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RENAL FUNCTION IN LEAD POISONING

BY

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Structural changes in the kidneys of animals poisoned by lead, notably enlargement and tubular degeneration, have been described frequently (Ophüls, 1915; Dilling and Haworth, 1929; Finner and Calvery, 1939; Fairhall and Miller, 1941; and others). The work of Fairhall and Miller (1941) suggests that the changes are at least partially reversible. Changes in function have been investigated less thoroughly. Albuminuria has been observed experimentally (Rambousek, 1909; Sumegi and Putnoky, 1939) and clinically (see Cantarow and Trumper, 1944, p. 94, for references), and Chiodi and Sammartino (1947) have described an increase in the effective tubular mass as estimated by the diodone clearance. The present work was performed in order to obtain further information about the changes in renal function which occur after the administration of lead salts.

METHODS

Albino rats, of initial weight about 250 g., were used. Eight females were used in a pilot experiment and thirty males in the main study. The rats were divided into two groups by taking alternate rats for each group. One group received lead acetate and the other sodium acetate by stomach tube. Initially, a dose of 35 mg. Pb (0.33 M.equiv.) per 100 g. as lead acetate was given three times a week, and an equivalent dose of sodium acetate was given to the control group; after 63 days (total 9.0 M.equiv./100 g.) these doses were increased fourfold and continued for a further 72 days. Eighty-nine days after the last dose of lead, dimercaprol was given to four rats of the group treated with lead; the dose used was 4 mg. (0.064 M.equiv.)/100 g., freshly dissolved in 0.9 per cent (w/v) sodium chloride solution, and it was injected intraperitoneally twice daily for five successive days. The four remaining animals in the same group were given control injections of sodium chloride solution.

Creatinine and p-*aminohippurate clearances*

Creatinine clearances were used as estimates of glomerular filtration rate, and *p*-aminohippurate (PAH) clearances at high plasma concentrations as an index of tubular excretory mass (Tm_{PAH}). A neutralized solution containing 4 per cent creatinine hydrochloride and 6 per cent PAH was injected subcutaneously. The dose given was 1.7 ml./100 g., and of this about half was injected into each flank. In order to ensure an adequate flow of urine during the collecting period, the rats were hydrated with 3 ml. of warm tap water per 100 g. body weight by stomach tube, 15 minutes before the injection of the clearance solution. Urine was collected for a period of 30 minutes, beginning 15 minutes after the injection of the clearance mixture; each rat was placed in a glass funnel from which urine drained into a graduated tube. At the beginning of the collecting period, the rats were stimulated to empty their bladders by blowing ether vapour at them. A single sample of 0.5 to 1.0 ml. blood was taken from the tail of each rat at the end of the collecting period.

Clotting was prevented with solid heparin. Creatinine concentrations in urine and in protein-free plasma were determined by Peters's modification (1942) of the alkaline picrate method. *p*-Aminohippurate concentrations were estimated and calculated by the method described by Goldring and Chasis (1944), based on the Bratton and Marshall reaction, in which method plasma proteins are precipitated with cadmium sulphate.

Measurement of arterial blood pressure

The systolic blood pressure was measured indirectly by the plethysmographic method of Byrom and Wilson (1938), using a modification of the apparatus, in which the plethysmograph and occluding cuff were incorporated in a single unit, ensuring a more satisfactory seal than the soft soap gland originally used. Since the slightest movement of the tail is transmitted to the water column whose sudden rise marks the end-point of the measurement, blood pressures were measured in the rats during light ether anaesthesia, just deep enough to allow two successive readings to be made before the righting reflex returned.

RESULTS

General condition

The animals in the group receiving lead continued to increase in weight while the lower dose was used, and after 63 days at this level of dosing, in which a total of 0.93 g. Pb was given per 100 g. body weight, the only evidence of absorption of lead was a raised porphyrin excretion in the urine of rats in this group. Increased porphyrin excretion has long been recognized as a feature of lead poisoning, and its appearance as the earliest clinical symptom in chronic lead poisoning was demonstrated by de Langen and ten Berg (1948) in human volunteer subjects. On the increased dose of lead, the rats became pale, and their haematocrits decreased. In a small number, a sharp fall in weight, accompanied by diarrhoea and a further fall in haematocrit, were observed. None of the rats died of lead poisoning, and after 72 days at this increased level of dosing, in which a further 4.2 g. Pb per 100 g. was given, dosing was discontinued.

Anatomical changes

Twelve rats, six from each group, were killed during the period of dosing for comparison of organ weights and for histological examination of the kidneys.

TABLE I

INCREASE IN THE FRESH WEIGHT OF SOME ORGANS IN ADULT MALE RATS TREATED WITH LEAD FOR 135–152 DAYS

Group	No. of rats	Mean wt. in g.	Fresh weight in g. per 100 g. body weight ± S.E. (adrenals in mg. per 100 g.)					
			Kidneys	Liver	Heart	Spleen	Lungs	Adrenals
Lead acetate	4	263	1.332 ±0.079	4.59 ±0.18	0.323 ±0.010	0.289 ±0.025	0.412 ±0.007	18.5 ±1.6
Sodium acetate	4	290	0.762 ±0.033	3.34 ±0.19	0.286 ±0.011	0.227 ±0.017	0.406 ±0.020	16.2 ±0.7
Significance of the increase in organ weights*		*t*	6.3	4.51	2.43	1.95	0.26	1.27
		P	<0.001	<0.01	>0.05	$\leqslant 0.1$	$\geqslant 0.8$	<0.3

* Smaller differences in the same direction were observed in the organ weights of two pairs of female rats killed 84 days after the first dose of lead.

These rats were killed in sets containing two treated and two control animals, one set 84 days after, the second 135 days after, and the third 152 days after the first dose of lead. The only gross differences in the two groups *post mortem* were the pale anaemic appearance of the viscera of the leaded animals, the blackness of the faecal material in their recta and colons, the enlargement of their kidneys, and, in the last four lead-treated animals, some enlargement of their livers. The fresh weights of the principal organs are given in Table I. The differences between the mean kidney weights in the two groups, and between the mean liver weights, are both highly significant. The mean weights of the hearts, adrenals, and spleens also showed a tendency to enlargement of these organs in the lead-poisoned rats, but only in the hearts are the differences significant. Similar, but slighter, changes were observed in two pairs of female rats killed after 84 days' exposure to lead.

Pathology of the kidneys

Early stage.—After lead-treatment for 84 days the kidneys were diffusely enlarged, but microscopic examination revealed little change. The tubules in the lower two-thirds of the cortex often showed some degree of epithelial swelling, the cytoplasm being unevenly vacuolated while the nuclei in the more severely affected tubules were in stages of karyolysis. These tubules appeared, for the most part, to be the descending parts of the first convoluted tubules in Henle's loops. The second convoluted tubules were sometimes similarly affected. No further changes were observed at this stage.

Later stage.—At 135 and 152 days the histological changes were advanced and similar at both periods of time. The glomeruli and blood vessels were normal. Severe degeneration of the tubules was evident, principally in the deep cortex towards the boundary zone. The first convoluted tubules were not obviously affected, but their straight portions forming the proximal limbs of loops of Henle were often grossly altered (Fig. 1). Stages of degeneration, even necrosis involving

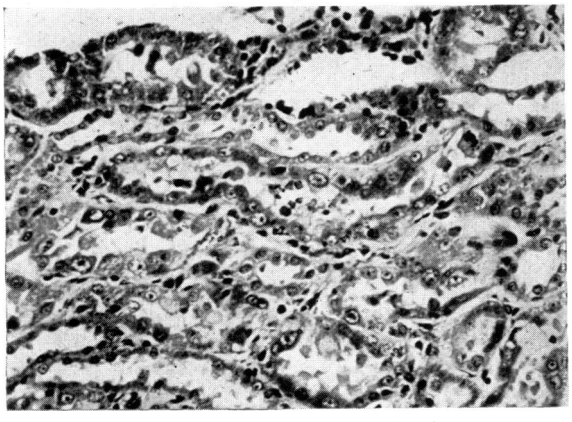

FIG. 1.—Kidney of rat killed after 135 days' exposure to lead. Loops of Henle, to show irregularity of tubular epithelium, disparity in size of nuclei, and small granules (dark) of refractile material in lumen of central tubule. H. and E., × 275.

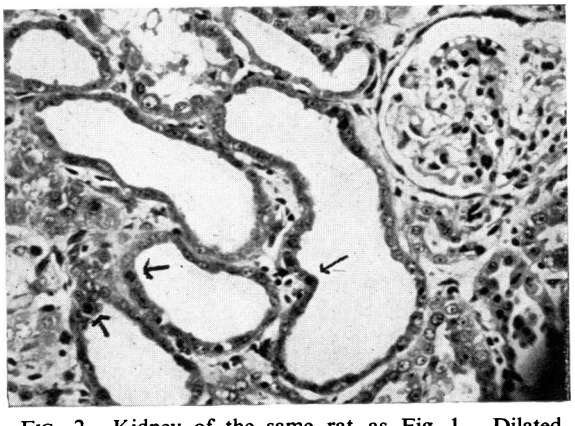

FIG. 2.—Kidney of the same rat as Fig. 1. Dilated second convoluted tubules with regenerating epithelium. Mitoses indicated by arrows. H. and E., × 290.

short strips of epithelium, were associated with evidence of regeneration (mitoses). The lumina contained desquamated cells and debris. The cells were sometimes heaped up upon one another in a disorderly fashion so that the lumen was indistinct. From the loss of eosinophilia in these cells in addition to the other abnormal features it was often impossible to determine which part of the loop of Henle was involved. The nuclei of these cells were often abnormally large, and some contained eosinophil inclusions of Cowdry's type B.

The second convoluted tubules were often similarly altered. In places they were greatly dilated, with a flattened regenerating epithelium (Fig. 2). Both in these cortical parts of the nephron and also in collecting tubules there were frequently groups of small, brightly refractile yellowish concretions of apparently inorganic deposits. The smallest of these sometimes occupied the cytoplasm of the epithelial cells; the larger, which were often concentrically laminated, lay in the lumina. The possibility that these contained lead was tested by fixing the kidney in Regaud's fluid, but no specific yellow coloration was obtained. By von Kossa's silver nitrate method they were negative for calcium.

No changes were found in the interstitial tissue apart from focal infiltration with small lymphocytes in relation to the most damaged tubules.

Blood pressure

The blood pressures of thirteen rats in each of the two treatment groups were measured at intervals during the experiment, twice before giving the first dose of lead and six times during the period of giving lead. The last set of measurements was taken 115 days after the first dose was given and 127 days after the first set of measurements. Table II gives the means and standard deviations of both groups

TABLE II

BLOOD PRESSURE OF RATS TREATED WITH LEAD ACETATE

Group of rats	Number of animals	Mean and standard deviation of the arterial blood pressures, in mm. Hg							
		Before treatment		Days from first dose					
				3	15	30	49	74	115
Lead acetate	13	116 ±11.5	112 ±7.1	114 ±19.1	122 ±15.6	119 ±18.3	126 ±9.9	117 ±13.6	112 ±11.7
Sodium acetate	13	113 ±10.3	109 ±12.5	120 ±7.4	123 ±10.5	125 ±13.5	126 ±14.7	115 ±15.3	119 ±9.4

of thirteen rats on each of the eight occasions that blood pressures were measured in the complete groups. The mean blood pressures rose slightly in both groups, from the average value before treatment of 115 mm. to 126 mm. by the 49th day of dosing. This increase was statistically significant ($t=3.56$, $P<0.01$), but it was seen in both groups, and there was no appreciable difference in the rate of increase in the two groups. It was therefore a non-specific effect, possibly due to handling or other parts of the experimental procedure. The pressure did not rise further, and after another 66 days of treatment the mean blood pressures were returning towards their original levels.

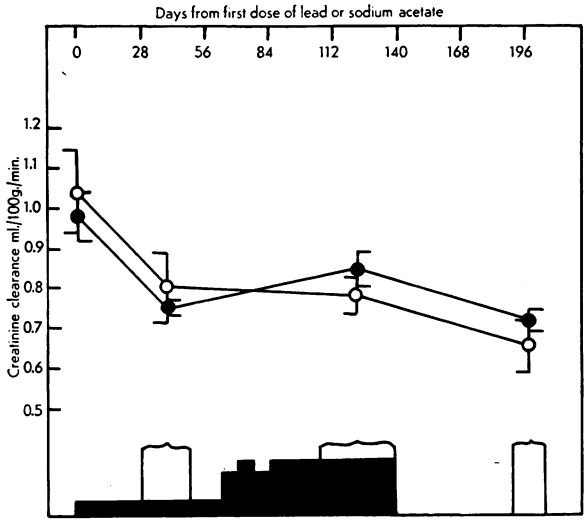

FIG. 3.—Creatinine clearances in rats before, during, and after administration of lead acetate, and in controls which received sodium acetate. Ordinates: Creatinine clearance ml./100 g./min. Abscissae: Days from first dose of lead or sodium acetate. ● Group of rats treated with lead acetate. ○ Group of rats treated with sodium acetate. Each point represents the mean and S.E. of estimations on 9–13 rats during the period indicated by the corresponding bracket on the abscissa. The solid black area represents the dose of lead acetate per week, and the period in which it was given.

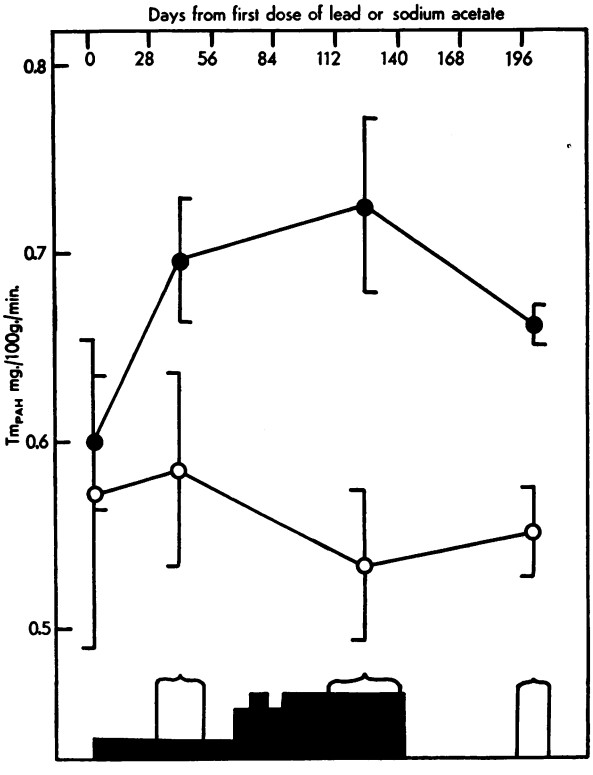

FIG. 4.—Tm_{PAH} in rats before, during, and after administration of lead acetate, and in controls which received sodium acetate. Ordinates: Tm_{PAH} mg./100 g./min. Abscissae: Days from first dose of lead or sodium acetate. Symbols as in Fig. 3.

Estimations of creatinine and p-*aminohippurate clearances*

Simultaneous creatinine and PAH clearances were estimated for each rat before giving lead, at intervals during both early and late phases of the period of dosing, and in the recovery period. In the earliest estimations, four rats were used in each experiment; in the later ones, six or eight were handled together, but in every one equal numbers of rats were taken from each group, each lead-poisoned rat being allotted a corresponding control rat for the whole series of observations. For each group, mean values have been calculated of those estimates made during each of the four periods, and the results are summarized in Figs. 3 and 4.

Creatinine clearances.—Before treatment with lead or sodium acetate the mean creatinine clearances of the two groups of twelve rats were 0.964 ml./100 g./min. and 1.029 ml./100 g./min. respectively. Subsequent estimates made while and after the animals were exposed to lead or sodium acetate were all lower (Fig. 3), but there was no systematic difference between the two groups and at no time was the difference between the two groups significant. These values were generally higher than those found in the literature (Smith, 1951). The reasons for this discrepancy are not certainly known, but since the control and lead-poisoned animals reacted similarly throughout, and since the negative results obtained are consistent with all the other available evidence that lead does not interfere with normal glomerular function, the matter has not been explored further.

After the 5-day course of dimercaprol, the mean creatinine clearance of four rats from the lead-treated group had risen to 0.941 ± 0.137 (S.D.). The treatment with dimercaprol, however, produced no significant effect on creatinine clearances, since in the remaining four rats of the same group at that time the mean clearance value was 0.848 ± 0.234 (S.D.). Unfortunately, animals of the control group which had received no lead were not examined at the same time.

PAH clearances.—In the control group, the tubular excretory mass was relatively constant throughout the series of experiments (Fig. 4); no significant differences were seen in the mean values estimated at intervals during, and after, treatment with sodium acetate. When lead acetate was given to the other group, the Tm_{PAH} value increased. By the end of the period of exposure to lead, the mean Tm_{PAH} was 21 per cent higher than its original value; the difference at this time between the mean Tm_{PAH} of the group treated with lead acetate and that treated with sodium acetate was highly significant ($P<0.01$). When the treatment with lead was discontinued, the increase in Tm_{PAH} was not maintained. After 55–71 days without lead, although the mean Tm_{PAH} in the lead-poisoned group was still higher than in the control group ($P<0.01$), the increase over the initial value was already approximately halved. This return to the original tubular excretory mass appeared to be complete in the four rats examined 100 days after the last dose of lead; their mean Tm_{PAH} was then 0.584 ± 0.058 (S.D.). Treatment with dimercaprol of the remaining four rats of the lead-poisoned group led to a more marked fall in Tm_{PAH}; the mean for these animals 100 days after the last dose of lead was 0.526 ± 0.063.

Changes in water diuresis

The water reabsorbing capacity of the tubules in water diuresis, and in response to vasopressin, was not examined in the rats of this series until the end of the period of treatment with lead. The results are reported separately and are included in

a more detailed examination of these functions in a further series of lead-poisoned rats (Pardoe and Weatherall, 1952).

Discussion

The kidneys in this series of lead-poisoned rats showed histological changes essentially similar to those reported for this species by earlier workers, i.e., the blood vessels in the organ, and the glomeruli, appeared to be normal, while there was evidence of damage to the tubular cells, particularly in the proximal limb of the loop of Henle, and in the distal convoluted tubules. Comparable changes were seen in functional activity; no hypertension or change in the glomerular filtration rate was detected, but there was an increase in tubular excretory capacity.

Where histological lesions have been examined in rats' kidneys in experimental lead poisoning the blood vessels in the organ have been found undamaged, even when hypertension was reported (e.g., Diaz-Rivera and Horn, 1945). The integrity of the vascular supply of the kidney in chronic lead poisoning is confirmed in this limited study, but the hypertension observed by Griffith and Lindauer (1944) and by Diaz-Rivera and Horn (1945) was not reproduced, even after four months, poisoning. Since these authors do not report the blood pressures of any control animals during the course of their experiments, it is impossible to assess from their figures how far the recorded rise is attributable to the administration of lead rather than to, e.g., handling of the animals. The relevance of changes produced by experimental procedure alone is suggested by the results in this series, where a small rise in blood pressure was seen after seven weeks' dosing, in both the lead-treated group and the control group. Some increase in weight of the heart was seen after chronic lead poisoning, but this appeared to be part of a generalized splanchnomegaly, as similar increases were found in the weights of the kidneys, liver, adrenals, and, in a later study (Pardoe and Weatherall, 1952), the alimentary canal.

After five months' continuous exposure to lead, no structural changes were observed in the glomeruli. This was consistent with the absence of an effect of lead on the creatinine clearance throughout this period. The method of measurement of creatinine clearance was not sufficiently sensitive to detect small differences in function, but was adequate to show that there was no serious impairment of filtration.

Tubular excretory activity of the kidneys, estimated as Tm_{PAH}, increased while lead was being administered to the rats. This extends the observation by Chiodi and Sammartino (1947) that, after removal of one kidney in rats, treatment with lead causes an exceptional increase in the tubular excretory mass of the remaining kidney, measured by diodone clearance. The reversibility of the change in tubular excretory function is shown by the fact that when administration of lead was discontinued the Tm_{PAH} value slowly returned to the pretreatment level. When this recovery was already well established, treatment with dimercaprol appeared to accelerate the process. However, the number of animals available for this study was small, and the treatment was given too late to warrant a more definite conclusion to be drawn from this indication.

It is not clear whether the increased Tm_{PAH} in lead poisoning was produced by an increased capacity of the transport mechanism in the existing tubular cells, or

by an increase in the number of functioning cells. Associated with the increase in tubular excretory function was an increase in the weight of kidneys. This increase in kidney weight in lead-poisoned rats is a consistent finding, and applies to both the wet and the dry weights. It is an early response of the organ to lead, as the increase in kidney weight reported by Chiodi and Sammartino appeared after only nine days' poisoning, and it has been found in acute lead poisoning in rats (Pardoe and Weatherall, 1952). The change in weight, like the change in tubular function, is reversible. The significance of the increase in kidney size, weight, and tubular function is difficult to assess; the change in weight, at least, has been shown by Chiodi and Sammartino to occur in adrenalectomized and hypophysectomized rats. Increase in kidney weight in the present chronically lead-poisoned rats was associated with a general increase in weight of several visceral organs, and may be the result of a less specific effect of lead on tissues.

Eosinophil inclusion bodies, which were seen in the nuclei of some of the renal tubular cells of the lead-poisoned rats, have been described before by Blackman (1936) in children and sometimes in experimental animals, and also by Dalldorf and Williams (1945) in rats. Despite their similarity to those found in virus infections, they are apparently not transmissible (Dalldorf and Williams, 1945), and their significance is doubtful.

No further information was obtained on the composition of the concretions seen in some of the cells and lying in the lumina of the tubules (Fig. 1). They resemble those described before in lead-poisoned rats by Diaz-Rivera and Horn (1945) and Eger (1937). Their appearance suggested that they were mineral, but they did not contain either calcium or lead. They were not seen in the kidneys of the rats killed after 84 days' exposure to lead, and as these kidneys were already enlarged it is unlikely that the concretions contributed to the changes in structure or function.

The segments of the kidney tubules which show the most marked histological changes after lead were the proximal limb of the loop of Henle and the distal convoluted tubules. Since the micropuncture studies of Walker, Bott, Oliver, and MacDowell (1941) have shown that tubular fluid in the proximal tubule is still in osmotic equilibrium with plasma, the function of facultative reabsorption of water has been attributed to some part of the tubule beyond this region, i.e., that part of the nephron which shows the most marked pathological changes in chronic lead poisoning. Although the actual segment of the distal tubule responsible for regulating water reabsorption is still disputed, it seemed likely from the pathological picture that the capacity for water reabsorption might be affected in lead poisoning. The clearance experiments of this series do not provide data by which this function can be measured, and studies on this subject are reported separately (Pardoe and Weatherall, 1952).

SUMMARY

1. Measurements of blood pressure, glomerular filtration rate, and tubular excretory mass have been made in a group of rats during prolonged treatment with lead acetate, and compared with measurements made in a control group receiving sodium acetate.

2. A comparison of the fresh weights of various organs of rats from the lead and control groups showed marked enlargement of the kidneys and livers, and some increase in weight of the hearts, spleens, and adrenals in the lead-poisoned rats.

3. Histological lesions were observed in the kidneys of the lead-poisoned rats, and appeared to affect mainly the proximal limb of Henle's loops and the distal convoluted tubules.

4. No significant rise in arterial blood pressure was observed in lead-poisoned rats, even after four months' exposure to lead. Reports of positive results are criticized on the ground of inadequately controlled observations.

5. Glomerular filtration rates showed no significant difference between the groups receiving lead acetate and the group receiving sodium acetate, throughout the period of dosing.

6. Tubular excretory capacity, assessed as Tm_{PAH}, rose in the group receiving lead, showing a statistically significant increase after prolonged dosing. This effect appeared to be reversible; 100 days after dosing with lead had ceased, the Tm_{PAH} had fallen to the pretreatment level. Treatment with dimercaprol possibly accelerated this recovery.

I am greatly indebted to Professor D. S. Russell for preparing the histological sections of the kidneys and for the account of their pathology. I should like to express my gratitude to Dr. M. Weatherall for his guidance in this work and to Miss H. Moore for technical assistance. The Hilger Spekker absorptiometer which was used for determinations of creatinine and *p*-aminohippurate was purchased with the aid of a grant (to Dr. M. Weatherall) from the University of London Central Research Fund.

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