Br. J. exp. Path. (1977) **58**, 101

HISTOCHEMICAL AND HISTOLOGICAL EFFECTS OF LEAD ON THE LIVER AND KIDNEY OF THE DOG

D. J. WHITE*

From the Wellcome Research Laboratories, Langley Court, Beckenham, Kent

Received for publication September 22, 1976

Summary.—In a series of 3 experiments, beagle dogs were dosed orally with lead carbonate and the histochemical and histological changes in the liver and kidney assessed. Dosing at 50 mg/kg per day for 5 weeks resulted in well documented histological changes in the kidney and hydropic degeneration in the liver; significant alterations in the activity of the majority of enzymes studied were also seen in both organs. In dogs dosed for one week at 50 or 100 mg/kg no histological changes were seen and histochemical alterations were mainly confined to the dehydrogenases and NADPH diaphorase. A third group of dogs was dosed for 3 weeks; during a subsequent recovery period of almost 2 months the mild clinical effects produced by lead during the dosing period were quickly reversible except in 2 dogs. At the end of the recovery period histochemical alterations were evident in both organs of these 2 dogs principally shown by a reduction in the dehydrogenases of the liver.

The findings are interpreted as an effect by lead on a range of cellular enzymes particularly those involved in energy production, these effects being still demonstrable after an extended recovery period.

OVERT pathological effects of lead poisoning are mainly confined to the kidneys, brain, erythrocytes and haem synthesis (Goyer and Rhyne, 1973). At least some of the effects of lead are known to be due to enzyme inhibition and lead appears to act at a large number of biochemical sites (Ulmer and Vallee, 1969). For most enzyme systems, however, the concentrations of lead required for inhibition *in vitro* are reported to greatly exceed those found in the blood of individuals with clinical lead poisoning (Vallee and Ulmer, 1972). Histochemical observations on tissues of lead poisoned experimental animals provide an opportunity to examine effects on enzyme systems *in vivo*. The few reports of this type of investigation include studies on striated muscle and myocardium (Kosmider, Enek and Gzhibek, 1964), liver and kidney (Sroczynski and Zajusz, 1966) and liver (Zegarska and Zegarski, 1968).

The present series of experiments, using the dog, was designed to further investigate the effects of differing conditions of lead intoxication on a number of liver and kidney enzymes using histochemical methods. Provision was made for a study of the reversibility of changes induced by lead and, by using routine histological examination, a correlation was attempted between morphological and histochemical changes in these tissues.

MATERIALS AND METHODS

Three experiments were performed using 18 beagle dogs of less than 1 year of age. The animals were housed individually and fed a proprietary tinned dog food and biscuit meal and supplied with water *ad libitum*.

In Experiment A, 2 dogs (numbered 1♂ and 2♀) were dosed orally each day for 7 days with lead carbonate at a dose level of 50 mg of lead/kg of bodyweight. A further 2 dogs (3♂ and 4♀) were dosed for 7 days at a level of 100 mg of lead/kg of bodyweight. Two dogs (5♂ and 6♀) acted as undosed controls.

In Experiment B, chronic oral dosing of 2 dogs (7♂ and 8♀) was carried out with lead

* Present address: Beecham Pharmaceuticals (Research Division), Harlow, Essex.

carbonate at a daily dose level of 50 mg of lead/kg of bodyweight; because of marked deterioration in bodily condition they were killed after 5 weeks' dosing. Two dogs (9♂ and 10♀) acted as undosed controls.

In Experiment C, 4 dogs (11♂, 12♂, 13♀ and 14♀) were dosed orally with lead carbonate at a daily dose level of 50 mg of lead/kg of bodyweight for 3 weeks; a period of 7½ weeks was then allowed to elapse during which the dogs were not treated and they were killed at the end of this period. Four dogs (15♂, 16♂, 17♀ and 18♀) were left undosed as controls and killed at the same time.

All dogs were weighed once each week and their clinical condition was recorded daily.

At the termination of each experiment the dogs were deeply anaesthetized by intravenous injection of pentobarbitone and killed by section of the cervical blood vessels followed by exsanguination. A small piece of liver and kidney was removed and quenched in a mixture of iso-pentane and solid carbon dioxide. These tissues were sectioned immediately using a cryostat microtome and the sections stained for demonstration of the activity of the following enzymes: acid phosphatase (AcP) by a modified Gomori method with incubation for 20 min (Pearse, 1968*a*) and alkaline phosphatase (AlkP) by the Gomori method with an incubation time of 15 min (Pearse, 1968*b*); adenosine triphosphatase (ATPase) using lead nitrate with an incubation time of 30 min (Pearse, 1968*c*); glucose-6-phosphatase (G6Pase) by the method of Zugibe (1970) with glucose-6-phosphate as substrate and using lead nitrate with an incubation time of 30 min. The activity of isocitric, glucose-6-phosphate (G6P), β hydroxy butyric (βHB) and succinic dehydrogenases (dh) was demonstrated by the methods of Zugibe (1970) employing Nitro BT with NAD or NADP as co-enzyme and the appropriate substrate; the incubation time was 20 min in each case. In addition, sections were stained for NADH and NADPH diaphorase activity by the methods described by Zugibe (1970).

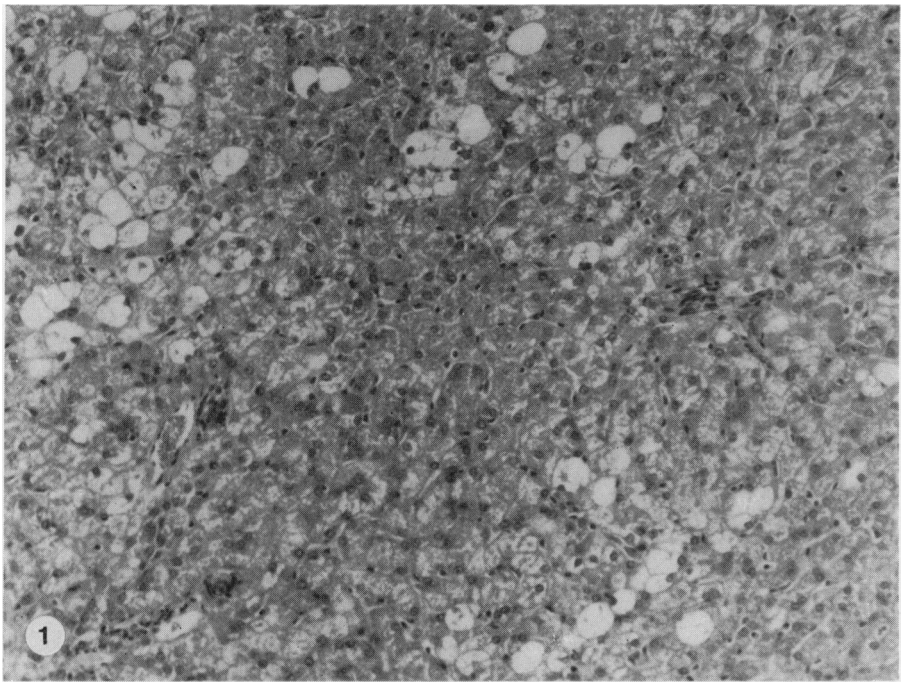
Cryostat sections were also stained for fat with Oil-red-O and for glycogen with periodic acid-Schiff. Specimens of liver and kidney were also fixed in 10% buffered formol saline, routinely processed, sectioned at 5 μm and stained with haematoxylin and eosin (H. and E.).

RESULTS

The clinical condition and bodyweight of the dosed dogs in Experiment A did not differ significantly from the controls. In Experiment B the bodyweight of the dosed dogs declined as the treatment period progressed and this was accompanied by a pronounced fall in bodily condition; at the end of the experiment each dosed animal had an emaciated appearance and weighed only just over half as much as its respective control. In Experiment C all dosed dogs suffered a slight check in bodyweight gain during the dosing period with some loss of condition. During the ensuing "recovery" period the bodyweight and appearance of 2 dogs (11♂ and 13♀) became similar to the controls; in the case of 12♂ and 14♀ slow recovery occurred but neither dog had fully regained its bodily condition by the end of the experiment.

The histochemical results for each experiment expressed as a comparison of the intensity of the reaction with the respective controls are shown for each dosed dog in Table I for the liver and in Table II for the kidney.

The only evidence of pathological change to the liver and kidney, as shown in H. and E. sections of fixed material, was seen in the dogs of Experiment B. In the livers the change consisted of the presence of groups of distended, vacuolated hepatocytes often with pyknotic nuclei; these cells were predominantly located in the midzone of the lobule and were considered to be undergoing hydropic degeneration since no fat was demonstrable. These cells also lacked enzyme activity; however, it was noteworthy that the increased activity of all enzymes in the liver, with the exception of NADPH diaphorase where a reduction was seen, was most marked in the cells adjacent to those showing degenerative changes; the increased activity of glucose-6-P dh and β hydroxy butyric dh was especially marked. Increases in liver acid and alkaline phosphatases were only recorded in this experiment, the former being mainly seen as an increase in Kupffer cells showing activity and the latter as widespread canalicular, and occasionally cellular, activity especially in periportal and midzone areas. In H. and E. sections of the kidneys of the dogs in this experiment



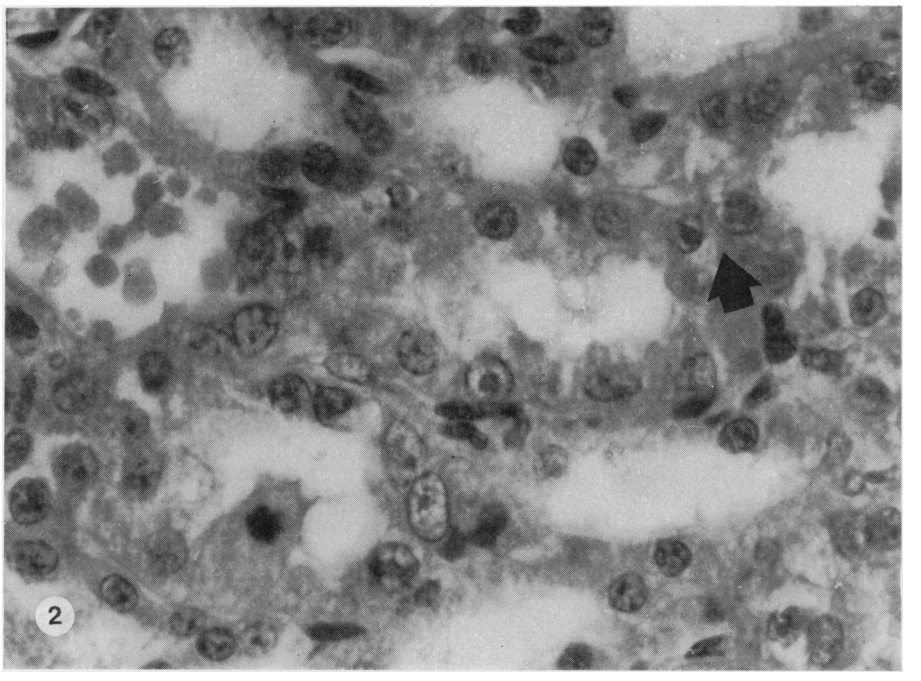


FIG. 1.—Liver of dog 7♂ (50 mg/kg of lead for 5 weeks) showing hydropic degeneration predominantly in midzone. H. and E. × 200.
FIG. 2.—Kidney of same dog showing degenerative changes in the straight proximal tubules. Arrow indicates nuclei with inclusions. H. and E. × 500.

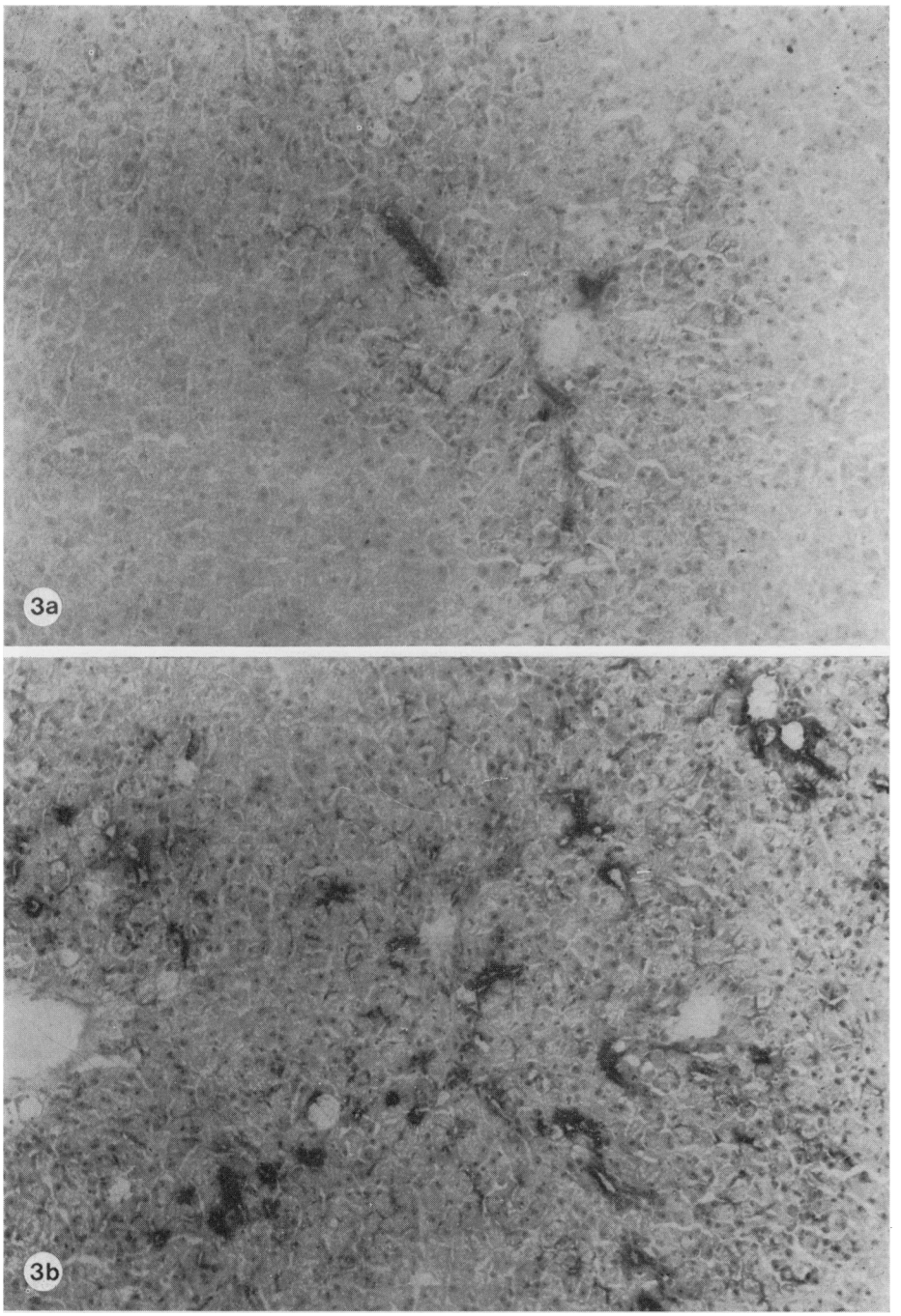


FIG. 3.—Activity of alkaline phosphatase in the liver. × 125. (a) Dog 10♀ (control). Activity virtually confined to vessels in periportal area. (b) Dog 8♀ (50 mg/kg of lead for 5 weeks). Increased activity especially in periportal area.

TABLE I.—*Summary of Histochemical Changes in the Livers of Dogs Dosed with Lead Compared with Control Dogs*

	Expt. A Dosed for 1 week 50 mg/kg		Expt. A Dosed for 1 week 100 mg/kg		Expt. B 50 mg/kg for 5 weeks		Expt. C 50 mg/kg for 3 weeks and killed 7½ weeks later			
Enzyme	1♂	2♀	3♂	4♀	7♂	8♀	11♂	12♀	13♂	14♀
AcP	0	0	0	0	INC +	INC ++	0	0	0	0
AlkP	0	0	0	0	INC ++	INC ++	0	0	0	0
ATPase	0	0	0	0	INC +	INC ++	0	0	0	0
G6Pase	RED +	RED +	0	INC +	0	INC +	0	RED +	0	RED +
NADH diaphorase	0	0	0	0	0	0	0	RED ++	0	RED +
NADPH diaphorase	INC ++	0	INC +	0	RED +	RED +	0	RED +	0	0
Isocitric dh	INC +	INC +	INC ++	INC +	0	INC ++	RED +	RED +++	0	RED ++
G6P dh	0	0	INC +	INC +	INC +++	INC +++	0	RED ++	0	RED +
βHBdh	INC ++	RED +++	INC +++	INC +++	INC ++	INC ++	0	RED ++	0	RED +
Succinic dh	INC +	0	INC ++	INC +	ND	ND	0	RED +	0	RED +
Glycogen	0	0	0	0	0	0	0	INC ++	0	INC ++

Key to changes: INC = Increase in intensity of reaction compared to controls; RED = Reduction. Degree of change: 0 = no difference from controls; + = slight difference; ++ = moderate difference; +++ = marked difference. ND = Not done.

mild degenerative changes were seen in the straight proximal tubules of the inner cortex. The changes consisted of cytoplasmic vacuolation of the lining epithelial cells with small numbers of desquamated cells in the lumens; in many of the epithelial cells the nuclei were grossly enlarged with a vesicular appearance and the nuclei often contained large eosinophilic inclusion bodies. Histochemical findings consisted of a reduction in the activity of all enzymes, mainly affecting the proximal tubules and especially involving acid and alkaline phosphatases and G6P dehydrogenase.

In Experiment A, the effects on the liver of short term dosing of lead resulted mainly in increases in activity of the dehydrogenases, the changes in enzyme activity being more apparent in the dogs given the higher dose level. The alteration in activity of β hydroxy butyric dh was especially marked. In the case of glucose-6-phosphatase both dogs at the low dose level showed a slight decrease in activity but without any change in G6P dehydrogenase. In the kidneys, changes in activity were sporadic except that in all dosed dogs there was a slight reduction in NADPH diaphorase in the proximal tubules.

Significant changes in enzyme activity in the livers of the dogs of Experiment C were almost entirely confined to the 2 dogs (12♂ and 14♀) whose bodily condition had still not returned to normal at the end of the "recovery" period. The changes consisted of a reduction in the activity of the dehydrogenases and glucose-6-phosphatase and were accompanied by an increase in glycogen. The reduction in isocitric dh was marked and was also seen in a third dog. In the kidney no similar pattern of changes was seen and altera-

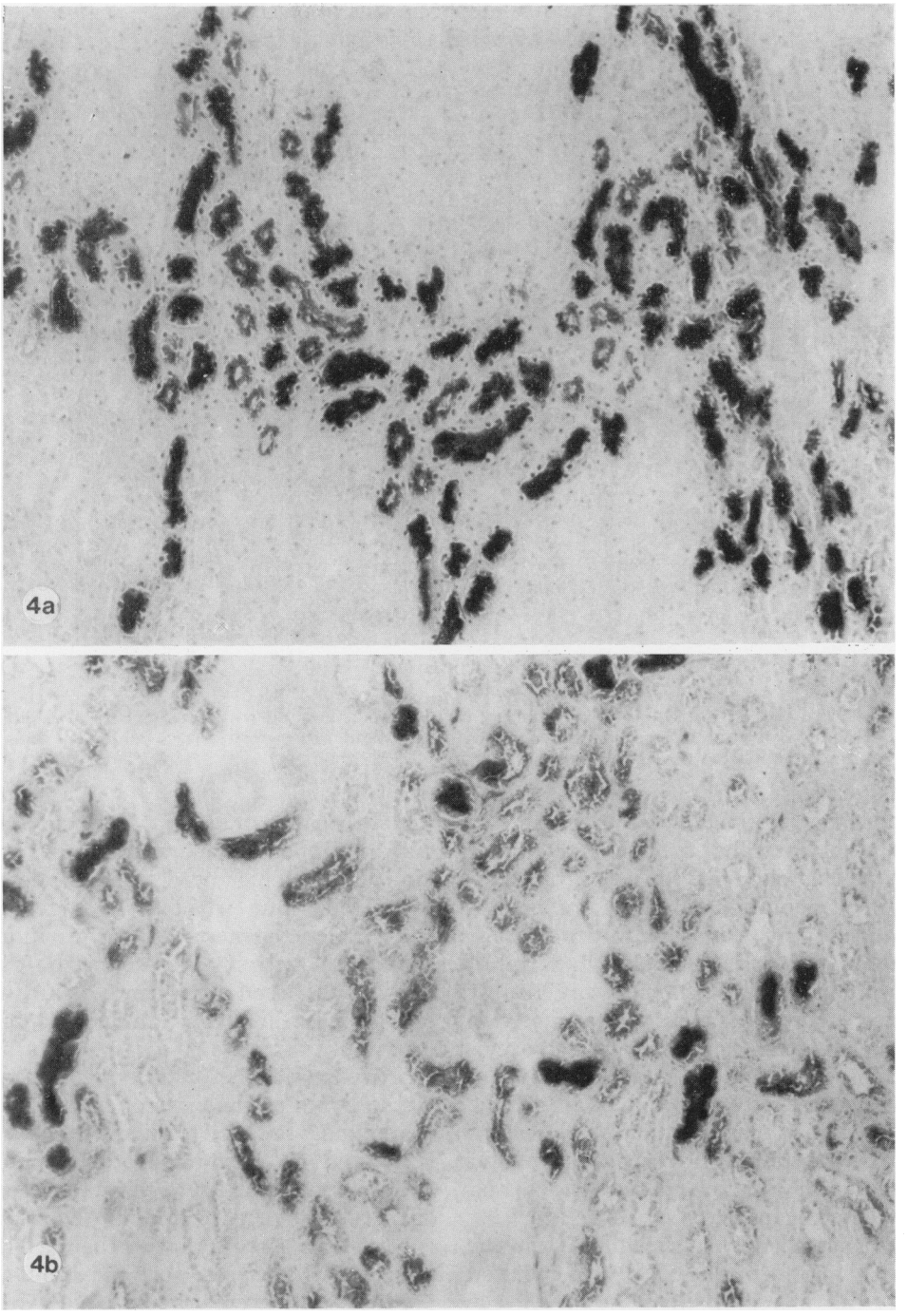


FIG. 4.—Alkaline phosphatase activity in the kidney. × 200. (a) Dog 10♀ (control). Inner cortex showing activity in brush borders of proximal tubules. (b) Dog 8♀ (50 mg/kg of lead for 5 weeks). Marked reduction in activity in the inner cortex.

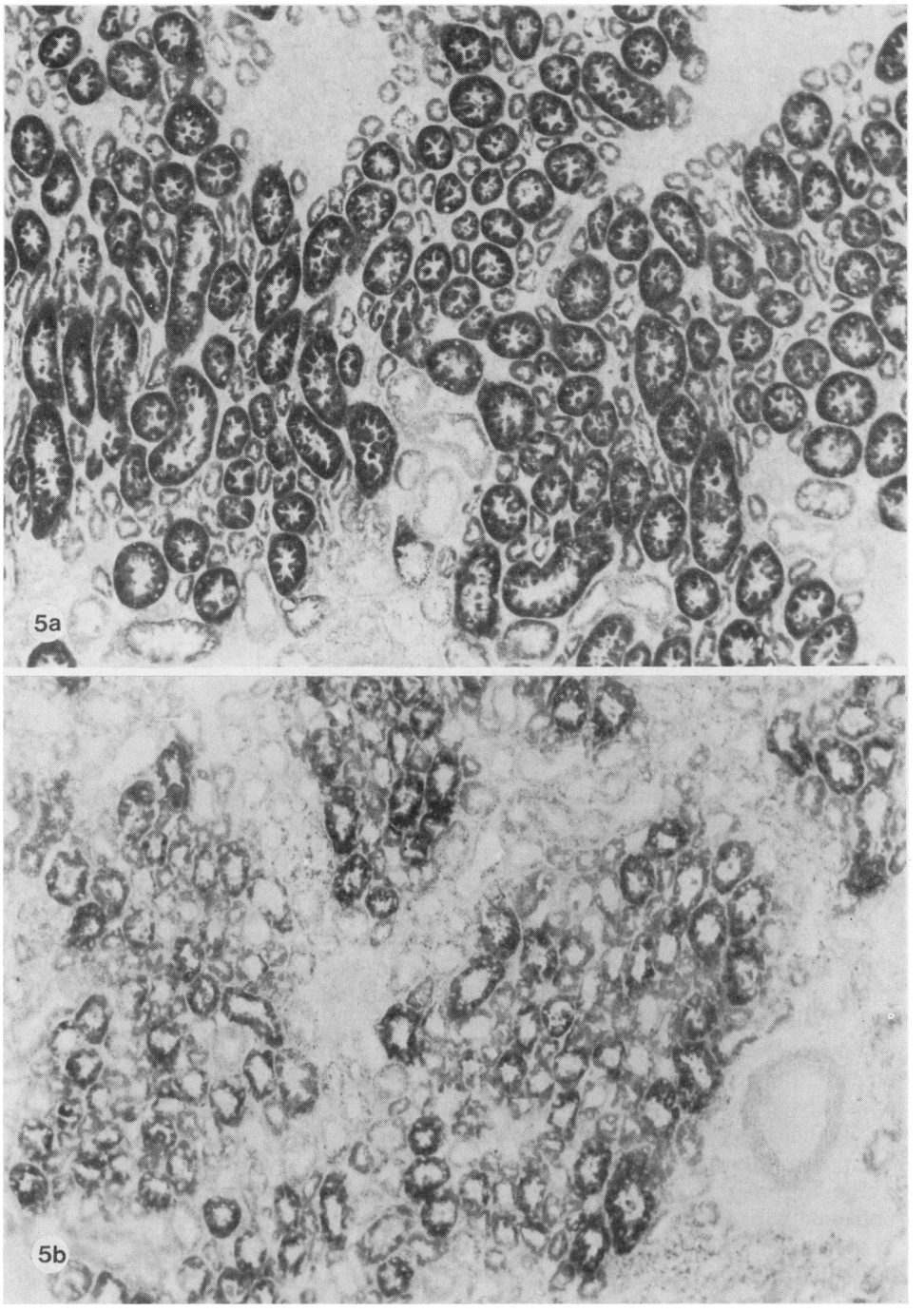


FIG. 5.—Activity of β hydroxy butyric dehydrogenase in the kidney. × 125. (a) Dog 9♂ (Control). Strong activity in proximal tubules of inner cortex. (b) Dog 7♂ (50 mg/kg of lead for 5 weeks). Proximal tubules showing marked reduction in activity.

TABLE II.—*Summary of Histochemical Changes in the Kidneys of Dogs Dosed with Lead Compared with Control Dogs*

	Expt. A Dosed for 1 week 50 mg/kg		Expt. A Dosed for 1 week 100 mg/kg		Expt. B 50 mg/kg for 5 weeks		Expt. C 50 mg/kg for 3 weeks and killed 7½ weeks later			
Enzyme	1♂	2♀	3♂	4♀	7♂	8♀	11♂	12♀	13♂	14♀
AcP	RED +	0	RED +	0	RED +++	RED ++	0	0	0	0
AlkP	0	0	0	0	RED ++	RED ++	0	INC +	0	0
ATPase	0	0	0	0	RED ++	RED ++	0	0	0	0
G6Pase	0	0	0	0	RED +	RED +	0	0	0	0
NADH diaphorase	0	0	0	0	0	0	0	0	0	RED +
NADPH diaphorase	RED +	RED +	RED +	RED +	RED +	RED +	0	0	0	0
Isocitric dh	RED ++	0	0	0	RED ++	RED +	0	0	0	0
G6P dh	0	INC +	0	INC +	RED ++	RED ++	INC +	INC +	0	RED +
βHBdh	RED +	0	0	RED +	RED ++	RED +	0	0	0	RED +
Succinic dh	0	0	0	0	ND	ND	0	0	RED +	RED ++

Key to changes: INC = Increase in intensity of reaction compared to controls; RED = Reduction. Degree of change: 0 = no difference from controls; + = slight difference; ++ = moderate difference; +++ = marked difference. ND = Not done.

tions in activity were sporadic and generally slight except that dog 14 ♀ showed a reduction in 3 of the dehydrogenases.

DISCUSSION

This series of experiments has shown that significant changes in enzyme activity occurred in the liver and kidney of dogs poisoned with lead not only where histopathological alterations were present but also in the absence of any changes observable with the light microscope. It has also been demonstrated that alterations in enzyme activity were still present in dogs poisoned subacutely almost 2 months after cessation of lead intake.

Frank histopathological effects on the liver and kidney were seen only in the chronic experiment and these were accompanied by the most pronounced changes in histoenzymatic activity. Increases in hepatic acid and alkaline phosphatases were only seen in this study thus providing further evidence for a toxic effect of lead (Thorbecke *et al.*, 1960). The much reduced activity of the phosphatases in the kidneys of these dogs formed only part of the generalized reduction in all enzymes and confirmed the histological observations of toxic effect on the cortical tubules. These findings are not fully in accord with those of Sroczynski and Zajusz (1966) and Zegarska and Zegarski (1968) but this probably reflects the importance of the timing of examination for activity in relation to treatment. The former dosed rabbits for 3 months and found that the activity of acid phosphatase in the liver, especially of emaciated rabbits, was much reduced and the activity of alkaline phosphatase showed no change; Zegarska and Zegarski found an increase in acid phosphatase and a marked reduction in alkaline phosphatase in rabbits dosed

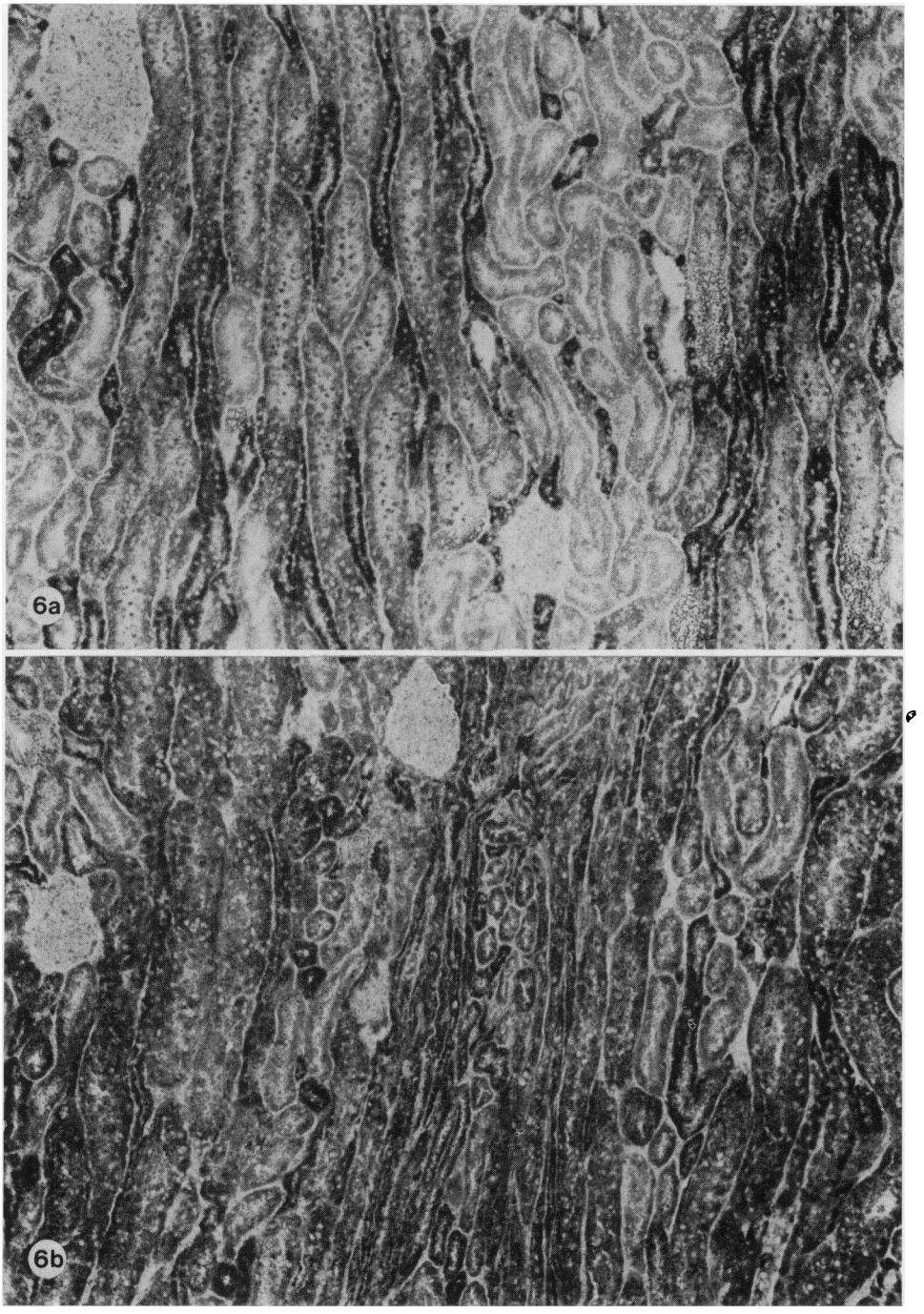


FIG. 6.—Activity of glucose-6-phosphate dehydrogenase in the kidney. × 125. (a) Dog 15♂ (Control). Activity mainly seen in straight tubules of the inner cortex. (b) Dog 11♂ (50 mg/kg of lead for 3 weeks, killed 7½ weeks later). Overall increase in activity in the inner cortex.

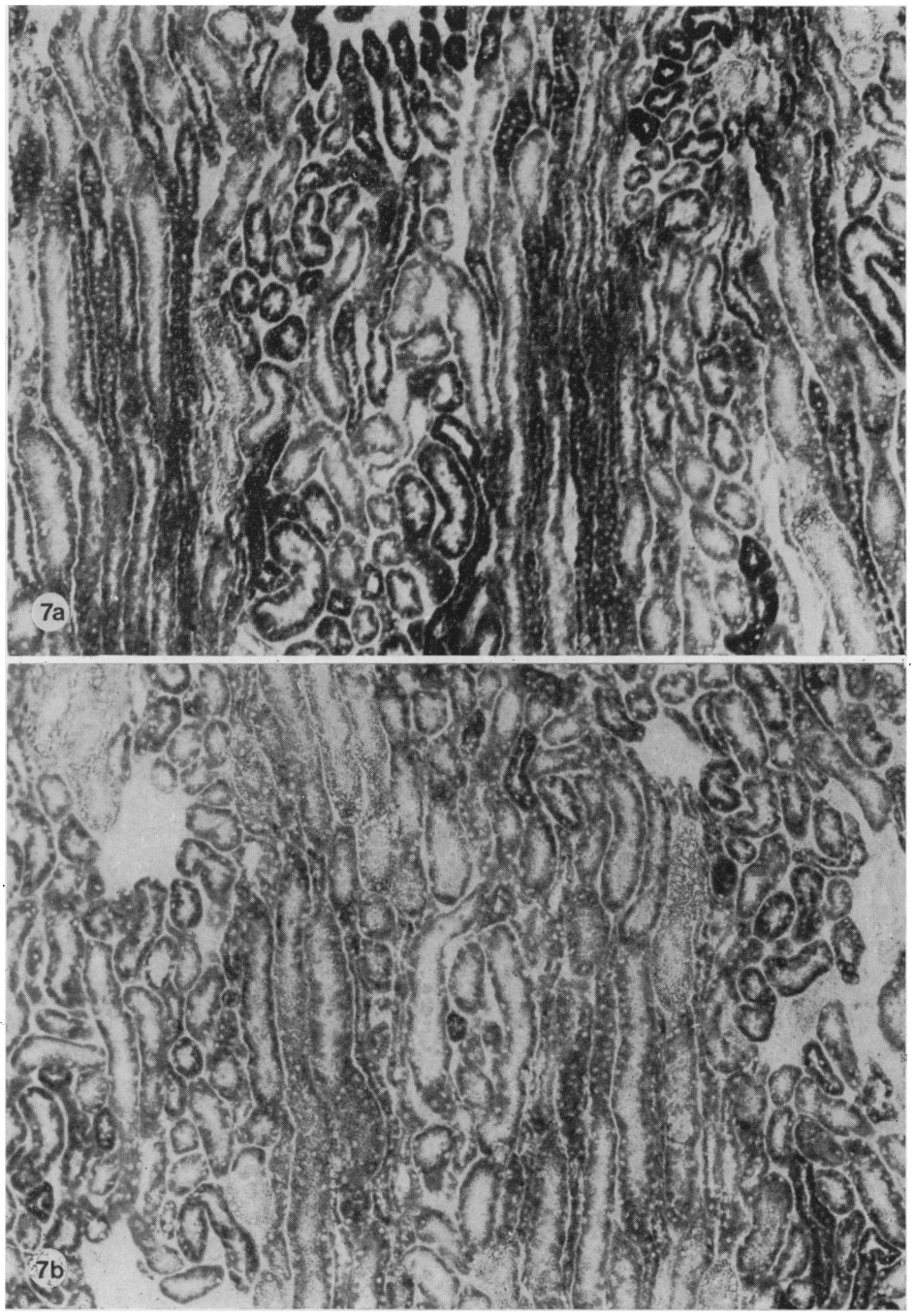


FIG. 7.—Activity of succinic dehydrogenase in the kidney. × ¡125. (a) Dog 18♀ (Control). Activity in the inner cortex mainly seen in the straight tubules. (b) Dog 15♀ (50 mg/kg of lead for 3 weeks, killed 7½ weeks later). Inner cortex showing overall reduction in activity.

intravenously with lead acetate for 10 days in the absence of histological damage. The observations by Sroczynski and Zajusz of a reduction of both enzymes in the kidney is, however, in agreement with the present findings.

The most consistent trend in all 3 experiments was the alteration in the liver of the activity of the dehydrogenases. In the 1-week and chronic experiments this took the form of an increase which, with β hydroxy butyric and G6P dehydrogenases, was sometimes marked; in the " recovery " experiment the dehydrogenases showed a reduction in activity particularly in isocitric dh. It is known that lead binds to and damages mitochondria (Goyer, 1968) leading to decreased respiration and partial uncoupling of oxidative phosphorylation (Teras and Kahn, 1966; Cardona, Lessler and Brierley, 1971) and there is also evidence for uptake of lead by other cell fractions with effects on microsomal enzymes (Castellino and Aloj, 1969; Alvares *et al.*, 1972). Other studies have demonstrated that lead is only slowly removed from these fractions and that it is particularly strongly bound to mitochondria (Barltrop, Barrett and Dingle, 1971). The most significant alterations in the present experiments have been seen in those enzymes predominantly located in the mitochondria and the effect of lead in the acute and chronic experiments can be interpreted as leading to an increase in oxidative mechanisms *via* the citric acid cycle with an increase in fatty acid oxidation. These changes were more marked at the dose level of 100 mg/kg compared with 50 mg/kg and were also marked in the chronic experiment. In this latter study it is difficult to dissociate the effects of cachexia from those of lead particularly in the case of G6P dehydrogenase which showed a marked increase; an increase in this enzyme mainly reflects the use of alternative pathways for energy production but its activity is also known to increase in liver damage with persistence of alterations long after the insulting agent is no longer present (Jones and Cohen, 1962). This persistence of effect was particularly evident in the kidneys of dogs on the " recovery " experiment. The evidence from these experiments suggests that lead exerted a primary effect on the mitochondrial enzymes but it cannot be said with certainty whether the generally mild effects on enzymes of the microsomal and soluble fractions, glucose-6-phosphatase and NADPH diaphorase, occurred secondarily to the effects on the mitochondrial enzymes or as a result of direct contact with lead. Alterations in hepatic glucose-6-phosphatase were slight and variable and in the subacute and chronic experiments showed no correlation with the activity of other enzymes or with any alteration in glycogen. In Zegarska and Zegarski's subacute experiments with rabbits, this enzyme increased slightly in the liver, but there was a concomitant marked reduction in glycogen and succinic dh. These authors also examined hepatic enzyme activity in rabbits allowed a 10 to 14-day recovery period and found that almost all enzymes had returned to normal. This is in contrast to the present study where after almost 2 months recovery, 2 dogs showed a reduction in all liver enzymes, except acid and alkaline phosphatases and ATPase, in the presence of an increase in glycogen. Neither of these animals had fully regained its bodily condition and these findings indicate the presence of a long lasting effect of lead on cellular metabolic pathways and thus on energy production. These observations were made in dogs which had been given moderately high doses of lead for a short period sufficient only to cause very mild clinical signs of intoxication. The possibility exists that a lower intake of lead over a longer period could result in similar effects especially as evidence was produced for a more marked effect on enzymes with an increasing dose of lead.

I would like to thank Miss D. Creasey for technical assistance and Mr D. Farebrother for helpful advice.

REFERENCES

ALVARES, A. P., LEIGH, S., COHN, J. & KAPPAS, A. (1972) Lead and Methyl Mercury: Effects of Acute Exposure on Cytochrome P-450 and the Mixed Function Oxidase System in the Liver. *J. exp. Med.*, **135**, 1406.

BARLTROP, D., BARRETT, A. J. & DINGLE, J. T. (1971) Subcellular Distribution of Lead in the Rat. *J. Lab. clin. Med.*, **77**, 705.

CARDONA, E., LESSLER, M. A. & BRIERLEY, G. P. (1971) Mitochondrial Oxidative Phosphorylation: Interaction of Lead and Inorganic Phosphate. *Proc. Soc. exp. Biol. Med.*, **136**, 300.

CASTELLINO, N. & ALOJ, S. (1969) Intracellular Distribution of Lead in the Liver and Kidney of the Rat. *Br. J. ind. Med.* **26**, 139.

GOYER, R. A. (1968) The Renal Tubule in Lead Poisoning. I. Mitochondrial Swelling and Aminoaciduria. *Lab. Invest.*, **19**, 71.

GOYER, R. A. & RHYNE, B. C. (1973) Pathological Effects of Lead. In *International Review of Experimental Pathology*, Vol. 12. Ed. G. W. Richter and M. A. Epstein. New York: Academic Press. p. 1.

JONES, W. A. & COHEN, R. B. (1962) The Effect of a Murine Hepatitis Virus on the Liver. An Anatomic and Histochemical Study. *Am. J. Path.*, **41**, 329.

KOSMIDER, S., ENEK, Y. & GZHIBEK, G. (1964) Histochemical Investigations of Alkaline Phosphatase, Acid Phosphatase, Adenosintriphosphatase and Diaphorase in Striated Muscles and in Myocardium During Acute Lead Poisoning. *Ghig. Trud. Prof. Zabolot*, **11**, 25.

PEARSE, A. G. E. (1968*a*) *Histochemistry: Theoretical and Applied.* 3rd ed., Vol. 1. London: J. & A. Churchill Ltd. p. 728.

PEARSE, A. G. E. (1968*b*) *Histochemistry: Theoretical and Applied.* 3rd ed., Vol. 1. London: J. & A. Churchill Ltd. p. 170.

PEARSE, A. G. E. (1968c) *Histochemistry: Theoretical and Applied.* 3rd ed., Vol. 1. London J. & A. Churchill Ltd. p. 721.

SROCZYNSKI, J. & ZAJUSZ, K. (1966) Histoenzymatic Reactions in Experimental Lead Poisoning in Rabbits. *Arch. Immunol. Ther. Exp.*, **14**, 391.

TERAS, L. & KAHN, H. (1966) Oxidation and Phosphorylation in the Liver in Lead Poisoning. *Vop. med. Khim.*, **12**, 41.

THORBECKE, G. J., OLD, L. J., BENACERRAF, B. & CLARKE, D. A. (1960) Acid and Alkaline Phosphatase in Mouse Livers during Various Conditions Modifying Activity of the Reticuloendothelial System. *Fed. Proc.*, **19**, 134.

ULMER, D. D. & VALLEE, B. L. (1969) Effects of Lead on Biochemical Systems. *Trace Subst. Environ. Hlth., Proc. Univ. Mo. Ann. Conf.*, 2nd, p. 7.

VALLEE, B. L. & ULMER, D. D. (1972) Biochemical Effects of Mercury, Cadmium and Lead. *Ann. Rev. Biochem.*, **41**, 91.

ZEGARSKA, Z. & ZEGARSKI, W. (1968) The Behaviour of some Enzymes in the Liver in the Course of Subacute Experimental Lead Poisoning. *Acta med. pol.*, **IX**, 119.

ZUGIBE, F. T. (1970) In *Diagnostic Histochemistry.* Saint Louis: The C.V. Mosby Company. p. 260.