (SD ± 2.3)	23 (SD ± 2.6)	18 (SD ± 3.0)	10 (SD ± 2.4)

experimental groups. However, a greater increase was observed in the lead-treated animals than in guinea pigs subjected to partial constriction of the renal artery (Table I). The average values obtained were 6.8 μmoles NADH/min/mg protein in the normal kidneys; 9.5 μmoles NADH/min/mg protein in the kidneys of the lead-poisoned guinea pigs; and 7.4 μmoles NADH/min/mg protein in the ischemic kidneys. The percentage distribution of this enzymatic activity in the cortex and medulla (Table I), was not modified with respect to controls.

In normal guinea pigs, the electrophoretic characterization of LDH isoenzyme fractions showed a prevalence of H-type isoenzymes (LDH_1 and LDH_2) in the kidney tissue *in toto*: in fact the fractions LDH_1 and LDH_2 accounted respectively for 37.2% and 24.2% of total activity; the LDH_3 fraction corresponded to 19.6% of total, while the fractions LDH_4 and LDH_5, were present in percentages of 13.5% and 5.1% respectively (Table II and Fig. 1). The distribution of isoenzymes of LDH in the

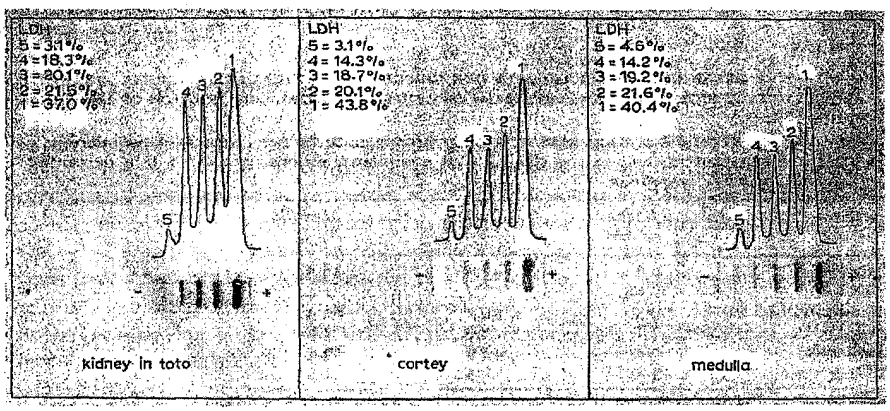

Fig. 1. Electrophoretic characterization of lactate dehydrogenase isoenzymes in kidneys of normal guinea pigs.

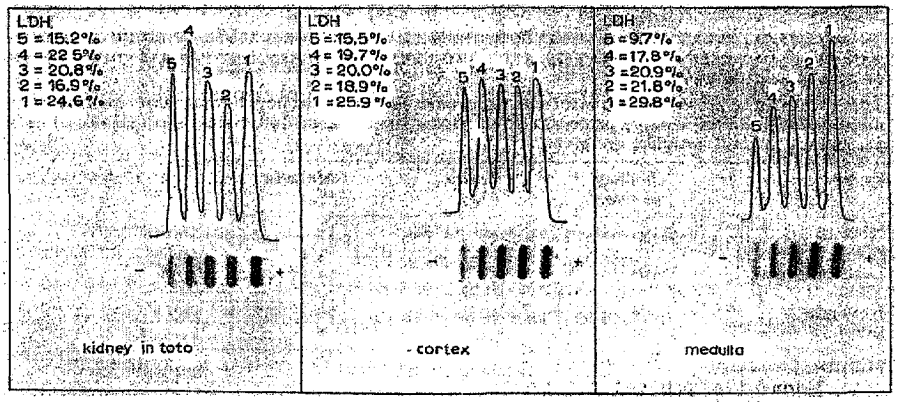

Fig. 2. Electrophoretic characterization of lactate dehydrogenase isoenzymes in kidneys of lead-poisoned guinea pig.

cortex and medulla was substantially the same as that observed in the kidney tissue *in toto*.

In the kidneys of the lead-poisoned guinea pigs we observed a marked increase in the activity of the M-type isoenzymes (LDH_4 and LDH_5); the LDH_4 activity was 18.5% and the LDH_5 activity was 12% of total activity. The increase in the activity of M-type LDH isoenzymes was of the same order in the cortex and medulla (Table II and Fig. 2).

In the clamped kidneys, a similar alteration in the pattern of LDH isoenzymes could be recognized, with a significant rise of M-type isoenzymes. In fact, in this group of animals LDH_4 activity in the kidney tissue *in toto* amounted to 18.8% and that of LDH_5 to 10.7% of total activity. As in lead-treated animals, the increase of LDH_4 and LDH_5 was of the same amount in cortex and in medulla (Table II and Fig. 3).

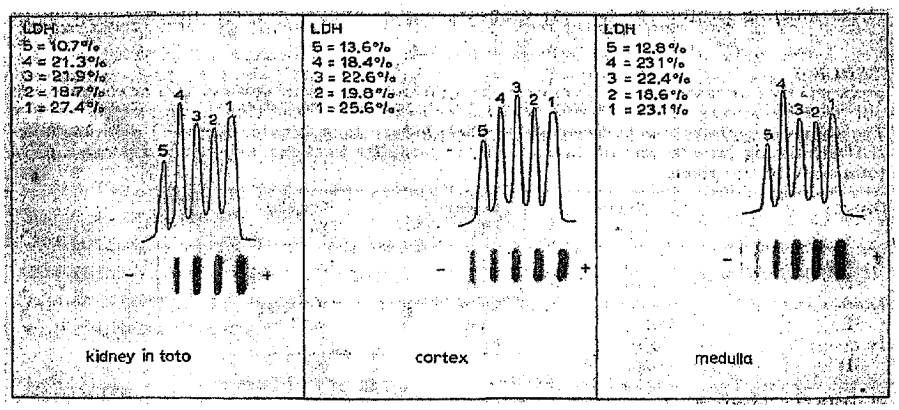

Fig. 3. Electrophoretic characterization of lactate dehydrogenase isoenzymes in guinea pigs' kidneys made ischemic by constriction of the renal artery.

TABLE III

QUANTITATIVE DETERMINATION OF GLUCOSE-6-PHOSPHATE DEHYDROGENASE IN KIDNEYS OF NORMAL AND LEAD-POISONED GUINEA PIGS, AND IN KIDNEYS MADE ISCHEMIC BY PARTIAL CONSTRICTION OF THE RENAL ARTERY

The values of glucose-6-phosphate dehydrogenase of whole kidney homogenate expressed as micromoles NADPH/min/mg protein, and of the cortical and medullar fractions, expressed as a percentage of total activity, are given.

Groups	*Kidney in toto*	*Cortex (%)*	*Medulla (%)*
Normal	51	43	57
SD ±	5.4	1.3	1.3
Lead-poisoned	107	48	52
SD ±	12.7	0.8	0.8
Ischemic	76	50	50
SD ±	11.4	2	2

Statistical evaluation:
Normal/Lead-poisoned $p < 0.001$.
Normal/Ischemic $p < 0.001$.

Glucose-6-phosphate dehydrogenase

G6PD evaluation in kidney tissue *in toto* showed a marked increase of this enzymatic activity in the kidneys of lead-poisoned guinea pig and in the kidneys made ischemic by constriction of the renal artery. The values obtained were $50 \cdot 10^{-3}$ μmoles NADPH/min/mg protein in normal kidneys, $107.2 \cdot 10^{-3}$ μmoles NADPH/min/mg protein in kidneys of lead-poisoned guinea pigs, and $75 \cdot 10^{-3}$ μmoles NADPH/min/mg protein in ischemic kidneys (Table III). As can be seen from Table III, in both experimental groups the rise in activity was present in the cortex and in medulla; however the increase was clearly higher in the cortex.

Glutamate dehydrogenase

GlDH evaluation in the kidney tissue *in toto* showed a significant reduction of this enzymatic activity both in the kidneys of lead-poisoned guinea pigs and in the kidneys made ischemic by arterial stenosis. The values obtained were 0.631 μmoles NAD/min/mg protein in normal kidneys, 0.484 μmoles NADH/min/mg protein in

TABLE IV

QUANTITATIVE DETERMINATION OF GLUTAMATE DEHYDROGENASE IN NORMAL AND LEAD-POISONED GUINEA PIGS, AND IN KIDNEYS MADE ISCHEMIC BY PARTIAL CONSTRICTION OF THE RENAL ARTERY

The values of glutamate dehydrogenase of whole kidney homogenate, expressed as micromoles NADH/min/mg protein, and of the cortical and medullar fractions, expressed as percentage of total activity, are given.

Groups	*Kidney in toto*	*Cortex (%)*	*Medulla (%)*
Normal	0.63	57.2	43
SD ±	0.19	5.5	5.5
Lead-poisoned	0.48	60.5	40
SD ±	0.15	3.5	3.5
Ischemic	0.48	64.2	36
SD ±	0.14	4.5	4.5

Statistical evaluation:
Normal/Lead-poisoned $p < 0.05$.
Normal/Ischemic $p < 0.05$.

kidneys of lead-treated guinea pigs and 0.482 μmoles NADH/min/mg protein in ischemic kidneys. The percentage distribution of this enzymatic activity in the cortical and medullar fractions of the kidneys shows a more marked reduction in activity in the medullar fraction in kidneys of both experimental groups; a distinct reduction in activity is however also observed in the cortical zone (Table IV).

DISCUSSION

The experiments here described show that in the kidneys of lead-poisoned guinea pigs and in the kidneys made ischemic by constriction of the renal artery, the same kind of alteration in enzymatic activities occurs. There is a marked increase in G6PD and LDH_4, LDH_5 activities and an evident reduction in GlDH activity, in both experimental groups.

In clamped kidneys of rats, an increase in G6PD activity was demonstrated histochemically in the macula densa by several authors[16,17–21], and has been related with an increase in renin synthesis.

Recently, Ambrosi *et al.*[36,37] demonstrated a hyperplasia of the juxta-glomerular apparatus and an increase in G6PD activity of the macula densa, also in the kidneys of lead-poisoned rabbits.

In these experiments we have been able to demonstrate that the increase in G6PD activity is not exclusively found in the kidney cortex, where juxta-glomerular apparatus is localized, but can be shown also in the medulla, in both experimental groups. Since G6PD is the enzyme leading into the hexose monophosphate shunt, we interpret this finding as an expression of kidney adaptation to hypoxia. Therefore the conspicuous increase in G6PD activity might be considered not only as an index of renin synthesis but also as an indication of a more general metabolic disorder caused by hypoxia.

The increase in M-type isoenzymes of LDH that we have observed in lead-treated and in ischemic kidneys is consistent with this hypothesis. It is known that the M-type LDH isoenzymes catalyze the pyruvate → lactate reaction and that their optimum activity occurs in conditions of low O_2 tension. The increased activity of these isoenzymes in the kidneys of lead-poisoned guinea pigs and in the kidneys made ischemic by constriction of the renal artery, can also be considered as an expression of metabolic adjustment of the tissue to cellular hypoxia. In such a condition, because of the reduced activity of the Krebs cycle, there is an accumulation of pyruvate in the tissue, which only an increased activity of M-type LDH isoenzymes can adequately metabolize to lactate.

The suggestion that in the kidney tissue of lead-poisoned guinea pigs the enzymatic alterations are related to a condition of hypoxia, is further demonstrated by the behaviour of GlDH. A marked reduction in this activity was observed in both the kidneys of lead-poisoned animals and in kidneys made ischemic by constriction of renal artery, and suggests the existence of a mitochondrial involvement in both experimental situations.

In conclusion, as regards the enzymatic activities investigated, an identical behaviour was observed in the kidneys of lead-poisoned guinea pigs and in kidneys made ischemic by constriction of the renal artery. Therefore, these experiments indicate that renal hypoxia occurs in experimental lead poisoning.

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